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Alejandro Lucia, Eduardo Zerba & Hector Masuh

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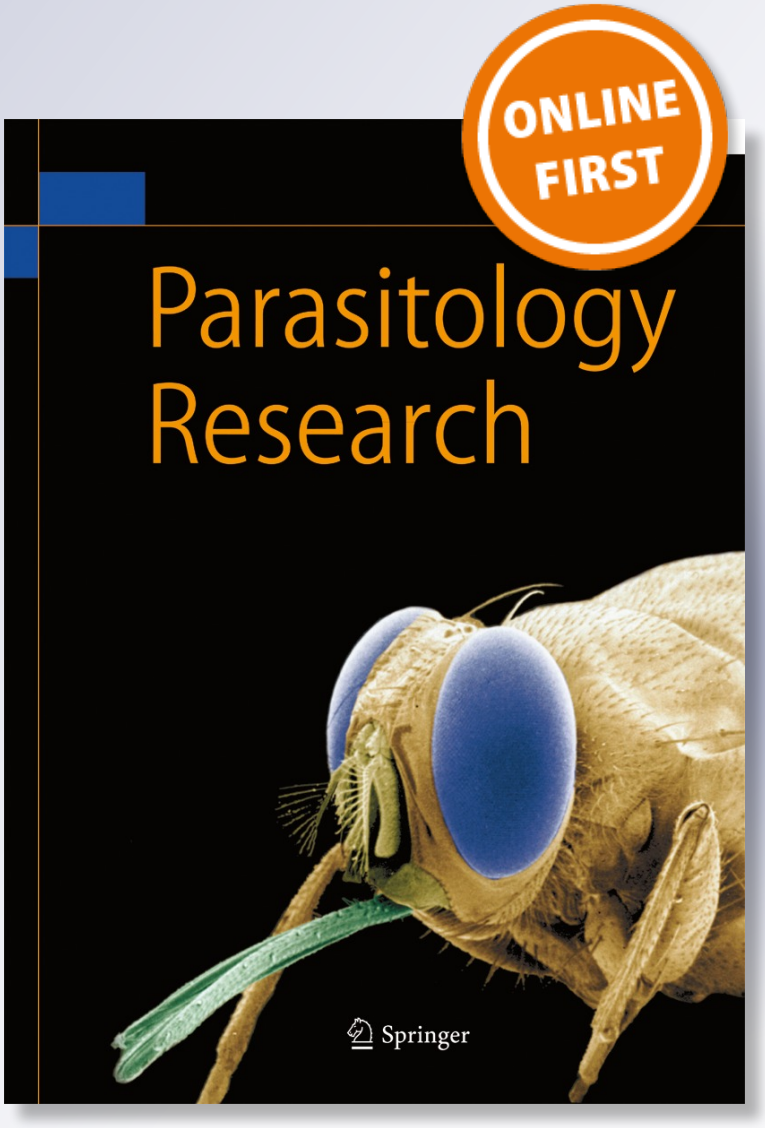

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ORIGINAL PAPER

Knockdown and larvicidal activity of six monoterpenes against *Aedes aegypti* (Diptera: Culicidae) and their structure-activity relationships

Alejandro Lucia · Eduardo Zerba · Hector Masuh

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Abstract The relationships between physicochemical parameters of majority components of *Eucalyptus* essential oils and their insecticide effect were evaluated on *Aedes aegypti* (L.) (Diptera: Culicidae). The octanol–water partition coefficients of the monoterpenes were estimated by the atom/fragment contribution method and the vapor pressures were determined by our laboratory in previous studies. The larvicidal activity ($LC_{50\ (ppm)}$) and knockdown effect ($KT_{50\ (min)}$) of each component was determined. The results show that the toxicity of EOs main components of *Eucalyptus* on adults and larvae of *A. aegypti* is strongly related to their physicochemical properties (vapor pressure and Log P). However, the interaction of both variables (vapor pressure * Log P) explains the toxicological phenomenon more precisely. The regression models were expressed as follows: $\mathbf{KT}_{\mathbf{50}(\mathrm{min})} = -10.9 + 3.7 * \mathrm{Log\ P} + 1.9 * 1/\mathrm{P_{vapor}}$ ($R^2 = 0.80$; $F = 42.5$) and $\mathbf{LC}_{\mathbf{50}(\mathrm{ppm})} = -94.3 + 438.6 * 1/\mathrm{Log\ P} + 2.8 * 1/\mathrm{P_{vapor}}$ ($F = 57.8$; $R^2 = 0.85$). The six evaluated components present different functional groups. Therefore, it was considered to evaluate the monoterpenes as a group and separated in two groups: oxygenated monoterpenes (α-terpineol, 4-terpineol, and 1,8-cineole) and terpene hydrocarbons (γ-terpinene, p-cymene, and α-pinene). The results show the regression models for each group as follows: (A) oxygenated terpenes: $\mathbf{KT}_{\mathbf{50}(\mathrm{min})} = -515.3 + 1613.2 * 1/\mathrm{Log\ P} + 5{,}2 * 1/\mathrm{P_{vapor}}$ ($F = 3176.7 R^2 = 0.99$) and $\mathbf{LC}_{\mathbf{50}(\mathrm{ppm})} = -1679.4 + 5402.1 * 1/\mathrm{Log\ P} + 12.7 * 1/\mathrm{P_{vapor}}$ ($F = 282.9$; $R^2 = 0.99$). (B) Hydrocarbons terpenes: $\mathbf{KT}_{\mathbf{50}(\mathrm{min})} = 18.2 - 58.3 * 1/\mathrm{Log\ P} + 2.7 * 1/\mathrm{P_{vapor}}$ ($F = 171.7$; $R^2 = 0.97$) and $\mathbf{LC}_{\mathbf{50}(\mathrm{ppm})} = -21.1 + 174.9 * 1/\mathrm{Log\ P} - 14.3 * 1/\mathrm{P_{vapor}}$ ($F = 410.0$; $R^2 = 0.99$). The association between the toxic effect of the evaluated monoterpenes against *A. aegypti* and the physicochemical properties can be better described when they are separated into functional groups (hydrocarbons vs. oxygenated terpenes).

Introduction

Aedes aegypti (L.) (Diptera: Culicidae) is an urban mosquito that feeds almost exclusively on humans (Christophers 1960). It is one of the most important species in the world, since it is the main vector of the yellow fever virus and the primary vector of dengue viruses (Eldridge 2005).

The insecticides usually used in the control of this mosquito were temephos for larvicidal treatment in water containers (focal treatment) and pyrethroids as adulticidal ultra low volume formulations (spatial treatment) in the event of an outbreak (Chavasse and Yap 1997). Several substances of plant origin have been identified as having toxic, repellent, antifeedant, and/or growth and development inhibiting potential on arthropod pests (Coats 1994). Essential oils are particular plant products made up of volatile substances found in a variety of species (Weinzieri et al. 1994; Weinzieri 2000). The main characteristics of the essential oils are that they are easily extractable, ecofriendly, biodegradable, possess low or no toxicity against mammals, and are very effective against wide spectra of insect pests (Tisserand and Balacs 1995; Isman 2000; Amer and Mehlhorn 2006; Elango et al. 2009; Govindarajan et al. 2008; Rahuman et al. 2009; Michaelakis et al. 2009).

A. Lucia · E. Zerba · H. Masuh (✉)
Centro de Investigaciones de Plagas e Insecticidas (CIPEIN) (UNIDEF (MINDEF-CONICET), Juan Bautista de La Salle 4397, 1603Buenos Aires Province Villa Martelli, Argentina
e-mail: hmasuh@gmail.com

A. Lucia
e-mail: luciaalejandro@yahoo.com.ar

E. Zerba
e-mail: ezerba@citedef.gob.ar

E. Zerba · H. Masuh
Instituto de Ingeniería e Investigación Ambiental, Universidad Nacional de General San Martín (UNSAM), Belgrano 3563 1 piso, San Martin, Provincia de Buenos Aires B1650ANQ, Argentina

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There are several reports showing the insecticidal activity of Eucalyptus essential oils and their main components in different species (Lee et al. 2001; Papachristos and Stamopoulos 2002; Lucia et al. 2007, 2008, 2009, 2012; Batish et al. 2008). Previous works at our laboratory showed significant relationships between component concentration of Eucalyptus essential oils and their insecticide effect on insect pests. The *p*-cymene, 1,8-cineole, α-pinene, γ-terpinene, 4-terpineol, and α-terpineol are the majority components of *Eucalyptus* essential oils. In this study, we present relationships between physicochemical parameters of majority components of *Eucalyptus* essential oils and their insecticide effect on *A. aegypti*.

Materials and methods

Insects

An insecticide-susceptible strain of *A. aegypti* (L.) (CIPEIN strain), reared according to previous reports (Lucia et al. 2007), was used in all bioassays. The laboratory colony has been kept in the laboratory since 1996, free of exposure to pathogens, insecticides, or repellents, at 25–30 °C, 80–90 % of RH, and a L_{12}/D_{12} photoperiod.

Chemicals

The following chemicals used for the bioassay were purchased from Sigma-Aldrich (Buenos Aires, Argentina): *p*-cymene (99 %), 1,8-cineole (99 %), α-pinene (97 %), γ-terpinene (97 %), 4-terpineol (96 %), and α-terpineol (99 %).

Physicochemical parameters

Octanol–water partition coefficients (log p) The octanol–water partition coefficients of the monoterpenes were estimated by the atom/fragment contribution method (Meylan and Howard 1995).

Determination of vapor pressures The vapor pressures of each monoterpene were determined by our laboratory in previous studies (Lucia et al. 2009) (Table 1). The vapor pressures values were calculated using chromatographic data as experimental inputs, as introduced by Van Roon et al. (2002) and refined by Hoskovec et al. (2005).

Larvicidal bioassay

The larvicidal bioassay was performed following the method from previous studies (Lucia et al. 2008). One milliliter of an acetone solution of the monoterpene to be tested was added to 224 ml of distilled water in a 500-ml plastic jar, which was shaken slightly to ensure a homogeneous test solution. Then, 20 late third or early fourth instars of *A. aegypti* in 25 ml of distilled water were transferred to that jar. The control solution was made with 1 ml of acetone mixed with 249 ml of distilled water, and the untreated solution contained 250 ml of distilled water only. No food was offered to the larvae. All bioassays were conducted in a 27±2 °C regulated chamber, with 80–90 % of relative humidity and a 12:12 h photoperiod. Mortality and survival were recorded after 24-h exposure. The moribund and dead larvae in five tests were combined and expressed as a percentage of larval mortality for each concentration. Larvae were considered dead when they failed to move. Moribund larvae were those incapable of rising to the surface (within a reasonable period of time) or showing the characteristic diving reaction when the water was disturbed. The monoterpenes were tested at a final concentration of 10, 20, 30, 40, 50, 60, and 100 ppm in 250 ml of water.

Table 1 Fumigant activity and vapor pressures of the different monoterpenes

Monoterpenes	$P_{vap.}$ (mmHg) [a]	Fumigant activity			
		$KT_{50\ (min)}$ [b]	Statistics [c]		
		(CI)	Slope±SE	X^2	df
α-Terpineol	0.09	26.38 (25.55–27.29)	10.83±0.68	1.39	18
γ-Terpinene	0.93	9.31 (8.94–9.67)	8.73±0.59	1.42	10
4-Terpineol	0.13	9.27 (8.86–9.68)	7.99±0.54	9.06	11
p-Cymene	1,35	5.82 (5.52–6.10)	7.16±0.56	4.73	8
α-Pinene	3.66	5.36 (4.95–5.73)	10.18±1.01	4.89	4
1,8-Cineole	1.34	(3.56–4.20)	8.26±0.75	5.80	5

[a] Data from Lucia et al. (2009)

[b] Time to 50 % knockdown with a 95 % confidence interval (*CI*). KT_{50} values are the means of four replicates using 13–15 adults (total 54–60 adults) for each essential oil, determined at 1-min intervals until 100 % of the adults were knocked-down. Data from Lucia et al. (2009)

[c] Statistics of the probit analysis of knockdown times

Bioassay for fumigant activity

The fumigant activity of monoterpenes against *A. aegypti* was determined in a previous study (Lucia et al. 2009) (Table 1). The bioassay fumigant tests developed in the laboratory were conducted in an enclosed specifically designed chamber that allowed concentration of the test vapors. Experimental units consisted of transparent acrylic tubes, 11.9 cm long, with an inner diameter of 4.4 cm, and 164.5 ml capacity (BioQuip, USA). Knockdown was considered as the inability of adult mosquitoes to fly.

Statistical analysis

Dose-mortality data from each *A. aegypti* pool was subjected to probit analysis (Litchfield and Wilcoxon 1949). A lethal

concentration of 50 % ($LC_{50\ (ppm)}$) with 95 % confidence intervals was obtained by means of Micro Probit 3.0 software and was expressed as parts per million in the final concentration.

$KT_{50\ (min)}$ values were calculated with their respective 95 % confidence intervals by using the statistical software for correlated data developed by Throne et al. (1995). Regression analysis was performed using Statgraphics Plus 4.0 software (Statistical Graphics Corporation, Herndon, VA).

Results and discussion

Table 2 shows the octanol–water partition coefficient values of the evaluated monoterpenes. The value ranges between 3.13 and 4.75. The oxygenated terpenes show the lowest values, whereas the terpene hydrocarbons the highest ones.

Additionally, this table shows that the larvicidal effect is greater on the components grouped as terpene hydrocarbons than on the oxygenated terpenes.

The toxicity of EOs main components of *Eucalyptus* on adults and larvae of *A. aegypti* is strongly related to their physicochemical properties (vapor pressure and Log P) ($P < 0.01$). However, the interaction of both variables (vapor pressure * Log P) explains the toxicological phenomenon more precisely. The six evaluated components present different functional groups, which not only do they provide the components with different physicochemical properties, but also they provide the molecule with the possibility of interacting with cellular or molecular whites, specific or general, in which to generate their toxic action.

Table 2 The octanol–water partition coefficient values and larvicidal activity of monoterpenes against *Aedes aegypti* larvae

Monoterpenes	Log P [a]	Larvicidal activity			
		$LC_{50\ (ppm)}$ [b]	Statistics [c]		
		(CI)	Slope±SE	X^2	df
α-Terpineol	3.33	76.68 (65.65–86.25)	2.80±0.63	3.76	4
γ-Terpinene	4.75	0.4 (0.34–0.48)	2.53±0.24	0.06	4
4-Terpineol	3.33	38.77 (25.07–52.66)	4.50±0.43	9.06	4
p-Cymene	4.00	12.49 (5.99–20.78)	2.17±0.18	4.73	4
α-Pinene	4.27	15.87 (12.43–19.73)	4.68±0.43	4.89	4
1,8-Cineole	3.13	53.63 (39.63–79.11)	3.00±0.27	5.80	4

[a] Estimating for atom/fragment contribution method (Meylan and Howard 1995)

[b] Each value of lethal concentration with a 95 % confidence interval ($LC_{50\ (ppm)}$) was obtained from the data from four independent replicates. After 24 h of exposure

[c] Statistics of the probit analysis of larvicidal activity

Therefore, it was considered to evaluate the monoterpenes as a group and separated in two groups as follows: oxygenated monoterpenes (α-terpineol, 4-terpineol, and 1, 8-cineole) and terpene hydrocarbons (γ-terpinene, p-cymene, and α-pinene).

A knockdown effect

Considering the total number of evaluated monoterpenes, the resulting model explains that as the vapor pressure of the components rises and the Log P decreases, their knockdown effect on the *A. aegypti* significantly increases; the following equation describes it:

$$\mathbf{KT_{50(min)}} = -10.9 + 3.7 * \mathrm{Log\,P} + 1.9 * 1/\mathrm{P_{vapor}}$$

$$(\mathbf{P < 0.01; F = 42.5; R^2 = 80.2; DF = 23})$$

By performing the analyses separately, the results show that in the case of oxygenated terpenes, as the vapor pressure and the Log P of the component rise, the knockdown time lowers, thus showing a greater toxicity on *A. aegypti*. ($\mathbf{KT_{50\ (min)}} = -515.3 + 1613.2 * 1/\mathrm{Log\ P} + 5{,}2 * 1/\mathrm{P_{vapor}}$)($\mathbf{P < 0.01}$; $\mathbf{F = 3176.7}$; $\mathbf{R^2 = 99.8}$; $\mathbf{DF = 11}$)).

In the case of terpene hydrocarbons, as the vapor pressure rises and the component Log P decreases, the knockdown time lowers, thus showing a greater knockdown effect on *A. aegypti*. ($\mathbf{KT_{50\ (min)}} = 18.2 - 58.3 * 1/\mathrm{Log\ P} + 2.7 * 1/\mathrm{P_{vapor}}$) ($\mathbf{P < 0.01}$; $\mathbf{F = 171.7}$; $\mathbf{R^2 = 97.4}$; $\mathbf{DF = 11}$)).

From all the data analyzed, it is deduced that the knockdown effect of the EOs main components on the evaluated *Eucalyptus* depends on the vapor pressure and the octanol–water partition coefficient. However, the knockdown effect of the components can be described more accurately by means of their physicochemical properties when the components are grouped considering their main functional group and not by evaluating the group itself.

All the models obtained show that as the vapor pressure of the component rises, its adulticide activity rises as well. These results match those of Toloza et al. (2006), who found a statistically significant relationship between the knockdown effect of at least ten monoterpenes against *Pediculus humanus capitis* with their corresponding vapor pressure and octanol–water partition coefficient values.

Rice and Coats (1994) evaluated the knockdown effect of 22 monoterpenes against *Musca domestica* and found a polynomial correlation between their fumigant toxicity and their corresponding volatility, this leading to the conclusion that as the monoterpene increases in volatility, its toxicity becomes greater.

When exposed to vapors, the main inlet channel into the organism is inhalation; the volatile substances enter through the spiracles as part of the respiratory process, and are

subsequently transported to the various tissues by the tracheas and tracheoles net, finally arriving at their action site (Mill 1985).

Therefore, the strong involvement of the vapor pressure variable in the knockdown effect for both groups evaluated (oxygenated monoterpenes and terpene hydrocarbons) can be explained by taking into account that the main inlet channel of the organism for essential oils or components is inhalation (through the trachea), since the knockdown effect was measured by means of vapor exposure.

On the other hand, and regardless of vapor pressure, the octanol–water partition coefficient (Log P) would bear a weaker involvement in the toxicity.

This association may be based on the fact that once the compound enters the organism through the tracheas and tracheoles, it penetrates it by circulating around them and through the cuticle invagination that constitutes them. It also circulates through the hemolymph, free of solution or absorbed by the hemolymph proteins, and spreads throughout the body of the insect (Welling and Paterson 1985). For such reason, the compound partition plays an essential role in the penetration, distribution, and/or circulation of the compound until it reaches the action site.

This possible explanation of the different contribution of the Log P of oxygenated compounds and terpene hydrocarbons can be supported by the two-phase model described for the correlation of this variable with the cuticular penetration and xenobiotic distribution in insects (Welling and Paterson 1985).

B-larvicidal effect

As regards the group of evaluated monoterpenes, the model explains that as the vapor pressure and the Log P of the components rise, their larvicidal effect against *A. aegypti* grows significantly; the following equation describes it:

$$\mathbf{LC_{50(ppm)}} = -94.3 + 438.6 * 1/\text{Log P} + 2.8 * 1/\mathrm{P_{vapor}}$$

$$\left(\mathbf{P < 0.01};\ \mathbf{F = 57.8};\ \mathbf{R^2 = 84.6}\ \mathbf{DF = 23}\right).$$

In this way, the larvicidal effect of all six monoterpenes evaluated could be explained by means of their physicochemical properties such as vapor pressure and the octanol–water partition coefficient.

For the oxygenated terpenes, results show that, as the vapor pressure and the Log P of the component rises, the toxicity on larvae of *A. aegypti* grows ($\mathbf{LC_{50\ (ppm)}}$=−1679.4+5402.1∗1/Log P+12.7∗1/$\mathrm{P_{vapor}}$ (**P <0.01**; **F = 282.9**; $\mathbf{R^2}$**=98.4**; **DF =11**))

In the case of the group of terpene hydrocarbons, as the vapor pressure decreases and the Log P of the component rises, the lethal concentration lowers, thus showing a greater toxicity on larvae of *A. aegypti* ($\mathbf{LC_{50\ (ppm)}}$=−21.1+174.9∗1/Log P−14.3*1/$\mathrm{P_{vapor}}$ (**P <0.01**; **F =410.0**;$\mathbf{R^2}$**=98.9**;**DF =11**)).

In general terms, it can be considered that the main inlet channel in mosquito larvae exposed to larvicides is the cuticular one. In this process, the polarity (expressed as the octanol–water partition coefficient) of the xenobiotic which enters through the tegument plays an essential role in determining the speed for entering the organism.

The cuticle of insects is made up of different regions, which differ remarkably with regards to their chemical composition and properties, being polarity the most significant of all. Many studies consider that there is an ideal polarity in xenobiotics for entering the organism. This polarity is considered by many authors as the medium polarity, which allows for optimal speed in cuticular penetration, taking into account a two-phase type of correlation (Welling and Paterson 1985).

The molecules of declining polarity penetrate more easily in membranes rich in lipids. Conversely, the higher the polarity, the faster they leave those barriers. This suggests that there must be a certain polarity in which the passage through the membranes is the highest (Welling and Paterson 1985).

The strong participation of the octanol–water partition coefficient can be explained, bearing in mind that the main inlet channel of the components into the organism is touch (outer cuticle), due to the fact that the larvicidal effect was evaluated through larvae immersion in a aqueous environment where the compound was applied. By means of the methodology used, we may consider that the partition occurs between the hydrophilic environment (water) and a lipophilic environment (larval epicuticle) (Fahmy et al. 1978). In this way, the same authors studied the toxicity of a series of insecticide derivatives of the methyl carbamate group on mosquito larvae, reaching the conclusion that toxicity on mosquito larvae increases systematically as the number of carbon atoms in aliphatic carbamate increments. The maximum toxicity was observed when the total number of carbon atoms was eight or nine, noticing a gradual decrease in the toxicity beyond this point. In the aquatic habitat of the larva, these carbofuran derivatives are distributed between the water and the hydrophobe epicuticular waxy layer of the larva, and in this process, those with more hydrophobicity must pass faster through the larval cuticle. In agreement with previous works, they found a strong trend to explain this phenomenon by means of a parabolic equation. These results suggest that molecule hydrophobicity plays an important role in the intoxication of the mosquito larva (Fahmy et al. 1978).

The involvement of vapor pressure in this model is not easy to explain and could be related to the displacement and progressive accumulation of the component in the water–air interface. The mosquito larva has the outer respiratory apparatus on the last portion of the abdomen. In the case of the *A. aegypti*, the spiracle where the gaseous exchange is performed is located at the end of the siphon. When the larva needs to

perform the gaseous exchange, it reaches the water–air interface with the tip of the syphon, so that the atmospheric air may enter the tracheal system (Corbet et al. 2000). Therefore, that is the moment in which a small fraction of the component in equilibrium with the vapor phase may enter though the inhaling channel.

As a precedent, the toxic effect of low molecular weight mineral oils on the lepidoptera *Epiphyas postvittana* has been studied. As regards this, it was postulated that the toxicity of these oils may be attributed to the displacement of the protective lipids due to solvent effect, which would affect the nervous activity by incrementing the membrane permeability to ionic exchange (Taverner et al. 2001).

With respect to the mode of action of the essential oil components, there are reports which indicate that essential oils or their monoterpene components produce neurotoxic intoxication, similar to that produced by organophosphates and carbamates, inhibiting the acetylcholinesterase enzyme (Isman 1999; Houghton et al. 2006). However, the inhibition produced was only found in in-vitro studies, but not in in-vivo. Moreover, the inhibition values were relatively weak to infer an inhibition of the acetylcholinesterase enzyme.

Additionally, some works were reported in which a possible action of some components of essential oils was determined on the octopamine (eugenol and α-terpineol) (Enan 2001). Octopamine is present in the nervous system of all insects and acts as a neurotransmitter, neurohormone, and neuromodulator (Evans 1980). Moreover, there is evidence suggesting that octopamine is a peripheral transmitter in superior invertebrates (Woodring et al. 1989). Finally, the only work in which it is inferred that the thymol terpene acts on the GABA receptors was carried out on *Drosophila melanogaster* (Priestley et al. 2003).

The association between the toxic effect of the evaluated monoterpenes and the physicochemical properties can be better described when they are separated into functional groups (hydrocarbons vs. oxygenated).

However, these results should be considered as preliminaries, due to the low number of compounds evaluated.

References

Amer A, Mehlhorn H (2006) Larvicidal effects of various essential oils against *Aedes, Anopheles*, and *Culex* larvae (Diptera, Culicidae). Parasitol Res 99:466–472

Batish DR, Singh HP, Kohli RK, Kaur S (2008) *Eucalyptus* essential oil as a natural pesticide. Forest Ecol Manage 256:2166–2174

Chavasse DC, Yap HH (1997) Chemical methods for the control of vectors and pests of public health importance. World Health Organization, Geneva

Christophers SR (1960) *Aedes aegypti* (L.), the yellow fever mosquito. Its life history, bionomics, and structure. Cambridge University Press, London, p 739

Coats JR (1994) Risks from natural versus synthetic insecticides. Annu Rev Entomol 39:489–515

Corbet SA, Tiley C, Moorhouse T, Giam C, Pursglove S, Raby J, Rich M (2000) Surface films as mosquito larvicides: partitioning the mode of action. Entomologia experimentalis et applicata 94:295–307

Elango G, Bagavan A, Kamaraj C, Abduz Zahir A, Abdul Rahuman A (2009) Oviposition-deterrent, ovicidal, and repellent activities of indigenous plant extracts against *Anopheles subpictus* Grassi (Diptera: Culicidae). Parasitol Res 105:1567–1576

Eldridge BF (2005) Mosquitoes, the culicidae. In: Marquardt WH (ed) Biology of disease vectors, 2nd edn. Elsevier Academic, Burlington, pp 95–113

Enan P (2001) Insecticidal activity of essential oils: octopaminergic sites of action. Comparative Biochemistry and Physiology Part C 130: 325–337

Evans P (1980) Biogenic amines in the insect nervous system. Adv. Insect Physiol 15:317–473

Fahmy MAH, Mllipudi NM, Roy Fukuto R (1978) Selective toxicity of N,N'-Thiodicarbamates. J Agric Food Chem vol. 26 (3)

Govindarajan M, Jebanesan A, Pushpanathan T (2008) Larvicidal and ovicidal activity of *Cassia fistula* Linn. leaf extract against filarial and malarial vector mosquitoes. Parasitol Res 102:289–292

Hoskovec M, Grygarová D, Cvacká J, Streinz L, Zima J, Pverevkin S, Koutek B (2005) Determining the vapor pressures of plant volatiles from gas chromatographic retention data. J Chromat A 1083:161–172

Houghton P, Ren Y, Howes M (2006) Acetylcholinesterase inhibitors from plants and fungi. Nat Prod Rep 23:181–199

Isman M (1999) Pesticides based on plant essential oils. Pesticide Outlook 10:68–72

Isman MB (2000) Plant essential oils for pest and disease management. Crop Prot 19:603–608

Lee B, Choi W, Lee S, Park B (2001) Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). Crop Prot 20:317–320

Litchfield JT, Wilcoxon FJ (1949) A simplified method of evaluating dose-effect experiments. J Exp Ther 96:99–100

Lucia A, Gonzalez Audino P, Seccacini E, Licastro S, Zerba E, Masuh HM (2007) Larvicidal effect of *Eucalyptus grandis* essential oil and turpentine and their major components on *Aedes aegypti* larvae. J Am Mosq Control Assoc 23:299–303

Lucia A, Juan LW, Zerba E, Harrand L, Marcó M, Masuh H (2012) Validation of models to estímate the fumigant and larvicidal activity of *Eucalyptus* essential oils against *Aedes aegypti* (Diptera: Culicidae). Parasitol Res 110:1675–1686

Lucia A, Licastro S, Zerba E, Gonzalez Audino P, Masuh H (2009) Sensitivity of *Aedes aegypti* adults (Diptera: Culicidae) to the vapors of *Eucalyptus* essential oils. Biores Technol 100:6083–6087

Lucia A, Licastro S, Zerba E, Masuh H (2008) Yield, chemical composition andbioactivity of essential oils from twelve species of *Eucalyptus* on *Aedes aegypti* (L.) larvae (Diptera: Culicidae). Ent Exp Appl 129:107–114

Meylan WM, Howard PH (1995) Atom/fragment contribution method for estimating octanol–water partition coefficients. J Pharm Sci 84: 83–92

Michaelakis A, Papachristos D, Kimbaris A, Koliopoulos G, Giatropoulos A, Polissiou MG (2009) Citrus essential oils and four enantiomeric pinenes against *Cx. pipiens* (Diptera: Culicidae). Parasitol Res 105(3):769–773

Mill PJ (1985) Structure and physiology of the respiratory system. In: En Kerkut GA, Gilbert LI (eds) Comprehensive insect physiology biochemistry and pharmacology, vol 3. Pergamon Press, Oxford, pp 517–593

Papachristos DP, Stamopoulos DC (2002) Toxicity of vapors of three essential oils to the immature stages of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). J Stored Prod Res 38:117–128

Priestley C, Williamson E, Wafford K, Satelle D (2003) Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABAA receptors and a homo-oligomeric GABA receptor from *Drosophila melanogaster*. Br J Pharmacol 140:1363–1372

Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA, Elango G (2009) Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol Res 104: 1365–13

Rice P, Coats R (1994) Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and Southern corn rootworm (Coleoptera: Chrysomelidae). J Econ Entomol 87:1172–1179

Taverner DP, Gunning RV, Kolesik P, Bailey TP, Inceoglu AB, Hammock B, Roush TR (2001) Evidence for direct neural toxicity of a "Light Oil" on the peripheral nerves of Light brown Apple Moth. Pestic Biochem Physiol 69:153–165

Throne JE, Weaver DK, Chew V, Baker JE (1995) Probit analysis of correlated data: multiple observations over time at one concentration. J Econ Entomol 88:1510–1512

Tisserand R, Balacs T (1995) Essential oils safety: a guide for health care professionals. Churchill Livingstone, London

Toloza AC, Zygadlo J, Mougabure Cueto G, Biurrun F, Zerba E, Picollo MI (2006) Fumigant and repellent properties of essential oils and component compounds against permethrin-resistant *Pediculus humanus capitis* (Anoplura: Pediculidae) from Argentina. J Med Entomol 43(5):889–895

Van Roon A, Parsons JR, Govers HAJ (2002) Gas chromatographic determination of vapor pressure and related thermodynamic properties of monoterpenes and biogenically related compounds. J Chromat A 955:105–115

Weinzieri RA (2000) Botanical insecticides, soaps, and oils. In: Rechcigl JE, Rechcigl NA (eds) Biological and biotechnological control of insect pests. Lewis, Boca Raton

Weinzieri RA, Palevitch D, Craker L (1994) Volatile oils as potential insecticides. Herb Spice Med Plant Dig 12:1–8

Welling W, Paterson GD (1985) Toxicodynamics of insecticides (Capitulo 16). In: En Kerkut GA, Gilbert LI (eds) Comprehensive insect physiology biochemistry and pharmacology. Volumen 12, Insect Control. Pergamon Press, Oxford, pp 603–646

Woodring J, Mcbride L, Fields P (1989) The role of octopamine in handling and exercise-induced hyperglycemia and hyperlipaemia in *Acheta domesticus*. J Insect Physiol 35:613–617