

Repellent Effects of Five Monoterpenoid Odours Against Two Stored Product Insect Pests.

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ABSTRACT. Five monoterpenoids, (*R*)-linalool, 1,8-cineole, (*S*)-2-heptyl acetate, (*S*)-2-heptanol, and citral, which are natural components of the essential oils of *Aframomum melegueta* (K. Schum) and *Zingiber officinale* (Roscoe), were tested at the ratios in which they occur naturally against *Tribolium castaneum* (Herbst.), and *Rhyzopertha dominica* (Fab.) in a 4-way olfactometer. The results showed the repellent properties of the compounds as both beetles spent less time in the olfactometer arm containing the test stimuli. Linalool and (*S*)-2-heptanol were stronger repellent compounds than the others. With linalool, *T. castaneum* significantly preferred the control arms (spending 2.78 ± 0.22 min in them) compared to the test arm (1.22 ± 0.22 min.). For *R. dominica* the times spent were 2.87 ± 0.26 min in the control arms and 0.89 ± 0.28 min in the test arm. With (*S*)-2-heptanol, *T. castaneum* spent 1.23 ± 0.27 min in the test arm compared to 2.83 ± 0.23 min in the control arms. *R. dominica* spent 1.61 ± 0.24 min in the test arm and 2.69 ± 0.23 min in the control arms. For the number of entries or visits made, *R. dominica* made significantly fewer visits to linalool-treated arm ($P=0.018$) and (*S*)-2-heptanol-treated arm ($P=0.038$) compared to the control arms. These compounds could be suitable as repellents or fumigants against stored product pests in a small-scale farmer level.

INTRODUCTION

Early attempts to control stored product pests relied on methods such as: (i) mixing dry soil and wood ash with the grains to cause lethal dehydration of insects, and (ii) fumigating with plant-derived fumigants (Levinson and Levinson, 1998). Over the past few decades, application of synthetic, broad-spectrum pesticides and fumigants to control stored product pest species has been a standard practice. However, because of the increasing problems associated with the use of toxic synthetic pesticides such as human

and eco-toxicity, there is a pressing need for the development of safer, alternative crop protectants such as botanical insecticides, repellents and antifeedants (Isman, 2006; Ukeh, 2008).

Plant secondary compounds have been the subject of thorough investigation for the past 20 years in an effort to discover new sources of botanical insecticides, repellents and antifeedants (Akhtar and Isman, 2004). Generally, botanical insecticides cause less damage to human and environmental health than conventional insecticides. Many of them degrade rapidly and do not accumulate in the body and environment while some are very pest specific and do little or no damage to other organisms (Rozman *et al.*, 2007).

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Essential oils of botanical origin and their major components, often various monoterpenoids, are among the best-known substances to have attracted attention in recent years as potential pest control agents due to their insecticidal, repellent, and/or antifeedant properties (Shaaya *et al.*, 1997; Stamopoulos, 1991; Ukeh, 2008).

Monoterpenoids are known neurotoxins (Stamopoulos *et al.*, 2007) and most of them are volatile and can act as fumigants, thus offering the prospect of use against stored product pests. Papachristos *et al.* (2004) reported that vapours of essential oils extracted from various parts of rosemary, *Rosmarinus officinale* (L.), and eucalyptus, *Eucalyptus macrorhyncha* (F. Muell), had strong insecticidal activity against *Acanthoscelides obtectus* (Say). Ukeh and Umoetok (2007) also reported that volatile odours from the seeds of *Piper guineense* and *Monodora myristica* alone, and the seeds admixed with winter wheat, *Triticum sativum* L. grains at the ratio of 1: 9 (% w/w), significantly repelled both sexes of the lesser grain beetle, *Rhyzopertha dominica* (Fab.) adults [Coleoptera: Bostrychidae]. Ukeh (2008), reported that vacuum distilled essential oils extracted from alligator pepper, *Aframomum melegueta*, and ginger, *Zingiber officinale*, (both Zingiberaceae), were repellent to *R. dominica* when tested alone and in combination with maize, *Zea mays* L., and winter wheat seeds. Correlating these observed toxicity and repellence of essential oils with their chemical composition revealed that their insecticidal properties depended on total oxygenated monoterpenoid content (Stamopoulos *et al.*, 2007).

The present study was undertaken to investigate the repellent effects of five naturally-occurring monoterpenoids: (*R*)-linalool, 1,8-cineole, (*S*)-2-heptyl acetate, (*S*)-2-heptanol, and citral (neral + geranial), in the essential oil of *A. melegueta* and *Z.*

officinale, against the red flour beetle, *Tribolium castaneum* (Herbst.) [Coleoptera: Tenebrionidae] and *R. dominica*. Both species are serious pests of stored cereals, flour and dry cassava roots in tropical and subtropical regions. Cereals constitute the most important component in the diet of the majority of the people in the tropics and are usually stored to provide a food reserve as well as seed for planting (Obeng-Ofori and Reichmuth, 1997).

MATERIALS AND METHODS

Essential Oils, Liquid and Gas Chromatography

Seeds of *A. melegueta* and rhizomes of *Z. officinale* were collected from fields around Akamkpa (situated between latitude 500 and 515 North and longitude 804 and 825 East) in Cross River State, Nigeria, and dried in the shade to approximately 15% moisture content. Essential oils of both were obtained by vacuum distillation (< 0.05 mm Hg for 24 h; ES50 Vacuum Pump) as described by Pickett and Griffiths (1980) and Ukeh (2008). Aliquots of the essential oils were subjected to liquid chromatography through Florisil[®] (100-200 mesh). Re-distilled hexane and diethyl ether were used sequentially as eluants, to obtain fractions containing non-polar and polar compounds, respectively.

The behaviourally active chromatography fractions were analysed using a 6890N gas chromatograph (GC) (Agilent Technologies) equipped with a split-splitless injector (230°C) and flame ionization detector (FID). Hydrogen was the carrier gas. The GC was equipped with HP-5 capillary column (30 m x 0.3 mm id, 0.25 µm film thickness). The stereochemistry of identified chiral compounds was determined by enantioselective gas chromatography using authentic compounds following techniques described in Birkett *et al.* (2008). The enantioselective GC of behaviourally active

compounds from *A. melegueta* and *Z. officinale* Florisil[®] diethyl ether fractions was performed on a 5890A GC equipped with a β -cyclodextrin (Supelco beta-DEX[™] 120; 30 m x 0.25 mm i.d., film thickness 0.25 μ m) capillary column. Hydrogen was the carrier gas. Initially, a 1- μ l aliquot that contained equal quantities of both enantiomers of the chiral compounds in re-distilled hexane was injected onto the chiral GC, to establish that successful separation of enantiomers took place. This was followed by co-injections of the vacuum-distilled essential oil sample, first with an authentic standard of one enantiomer and then with the second enantiomer. Peak enhancement with either enantiomer confirmed the presence of that enantiomer in the vacuum distilled essential oil sample.

Coupled Gas Chromatography- Mass Spectrometry (GC-MS)

The GC-MS analyses of behaviourally active components of *A. melegueta* and *Z. officinale* Florisil[®] diethyl ether fraction were performed using a fused silica capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μ m, DB-5), fitted with an on-column injector, which was directly coupled to a magnetic sector mass spectrometer (Autospec Ultima, Fisons Instruments, Manchester, UK). Ionization was by electron impact (70 eV, source temperature 250 °C), and the carrier gas was Helium. The oven temperature was maintained at 30 °C for 5 min, and then programmed at 5 °C/min to 250 °C. Tentative identifications were made by comparison of spectra with mass spectral databases (NIST, 2005), and confirmed by peak enhancement on GC using authentic compounds.

Chemical Compounds

The compounds used in this study are listed in Table 1.

Table 1. Synthetic compounds tested for repellent activity against *T. castaneum* and *R. dominica*.

Compound	Source	Purity by GC (%)
(S)-2-Heptanol	Sigma-Aldrich, Gillingham, Kent	>99
(S)-2-Heptyl acetate	Synthesized from (S)-2-heptanol by acetylation	>99
(R)-Linalool	Botanix Ltd, Paddock Wood, Kent	>99
Citral (neral + geranial)	Fluka	>95
1,8-Cineole	Fluka	>99

Test Insects

Tribolium castaneum was obtained from the laboratory colony in Arthropod Functional Ecology and Behaviour Laboratory, University of Aberdeen, UK. *R. dominica* was obtained from a culture maintained by Central Science Laboratory, Sand Hutton, York, UK. *T. castaneum* was reared on untreated yellow maize grains purchased from Akim Foodstuff Market in Calabar, Cross River State, Nigeria, and *R. dominica* on wheat flour purchased from food merchants in Aberdeen. The insects were cultured in medium-sized heating laboratory incubator (Gallenkamp, UK) maintained at 25 °C, and 65% RH.

Bioassay Method

Behavioural bioassays were performed in an olfactometer modified after Pettersson (1970). The olfactometer, consisting of three layers of 6 mm thick transparent Perspex screwed together, was purchased from Rothamsted Research, Harpenden, UK. A four-pointed star-shaped exposure chamber was milled into a circular plate measuring 12 x 12 x 1.2 cm, with a hole (3 mm diameter) drilled into the walls at each of the four cardinal points. Another plate (10.2 x 10.2 x 0.6 cm) served as the floor and the third plate of the same size but with a hole (4 mm diameter) in its centre, served as a cover. Since *T. castaneum* and *R. dominica* cannot walk on smooth surfaces a sheet of Fisherbrand QL 100 filter paper (Springfield Mill, Maidstone, Kent, England) was used as a floor covering. The olfactometer side arms made of socket glass were inserted through the holes of the chamber walls. The olfactometer was housed in the behaviour room running at 25C, 65 % RH, and 12: 12 LD cycle illuminated by

fluorescent tubes but with no natural lighting.

The air stream through the olfactometer was supplied by the air entrainment system (KNF Neuberger, Germany) through Teflon tubing (Camlab Ltd., UK). Immediately after the pump, the air was divided through two charcoal filters to purify it. From each charcoal filter, the air stream was then further divided and pushed through two flow meters (GPE Ltd., Leighton Buzzard, UK) to give a total of four air flows going into the behaviour chamber. The air stream passed through a glass test arm containing stimulus-impregnated filter paper disc or through the control arm containing hexane-impregnated filter paper disc. Each arm's inlet was covered with net to prevent insect entry. The rate of airflow through each side arm was set at 200 ml min⁻¹.

The air streams formed four distinct zones in the chamber and this was confirmed by smoke tests. The air was pulled from the chamber at the rate of 800 ml min⁻¹ through the central hole in the cover plastic plate. Each bioassay was run for 10 min using a stopwatch, and was replicated 12 times using a fresh insect, stimulus source, and olfactometer. Odour sources (10 µl each) were the single compounds identified from the diethyl ether fraction of *A. melegueta* and *Z. officinale* vacuum distilled oils. They comprised (*S*)-2-heptanol (0.1 mg/ml), (*S*)-2-heptyl acetate (0.6 mg/ml), (*R*)-linalool (0.3 mg/ml) (from *A. melegueta*) in their natural ratios of 1:6:3, and 1,8-cineole (0.548 mg/ml) and citral (0.313 mg/ml) (from *Z. officinale*) in their natural ratio of 5.48: 3.13 in the essential oils. Equal amounts (10 µl) of the solvent and solvent plus appropriate concentration of each synthetic compound were impregnated into filter papers in 4-way olfactometer semiochemical bioassays. In control experiments, 10 µl hexane was tested against control arms containing clean filter paper discs. These concentrations were tested for repellency against 7-d old *T. castaneum*

and *R. dominica* of mixed sexes.

Data Collection and Analysis

A computer programme for collecting and analysing behavioural data with the four-armed olfactometer (OLFA programme, Francesco Nazzi, 33100 Udine, Italy) was used. The data recorded included time spent by each beetle in the different areas of the olfactometer and the number of visits into each area or odour zone. The time spent in each olfactometer arm was tested using a one-way analysis of variance (ANOVA) followed by comparison of means by Tukey's 95% simultaneous confidence intervals (MINITAB 15 Statistical Software). The number of visits to the odour-treated arm was compared with the number of visits to control arms using a Chi-square contingency table (Zar, 1999).

RESULTS AND DISCUSSION

GC-MS analysis of the behaviourally active compounds in Florisil® diethyl ether fraction of *A. melegueta* identified (*S*)-2-heptanol, (*S*)-2-heptyl acetate and (*R*)-linalool in the ratio of 1:6:3, whereas *Z. officinale* had 1,8-cineole, nerol and geranial in the ratio of 5.5:1:2 (Fig. 1). The responses of walking adult *T. castaneum* and *R. dominica* to the identified bioactive chemical constituents of *A. melegueta* and *Z. officinale* vacuum distilled essential oils in terms of the mean time spent and mean number of visits are shown in Tables 2 and 3, respectively. There was significant repellent effect in terms of time spent (Table 2) and the mean number of visits to the arms (Table 3). Going by the time spent in olfactometer arms, *T. castaneum* was significantly repelled by 10 µl hexane containing 0.3 mg/ml (*R*)-linalool ($P < 0.001$), 0.6 mg/ml (*S*)-2-heptyl acetate ($P = 0.039$), 0.1 mg/ml (*S*)-2-heptanol ($P < 0.001$), and 0.313 mg/ml citral ($P < 0.001$) but not by 0.548 mg/ml 1,8-cineole ($P = 0.207$) when compared

GC/GC-MS Analysis

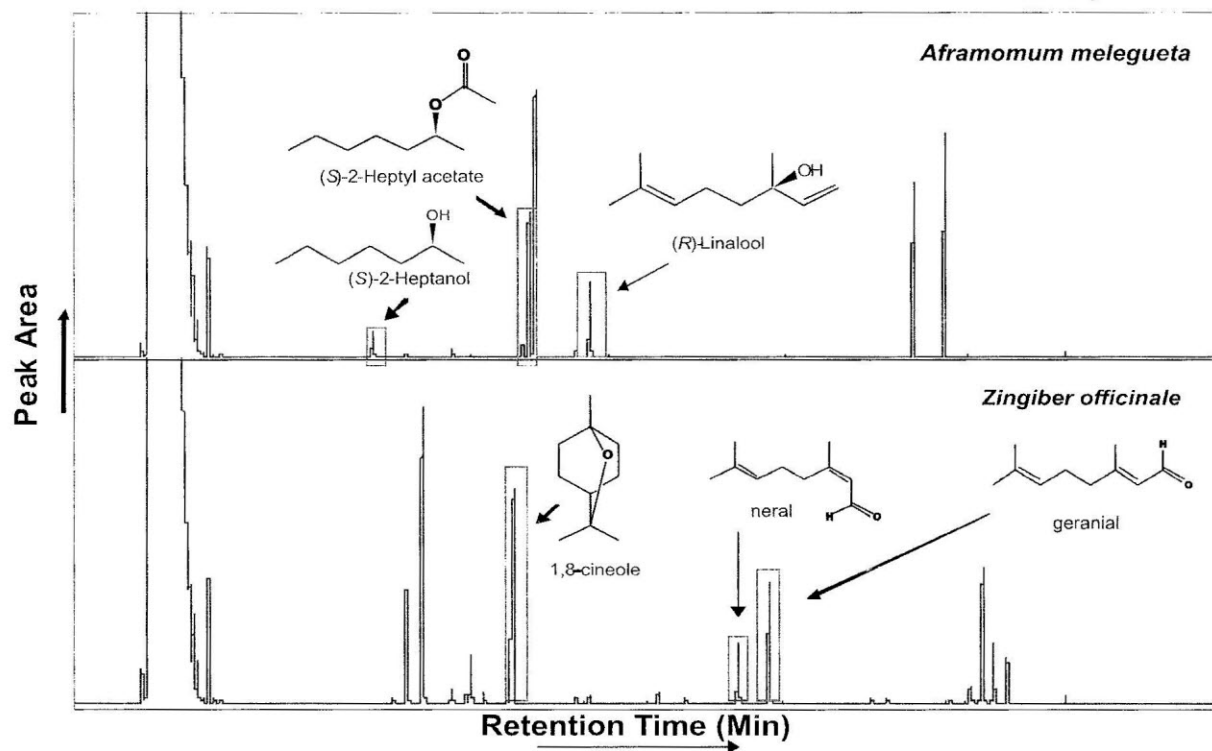


Fig. 1. Total ion chromatogram (TIC) showing bioactive compounds obtained by coupled GC-MS analysis of diethyl ether fractions of vacuum distilled *Aframomum melegueta* and *Zingiber officinale* essential oils.

with the control arms containing 10 μ l hexane. *R. dominica* was significantly repelled by all the synthetic organic compounds tested with the same concentrations of (*R*)-linalool ($P < 0.001$), (*S*)-2-heptyl acetate ($P < 0.001$), (*S*)-2-heptanol ($P = 0.002$), citral ($P = 0.047$) and 1,8-cineole ($P = 0.011$) when compared with the control arms (Table 2). For the mean number of visits, *R. dominica* significantly preferred the control arms to (*R*)-linalool ($\chi^2 = 10.1$, $df = 3$, $P = 0.018$) and (*S*)-2-heptanol ($\chi^2 = 8.42$, $df = 3$, $P = 0.038$) treated arms only. For all other bioassays, both *T. castaneum* and *R. dominica* did not show any significant ($P > 0.05$) preference for the test or control arms (Table 3).

Research has been undertaken in order to evaluate the potential of many local plant species to control insect pests during grain

storage in Africa (Bekele and Hassanali, 2001; Keita *et al.*, 2000; Tapondjou *et al.*, 2005). For example, the essential oil of *Xylopia aethiopica* at a concentration of 1 ml per 100 g maize seeds has been reported to cause 100% *S. zeamais* mortality in 24 h, while four of the major components, namely, α -pinene, β -pinene, δ -3-carene and terpinen-4-ol (according to their proportions in the essential oil) were responsible for 50% mortality (Kouninki *et al.*, 2005; 2007). Obeng-Ofori *et al.* (1997) found 1,8-cineole to be highly repellent and toxic to *S. granarius*, *S. zeamais*, *T. castaneum* and *Prostephanus truncatus* (Horn.). Plant oils have also been described as potentiation agents of monoterpenes such as 1,8-cineole, eugenol, and camphor for the protection of stored grains against damage by stored product beetle pests through mortality and inhibition of progeny production (Obeng-

Table 2. Behavioural responses of *T. castaneum* and *R. dominica* [measured as mean time spent in each arm (min \pm SE)], to bioactive compounds extracted from *A. melegueta* and *Z. officinale* in a 4-way olfactometer.

Test compound	Stimulus presentation	<i>T. castaneum</i>	<i>R. dominica</i>
Control	Solvent hexane	2.36 \pm 0.15a	2.34 \pm 0.15a
	Solvent control	2.58 \pm 0.23a	2.42 \pm 0.15a
	Solvent control	2.27 \pm 0.17a	2.48 \pm 0.16a
	Solvent control	2.21 \pm 0.21a	2.24 \pm 0.21a
(R)-linalool (0.3 mg/ml hexane)	Test	1.22 \pm 0.22a	0.89 \pm 0.28a
	Control 1	2.47 \pm 0.20b	2.58 \pm 0.26b
	Control 2	3.05 \pm 0.21b	3.35 \pm 0.30b
	Control 3	2.81 \pm 0.24b	2.68 \pm 0.23b
(S)-2-heptyl acetate (0.6 mg/ml hexane)	Test	1.81 \pm 0.28a	1.57 \pm 0.27a
	Control 1	2.65 \pm 0.29b	2.61 \pm 0.25b
	Control 2	2.62 \pm 0.22b	2.69 \pm 0.26b
	Control 3	2.46 \pm 0.21ab	2.80 \pm 0.25b
(S)-2-heptanol (0.1 mg/ml hexane)	Test	1.23 \pm 0.27a	1.61 \pm 0.24a
	Control 1	2.51 \pm 0.19b	2.73 \pm 0.23b
	Control 2	3.02 \pm 0.25b	2.55 \pm 0.19b
	Control 3	2.92 \pm 0.24b	2.80 \pm 0.28b
Citral (0.313 mg/ml hexane)	Test	1.18 \pm 0.24a	1.84 \pm 0.29a
	Control 1	2.75 \pm 0.22b	2.38 \pm 0.24b
	Control 2	2.92 \pm 0.23b	2.65 \pm 0.25b
	Control 3	2.75 \pm 0.24b	2.71 \pm 0.26b
1,8-Cineole (0.548 mg/ml hexane)	Test	2.09 \pm 0.24a	1.89 \pm 0.26a
	Control 1	2.42 \pm 0.22a	2.33 \pm 0.22ab
	Control 2	2.57 \pm 0.22a	2.72 \pm 0.22b
	Control 3	2.48 \pm 0.21a	2.67 \pm 0.23b

^a Means for each compound followed by the same letter in the same column are not significantly different at the 0.05 level as determined by Tukey's 95% Simultaneous Confidence Intervals.

Table 3. Mean number of visits to the olfactometer arms made by *S. zeamais* in response to bioactive compounds from *A. melegueta* and *Z. officinale* in a 4-way olfactometer.

Test compound	Species	Mean no. visits in		n	χ^2 ^b	P*
		T ^a	C			
Hexane	<i>T. castaneum</i>	4.08	3.72	12	1.02	0.796
	<i>R. dominica</i>	4.33	4.42	12	0.02	0.999
(R)-linalool (0.3 mg/ml)	<i>T. castaneum</i>	2.00	3.36	12	6.09	0.107
	<i>R. dominica</i>	1.58	3.00	12	10.1	0.018
(S)-2-heptyl acetate (0.6 mg/ml)	<i>T. castaneum</i>	2.17	2.86	12	2.19	0.534
	<i>R. dominica</i>	2.08	2.44	12	0.66	0.883
(S)-2-heptanol (0.1 mg/ml)	<i>T. castaneum</i>	3.00	2.67	12	3.98	0.264
	<i>R. dominica</i>	2.33	4.14	12	8.42	0.038
Citral (0.313 mg/ml)	<i>T. castaneum</i>	2.33	3.69	12	5.48	0.139
	<i>R. dominica</i>	2.75	3.42	12	1.38	0.710
1,8-Cineole (0.548 mg/ml)	<i>T. castaneum</i>	2.50	3.11	12	1.27	0.736
	<i>R. dominica</i>	2.17	2.89	12	2.06	0.56

(a) The test arm (T) contained 10 μ l hexane the stated concentration of a bioactive compound impregnated onto filter paper disc, while each control arm (C) contained 10 μ l hexane loaded on clean filter paper discs.

(b) χ^2 analysis was performed on the total number of visits (n = 12) into the test, control 1, control 2 and control 3 arms in a 4-way contingency table.

Ofori and Reichmuth, 1997).

All the five monoterpenoids elicited repellent activity against *T. castaneum* and *R. dominica* in olfactometry bioassays, with (R)-linalool and (S)-2-heptanol exhibiting more repellent activity. Bioactivities of some of the identified chemical components of *A. melegueta* and *Z. officinale* essential oils have been reported previously. For instance, linalool (Kessler and Baldwin, 2001), linalool, limonene, myrcene, (*E*)- β -ocimene (monoterpenes), (*E*)- β -farnesene, β -bisabolene and (*E*)- β -caryophyllene (sesquiterpenes) (Degenhardt *et al.*, 2003) have been shown as components of the volatile blends released after herbivore attack in some plant species to repel herbivores and attract parasitoids and predators of the herbivores. In choice field tests, *Myzus persicae* (Sulzer) was repelled from *Arabidopsis thaliana* (L.) plants that constitutively produce high levels of linalool (Aharoni *et al.*, 2003). (R)-Linalool proved to be the major component of the essential oils conferring repellent activity. Linalool is known to act as a reversible competitive inhibitor of acetylcholinesterase (Ryan and Byrne, 1988). Acetylcholinesterase is a key enzyme that terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter, acetylcholine, in the nervous system in various organisms (Nathan *et al.*, 2008; Wang *et al.*, 2004).

Secondary plant compounds are therefore recognised important components of plant defence system against herbivores and pathogens, as well as shaping the diet of herbivores. They are the by-products of plant metabolism and sometimes referred to as volatile plant secondary metabolites. Most essential oils comprise of monoterpenes, compounds that contain 10 carbon atoms often arranged in a ring or cyclic form, as well as sesquiterpenes which are hydrocarbons comprising of 15 carbons (Koul *et al.*, 2008). Terpenes are the most widespread and

important secondary plant compounds, and can exert toxic, deterrent, antifeedant and repellent effects on insect herbivores. They are the dominant components of many natural volatile blends and are responsible for many of the characteristic smells of plant oils, resins, fruits and flowers (Paré and Tumlinson, 1999). Terpenoid chemistry may vary among plants due to many factors which may include environmental and genetic influences (Langenheim, 1994; Powell and Raffa, 1999; Wang and Lincoln, 2004). In the future, with proper formulation and production technology, these essential oils could be exploited for use against insect infestation of stored-products at the small scale farmer's level especially in the developing world for a more sustainable food security. This study presents a rational approach to investigating the chemical basis of repellent non-host plant volatiles, for assessing the behavioural effects of such repellent volatiles on stored-product pests such as *T. castaneum* and *R. dominica* in small-scale farmer conditions in Africa.

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REFERENCES

- Aharoni, A., Giri, A. P., Deuerlein, S., Griepink, F., de Kogel, W. J., Verstappen, F. W. A., Verhoeven, H. A., Jongsma, M. A., Schwab, W. and Bouwmeester, H. J. 2003. Terpenoid metabolism in wild and transgenic *Arabidopsis* plants. *The Plant Cell* 115: 2866-2884.
- Akhtar, Y. and Isman, M. B. 2004. Comparative growth inhibitory and antifeedant effects of plant extracts and pure allelochemicals on four phytophagous insect species. *Journal of Applied Entomology* 128: 32-38.
- Bekele, J. and Hassanali, A. 2001. Blend effects in the toxicity of the essential oil constituents of *Ocimum kilimandscharicum* and *Ocimum kenyense* (Labiatae) on two post-harvest insect pests. *Phytochemistry* 57: 385-391.
- Birkett, M. A., Al Abassi, S., Kröber, T., Chamberlain, K., Hooper, A. M., Guerin, P. A., Pettersson, J., Pickett, J. A., Slade, R. and Wadhams, L. J. 2008. Antiectoparasitic activity of the gum resin, gum haggard, from the East African plant, *Commiphora holtziana*. *Phytochemistry* 69: 1710-1715.
- Degenhardt, J., Gershenzon, J., Baldwin, I. T. and Kessler, A. 2003. Attracting friends to feast on foes: engineering terpene emission to make crop plants more attractive to herbivore enemies. *Current Opinion in Biotechnology* 14: 169-176.
- Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology* 51: 45-66.
- Keita, S. M., Vincen, C., Schmit, J. P., Ramaswamy, S. and Belanger, A. 2000. Effect of various essential oils on *Callosobruchus maculatus* (F.) [Coleoptera: Bruchidae]. *Journal of Stored Products Research* 36: 355-364.
- Kessler, A. and Baldwin, I. T. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291: 2141-2144.

- Koul, O., Walia, S. and Dhaliwal, G. S. 2008. Essential oils as green pesticides: Potential and constraints. *Biopesticide International* 4(1): 63-84.
- Kouninki, H., Haubruge, E., Noudjou, F. A., Lognay, G., Malaisse, F., Ngassoum, M. B., Ngamo, L. S. T. and Hance, T. 2005. Potential use of essential oils from Cameroon applied as fumigant or contact insecticides against *Sitophilus zeamais* Motsch. [Coleoptera: Curculionidae]. *Communications in Agricultural and Applied Sciences* 70: 787-792.
- Kouninki, H., Hance, T., Noudjou, F.A., Lognay, G., Malaisse, F., Ngassoum, M. B., Mapongmetsem, P. M., Ngamo, L. S. T. and Haubruge, E. 2007. Toxicity of some terpenoids of essential oils of *Xylopi aethiopica* from Cameroon against *Sitophilus zeamais* Motschulsky. *Journal of Applied Entomology* 131(4): 269-274.
- Langenheim, J. H. 1994. Higher plant terpenoids: a phytocentric overview of their ecological roles. *Journal of Chemical Ecology* 20: 1223-1280.
- Levinson, H. Z. and Levinson, A. R. 1998. Control of stored food pests in the ancient Orient and classical antiquity. *Journal of Applied Entomology* 122: 137-144.
- Nathan, S. S., Choi, M.Y., Seo, H. Y., Paik, C. H., Kalaivani, K. and Kim, J. D. 2008. Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown planthopper *Nilaparvata lugens* (Stål). *Ecotoxicology and Environmental Safety* 70: 244-250.
- NIST 2005. NIST mass spectral search for the NIST/EPA/NIH mass spectral library version 2.0. Office of the Standard Reference Data Base, National Institute of Standards and Technology, Gaithersburg, Maryland.
- Obeng-Ofori, D. and Reichmuth, C. 1997. Bioactivity of eugenol, a major component of essential oil of *Ocimum suave* (Wild.) against four species of stored-product Coleoptera. *International Journal of Pest Management* 43: 89-94.
- Obeng-Ofori, D., Reichmuth, Ch., Bekele, J. and Hassanali, A. 1997. Biological activity of 1,8-cineole, a major component of essential oil of *Ocimum kenyense* (Ayobangira) against stored product beetles. *Journal of Applied Entomology* 121: 237-243.
- Papachristos, D., Karamanoli, K., Stamopoulos, D., Menkissoglu-Spirudi, U. 2004. The relationship between the chemical composition of three essential oils and their insecticidal activity against *Acanthoscelides obtectus* (Say). *Pest Management Science* 60: 514-520.
- Paré, P. W. and Tumlinson, J. H. 1999. Plant volatiles as a defence against insect herbivores. *Plant Physiology* 121: 325-331.
- Pettersson, J. 1970. An aphid sex attractant 1. Biological studies. *Entomologica Scandinavica* 1: 63-73.
- Pickett, J. A. and Griffiths, D. C. 1980. Composition of aphid alarm pheromones. *Journal of Chemical Ecology* 6: 349-360.
- Powell, J. S. and Raffa, K. F. 1999. Sources of variation in concentration and composition of foliar monoterpenes in Tamarack (*Larix laricina*) seedlings: roles of nutrient availability, time of season, and plant architecture. *Journal of Chemical Ecology* 25: 1771-1797.
- Rozman, V., Kalinovic, I. and Korunic, Z. 2007. Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored products insects. *Journal of Stored Products Research* 43: 349-355.

- Ryan, M. F. and Byrne, O. 1988. Plant-insect coevolution and inhibition of acetylcholinesterase. *Journal of Chemical Ecology* 14: 1965-1975.
- Shaaya, E., Kostjukovsky, M., Eilberg, J. and Sukprakarn, C. 1997. Plant oils as fumigants and contact insecticides for the control of stored-product insects. *Journal of Stored Products Research* 33: 7-15.
- Stamopoulos, D. C. 1991. Effects of four essential oil vapours on the oviposition and fecundity of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae): laboratory evaluation. *Journal of Stored Products Research* 27: 199-203.
- Stamopoulos, D. C., Damos, P. and Karagianidou, G. 2007. Bioactivity of five monoterpenoid vapours to *Tribolium confusum* (du Val) [Coleoptera: Tenebrionidae]. *Journal of Stored Products Research* 43: 571-577.
- Tapondjou, A. L., Adler, C., Fontem, D. A., Bouda, H. and Reichmuth, C. 2005. Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. *Journal of Stored Products Research* 41: 91-102.
- Ukeh, D. A. 2008. Bioactivity of essential oils of *Aframomum melegueta* and *Zingiber officinale* (both Zingiberaceae) against *Rhyzopertha dominica*. *Journal of Entomology* 5(3): 193-199.
- Ukeh, D. A. and Umoetok, S. B. A. 2007. Effects of host and non-hosts plant volatiles on the behaviour of the lesser grain borer, *Rhyzopertha dominica* (Fab.). *Journal of Entomology* 4(6): 435-443.
- Wang, M. and Lincoln, D. 2004. Effect of light intensity and artificial wounding on monoterpenes production in *Myrica cerifera* from two different ecological habitats. *Canadian Journal of Botany* 82: 1501-1508.
- Wang, J. J., Cheng, W. X., Ding, W. and Zhao, Z. M. 2004. The effect of the insecticide dichlorvos on esterase activity extracted from the psocids, *Liposcelis bostrychophila* and *L. entomophila*. *Journal of Insect Science* 4: 1-5.
- Zar, J. H. 1999. *Biostatistical Analysis*. 4th ed. Prentice Hall, Upper Saddle River, New Jersey, USA.

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