The African Journal of Plant Science and Biotechnology ©2013 Global Science Books

The Leaf Essential Oil Composition of *Eugenia javanica* from South West Nigeria and Insecticidal Activity against *Sitophilus zeamais*

Moses S. Owolabi[1*] • Wasiu Kazeem[1] • Noura S. Dosoky[2] • William N. Setzer[2]

[1] Department of Chemistry, Lagos State University, P.M.B 001, Ojo, Lagos, Nigeria
[2] Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA

Corresponding author: * **sunnyconcept2007@yahoo.com**

ABSTRACT

Volatile oils play an important role as natural insecticides for protection of stored food products. Members of the Myrtaceae have been used traditionally as insecticides and insect repellents. In this work we have examined the chemical composition of *Eugenia javanica* leaf oil, which was obtained in 0.63% yield, and the activity of the oil as a contact insecticide against the maize weevil, *Sitophilus zeamais*. *E. javanica* leaf oil was composed largely of α-terpineol (14.1%), terpinen-4-ol (7.2%), (*E*)-caryophyllene (6.6%), α-cadinol (12.2%), caryophyllene oxide (9.6) and 1-*epi*-cubenol (6.0%). The oil showed notable contact toxicity to *S. zeamais* (100% mortality after 96 h). The insecticidal activity of *E. javanica* oil is likely due to relatively high concentrations of known insecticidal components α-terpineol, terpinen-4-ol, and caryophyllene oxide, and is consistent with the traditional use of this plant family as an insecticide and insect repellant.

Keywords: caryophyllene oxide, contact toxicity, terpinen-4-ol, α-terpineol
Abbreviations: EIMS, electron impact mass spectrometer; **GC**, gas chromatograph/chromatography; **MS**, mass spectrometer/spectrometry; **MSD**, mass selective detector; **NIST**, National Institute of Standards and Technology

INTRODUCTION

The maize weevil, *Sitophilus zeamais* (Motschulsky) is a serious insect pest of stored grains throughout the world (Rees 2008). Current control protocols involve the use of fumigants such as methyl bromide or synthetic insecticides such as organochlorines and organophosphates. Subsistence farmers have utilized aromatic plants and essential oils to both protect stored food products from insect pests, and represent effective alternatives to environmentally adverse synthetic pesticides (Regnault-Roger *et al.* 2012). The rose apple (*Eugenia javanica* Lam., Myrtaceae) is indigenous to the East Indies and Malaya and is cultivated and naturalized in many parts of India, Southeast Asia and the Pacific Islands. This exotic fruit is very popular in Thailand. Its plantation in Thailand covers an area of 9,634 hectares, producing rose apple fruit with a market value of 31.5 million US $ (Department of Agricultural Extension 2003). Rose apple is a rich source of vitamins and minerals with the most popular variety being ‘Thongsamsri’ (Tungjatupohn 2000). Members of the Myrtaceae, e.g., *Eugenia aromatica* syn. *Syzygium aromaticum* and *Eucalyptus* spp. (Maia and Moore 2011; Pohlit *et al.* 2011), are still extensively used in the traditional way throughout the communities in the tropics as insect repellent against mosquitoes (Barnard 1999). In our search for viable alternatives to synthetic pesticides, we have examined the leaf essential oil of *E. javanica* for insecticidal activity against *S. zeamais*.

MATERIALS AND METHODS

Plant material

Fresh samples of *Eugenia javanica* leaves were collected from a single vegetative plant (about 15 years of age) in September, 2011, from Alimosho, Lagos state, Nigeria. The plant was taxonomically identified and authenticated at the Herbarium of the Department of Botany of the University of Lagos with a voucher specimen number LUH 4200. A sample (500 g) of *E. javanica* was subjected to hydrodistillation in a Clevenger-type apparatus (British Pharmacopoeia 1980) for 4 h. The yield of oil was 0.63% on a fresh weight basis. The oil was dried over anhydrous sodium sulfate and stored in a sealed vial under refrigeration prior to analysis.

Gas chromatographic – mass spectral analysis

The volatile oil sample was subjected to GC-MS analysis on an Agilent system consisting of an Agilent model 6890 gas chromatograph, an Agilent 5973 mass selective detector (EIMS, electron energy = 70 eV, scan range = 45-400 amu, and scan rate = 3.99 scans/sec) and an Agilent Chemstation data system. The GC column was a HP-5ms fused silica capillary with a (5% phenyl)-methyl polysiloxane stationary phase, film thickness 0.25 μm, length 30 m, and internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 7.07 psi and a flow rate of 1.0 mL/min. Inlet temperature was 200°C and MSD detector temperature was 280°C. The GC oven temperature program was used as follows: 40°C initial temperature, hold for 10 min, increased at 3°C/min to 200°C, increased 2°C/min to 220°C. The sample was dissolved in dichloromethane and a splitless injection technique was used. Identification of the constituents of the volatile oil was achieved based on their retention data (retention indices) determined with reference to C_{10}-C_{40} *n*-alkane homologous series, and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams 2007) and stored on the MS library [NIST database (G1036A, revision D.01.00) / ChemStation data system (G1701CA, version C.00.01.08)]. The chemical composition of *E. javanica* essential oil is summarized in **Table 1**.

Received: 30 June, 2012. Accepted: 25 July, 2012.

Research Note

Table 1 Chemical composition of the leaf essential oil of *Eugenia javanica* from Nigeria.

RI[a]	Compound	%	RI	Compound	%
941	α-Pinene	1.1	1524	δ-Cadinene	3.4
978	β-Pinene	1.0	1526	Zonarene	tr
1024	*p*-Cymene	1.1	1544	α-Calacorene	0.3
1028	Limonene	0.3	1570	Caryolan-8-ol	0.5
1058	γ-Terpinene	1.3	1574	Spathulenol	0.1
1088	Terpinolene	0.3	1583	Caryophyllene oxide	9.6
1100	Linalool	0.7	1586	Gleenol	0.9
1112	*endo*-Fenchol	2.6	1594	Cubeban-11-ol	0.6
1144	*neo*-Isopulegol	0.6	1602	Rosifoliol	0.6
1146	Camphene hydrate	0.5	1610	Humulene epoxide II	0.4
1176	Terpinen-4-ol	7.2	1613	Unidentified	2.7
1189	α-Terpineol	14.1	1615	1,10-di-*epi*-Cubenol	0.7
1227	Citronellol	0.3	1616	Unidentified	0.6
1353	Citronellyl acetate	0.2	1622	Unidentified	1.0
1375	α-Copaene	0.7	1628	1-*epi*-Cubenol	6.0
1418	(*E*)-Caryophyllene	6.6	1631	Unidentified	1.9
1453	α-Humulene	0.6	1635	Caryophylla-4(12),8(13)-dien-5-ol	0.4
1476	β-Chamigrene	0.3	1641	τ-Cadinol	2.3
1477	γ-Muurolene	1.2	1643	Cubenol	2.9
1486	β-Selinene	3.8	1645	α-Muurolol (= Torreyol)	1.4
1494	α-Selinene	4.2	1650	β-Eudesmol	0.7
1497	Indipone	tr[b]	1653	α-Eudesmol	tr
1501	α-Muurolene	0.2	1655	β-Cadinol	12.2
1515	γ-Cadinene	0.4	1658	Selin-11-en-4α-ol	1.0
				Total Identified	93.4

[a] RI = "Retention Index" on an HP-5ms column based on a homologous series of *n*-alkanes.
[b]tr = "trace" (< 0.5%).

Table 2 Insecticidal activity (% mortality) of *Eugenia javanica* leaf essential oil to *Sitophilus zeamais*.[a]

Concentration (%)	24 h	48 h	72 h	96 h	120 h
control	0.0 ± 0.0	0.0 ± 0.0	17.6 ± 0.3	36.8 ± 0.3	54.3 ± 0.9
5	3.3 ± 0.3	6.7 ± 0.3	20.0 ± 0.3	56.7 ± 0.3	70.0 ± 0.6
10	6.7 ± 0.3	13.3 ± 0.3	30.0 ± 0.0	66.7 ± 0.3	80.0 ± 0.6
20	13.3 ± 0.3	16.7 ± 0.3	36.7 ± 0.0	73.3 ± 0.3	83.3 ± 0.3
50	16.7 ± 0.3	20.0 ± 0.6	63.3 ± 0.3	83.3 ± 0.3	100.0 ± 0.0
75	20.0 ± 0.0	30.0 ± 0.5	70.0 ± 0.6	100.0 ± 0.0	100.0 ± 0.0
100	30.0 ± 0.6	53.3 ± 0.9	96.7 ± 0.7	100.0 ± 0.0	100.0 ± 0.0

[a] Averages and standard deviations based on triplicate determinations of10 insects each.

Insecticidal activity

The insecticidal activity of *E. javanica* essential oil against *Sitophilus zeamais* (maize weevil) was investigated. Insects were obtained from infested maize and cultured in the laboratory for seven days. Insecticidal activity was determined by treatment of Whatman No 1 filter paper discs (7 cm diameter) with the oil in several dilutions (5, 10, 20, 50, 75 and 100%, w/v) in hexane. The essential oil solution (1 mL) was applied to the filter paper disc; hexane alone was used as a negative control. The solvent was allowed to evaporate from the filter paper, which was then placed into a Petri dish. Ten well-fed live adult *S. zeamais* were added and the Petri dish was covered. Control experiments were set up as described above without the aliquots essential oil. Each treatment was repeated three times. Insect mortalities were investigated by observing the recovery of immobilized insects after a 24-h interval for up to 120 h to monitor the insecticidal activity of the essential oil, summarized in **Table 2**.

RESULTS AND DISCUSSION

Essential oil composition

Analysis of *E. javanica* (**Table 1**) resulted in the identification of 44 components comprising 93.4% of the composition. The oil was found to be dominated by α-terpineol (14.1%), terpinen-4-ol (7.2%), (*E*)-caryophyllene (6.6%), α-cadinol (12.2%), caryophyllene oxide (9.6) and 1-*epi*-cubenol (6.0%). The oil was characterized by the predominance of oxygenated monoterpenoids (26.2%) and oxygenated sesquiterpenoids (40.4%). Monoterpene and sesquiterpene hydrocarbons comprised 5.0% and 21.8% of the *E. javanica* oil, respectively. A previous examination of *E. javanica* from Thailand revealed the leaf oil to be composed largely of γ-terpinene (28.5%), α-pinene (18.2%), and *p*-cymene (13.7%) (Suksamrarn and Brophy 1987). Thus, *E. javanica* from Nigeria is a completely different chemotype.

Insecticidal activity

The insecticidal bioassay (**Table 2**) of the volatile oil of *E. javanica* against *S. zeamais* (maize weevil) showed that the activity of the oil was both dose dependent and exposure dependent. Thus, 75% of the essential oil gave 73.3% mortality after 72 h and 100% mortality after 96 h of exposure, while a dose of 100% of the oil gave 100% mortality after 96 h. Therefore, *E. javanica* essential oil may be considered to be a useful alternative to synthetic insecticides.

Essential oils and essential oil components have shown insecticidal activity against *Sitophilus zeamais* (Chu *et al.* 2010; Kerdchoechuen *et al.* 2010; Liu *et al.* 2010; Mendesil *et al.* 2012). Both terpinen-4-ol and α-terpineol have shown insecticidal activity against *Sitophilus oryzae* (Lee *et al.* 2001) and *Sitophilus granarius* (Kordali *et al.* 2006). In addition, caryophyllene oxide, but not (*E*)-caryophyllene, has demonstrated larvicidal activity toward *Aedes aegypti* (Silva *et al.* 2008). (*E*)-Caryophyllene was also inactive against the human head louse, *Pediculushumanus capitis* (Yang *et al.* 1009), but did exhibit some activity against adult mosquitoes *Anopheles tessellatus* and *Culex quinquefasciatus* (Samaraskera *et al.* 2008). The concentrations of terpinen-4-ol, α-terpineol, and caryophyllene oxide in the essential oil of *E. javanica* along with the previously demonstrated insecticidal activities of these compounds, suggest that they contribute to the insecticidal activity of *E. javanica* oil.

REFERENCES

Adams RP (2007) *Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry* (4^{th} Edn), Allured Publishing Corp, Carol Stream, Illinois, 804 pp

Barnard DR (1999) Repellency of essential oils to mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology* **36**, 625-629

British Pharmacopoeia (1980) (Vol II), H.M. Stationary Office, London, A 109

Chu SS, Hu JF, Liu ZL (2011) Composition of essential oil of Chinese *Chenopodium ambrosioides* and insecticidal activity against maize weevil, *Sitophilus zeamais*. *Pest Management Science* **67**, 714-718

Department of Agricultural Extension (2003) *Annual Statistics of Thai Fruit Production / Crop Year 2003* (in Thai)

Kerdchoechuen O, Laohakunjit N, Singkornard S, Matta FB (2010) Essential oils from six herbal plants for biocontrol of the maize weevil. *HortScience* **45**, 592-598

Kordali S, Aslan I, Çalmaşur O, Cakir A (2006) Toxicity of essential oils isolated from three *Artemisia* species and some of their major components to granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). *Industrial Crops and Products* **23**, 162-170

Lee BH, Choi WS, Lee SE, Park BS (2001) Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). *Crop Protection* **20**, 317-320

Liu ZL, Chu SS, Liu QR (2010) Chemical composition and insecticidal activity against *Sitophilus zeamais* of the essential oils of *Artemisia capillaris* and *Artemisia mongolica*. *Molecules* **15**, 2600-2608

Maia MF, Moore SJ (2011) Plant-based insect repellents: A review of their efficacy, development and testing. *Malaria Journal* **10 (Suppl 1)**, S11

Mendesil E, Tadesse M, Negash M (2012) Efficacy of plant essential oils against two major insect pests of coffee (Coffee berry borer, *Hypothenemus hampei*, and antestia bug, *Antestiopsis intricata*) and maize weevil, *Sitophilus zeamais*. *Archives of Phytopathology and Plant Protection* **45**, 366-372

Pohlit AM, Lopes NP, Gama RA, Tadei WP, Neto VF (2011) Patent literature on mosquito repellent inventions which contain plant essential oils – a review. *Planta Medica* **77**, 589-617

Rees DP (2008) *Insects of Stored Products*, CSIRO, Collingwood, Victoria, Australia, 180 pp

Regnault-Roger C, Vincent C, Arnason JT (2012) Essential oils in insect control: Low-risk products in a high-stakes world. *Annual Review of Entomology* **57**, 405-424

Samarasekera R, Weerasinghe IR, Hemalal KDP (2008) Insecticidal activity of menthol derivatives against mosquitoes. *Pest Management Science* **64**, 290-295

Silva WJ, Dória GAA, Maia RT, Nunes RS, Carvalho GA, Blank AF, Alves PB, Marçal RM, Cavalcanti SCH (2008) Effects of essential oils on *Aedes aegypti* larvae: Alternatives to environmentally safe insecticides. *Bioresource Technology* **99**, 3251-3255

Suksamrarn A, Brophy JJ (1987) The volatile leaf oil of *Eugenia javanica* Lamk. *Flavour and Fragrance Journal* **2**, 37-40

Tungjatupohn S (2000) *Rose Apple*, Chonburee Agriculture and Technology College, Department of Vocational School, Ministry of Education (in Thai)

Yang YC, Lee SH, Clark JM, Ahn YJ (2009) Ovicidal and adulticidal activities of *Origanum majorana* essential oil constituents against insecticide-susceptible and pyrethroid/malathion-resistant *Pediculus humanus capitis* (Anoplura: Pediculidae). *Journal of Agricultural and Food Chemistry* **57**, 2282-2287