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ORIGINAL PAPER

Comparative toxicity of oxygenated monoterpenoids in experimental hydroalcoholic lotions to permethrin-resistant adult head lice

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Abstract The use of botanical compounds such as essential oils has recently become the subject of great interest as a natural means of pest control because of their ovicidal, larvicidal, or adulticidal activity against various insect species including head lice. We tested and compared the efficacy of pure oxygenated monoterpenoids that are main ingredients of essential oils of good biological activity. We used pulegone and citral, components of *Aloysia citrodora*, and geraniol, citronellol, and linalool, components of *Geranium* sp. oil. We found that citronellol and geraniol showed the highest knockdown and mortality effect (>60%) on adults of both sexes (50:50%) and third-stage nymphs. Pulegone, linalool, and citral showed knockdown percentages between 42 and 55%, and mortality percentages between 47 and 53%. A simple linear regression analysis showed statistically significant relationships between the studied toxic effects and viscosity of the monoterpenoids ($p < 0.05$), but not with their partition coefficient (log P).

Keywords Head lice · Monoterpenoids · Permethrin resistant · Structure–activity correlation

Introduction

The infestation with head lice (*Pediculus humanus capitis* De Geer) (Phthiraptera: Pediculidae) is widespread throughout the world and is increasing due to the lack of effectiveness of pediculicides. This lack of efficacy is mainly due to the sale of ineffective products, the incorrect use of pediculicides, and that lice develop resistance to insecticides such as DDT, lindane, malathion, carbaryl, permethrin, and δ-phenothrin in several countries [5, 9, 11, 18]. In Argentina, field populations of *P. h. capitis* have developed resistance to permethrin and other pyrethroids after extensive use of insecticides since 1990 [14, 15, 17]. Recently, an extensive resistance survey in Buenos Aires showed significant levels of resistance in lice on children from schools [25], and seasonal fluctuations in head lice infestations are also common [4].

Insecticide resistance in *P. h. capitis* is a phenomenon of great concern because of the lack of novel insecticides introduced into the market as new safe products for chemical control of head lice. The use of essential oil constituents has recently become the subject of great interest as a natural means of pest control because of their repellent, ovicidal, adulticidal, and feeding inhibition activities against various insect species [10, 12, 20, 23] including head lice [19, 21, 24, 26–30].

Essential oils are the steam-distillable fraction of plant leaves which are often responsible for a plant's distinctive scent, odor, or taste. These oils are of rather complex composition, generally consisting of low molecular weight monoterpenes (10-carbon) and related phenols.

In this research, we tested and compared the efficacy of individual pure oxygenated monoterpenoids that are main ingredients of essential oils that have previously shown good biological activity. We used pulegone and citral, components of *Aloysia citrodora* [26], and geraniol, citronellol, and linalool, components of *Geranium* sp. oil [7]. Results from our lab showed very good fumigant activity for Aloysia oil [26] and for Geranium oil (A. Gallardo, unpublished results).

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We also performed a simple linear regression analysis to correlate toxic effects of the studied monoterpenoids with their physicochemical properties like octanol/water partition coefficient (log *P*) and viscosity.

Materials and methods

Biological material

Lice were collected from heads of infested children (6–12 years old) from three elementary schools in several areas of Buenos Aires, Argentina, using a fine-toothed anti-louse comb according to a protocol approved by ad-hoc committee of the Research Centre of Pests and Insecticides. After collection, the lice were sent to our laboratory where adults and third-stage nymphs were selected and immediately used in the bioassays. The resistance levels to permethrin of the studied populations ranged from 35 to 72 compared to the susceptible population [27].

Chemicals

The monoterpenoids pulegone, citral, geraniol, citronellol, and linalool were of more than 98% purity supplied by Fluka, Munich, Germany and by Sigma, St Louis, MO, USA. They are all ten-carbon unsaturated (1–3 double bonds) monoterpenoids, with one oxygenated function (alcohol or carbonyl). Among them, pulegone was chosen as the baseline comparator because screening results showed that it was the most effective by comparison of their knockdown activities.

Measurement of viscosity

The viscosity of monoterpenoids was measured with an adaptation of an Ostwald viscometer. The viscosity was expressed in millistokes and was calculated employing the equation $\eta_x = \eta_{H_2O}\rho_x t_x / \rho_{H_2O} t_{H_2O}$, where η_{H_2O} = water viscosity (millistokes), ρ_x = density of the monoterpenoid *x* (g/ml), ρ_{H_2O} = water density (g/ml), t_x = time taken by the level of the liquid to pass between the two marks of the viscometer (s) (t_x = monoterpenoid *x*, t_{H_2O} = water).

Bioassay

The toxic activities of experimental solutions were evaluated by the immersion method [6, 13]. Dilutions of ethanol in distilled water from 0 to 70% were evaluated in order to establish their toxic activity, and the optimal concentration of the ethanol used as diluent.

Dilutions of pulegone in the optimal ethanol–water solution ranging from 0.05 to 10% w/v were prepared in order to establish the optimal concentration for the evaluation of toxic activities of the monoterpenoids evaluated. The optimal concentration found for pulegone was used as a baseline comparator to evaluate the other monoterpenoids. Taking into account that 5 and 10% pulegone solution did not show significant differences, and that some of the other monoterpenoids tested are not completely soluble in 10% concentration, 5% w/v concentration was chosen as the standard concentration for comparisons. Batches of at least ten adults of both sexes (proportion of 50:50%) and third-stage nymphs were submerged for 2 min in 1 ml of each experimental lotion. At the end of the exposure period, the treated insects were placed onto a wire mesh and washed with 100 ml of water. The lice were then placed onto a piece of filter paper no. 1 (Whatman, Maidstone, UK) of 7 cm diameter moistened with 0.5 ml water that was placed in the bottom of a plastic Petri dish. Knockdown of exposed lice was recorded after 10 min, the necessary time for total recovery of control lice. Control lice were exposed to ethanol–water solution. The criterion for knockdown was inability of lice to walk over a filter paper and was recorded after 10 min, the necessary time for total recovery of control insects. The criterion for mortality was the inability to walk after 18 h. In all experiments, at least three replicates of each concentration were used, and data were corrected using the Abbott formula [1]. All treated insects were kept in the same experimental units and placed in an environmental chamber (Lab-Line Instruments, Melrose Park, IL, USA) at 18 ± 0, 5°C and 70–80% RH in the dark, in order to record mortality at 18 h posttreatment as reported previously [17].

Statistical analysis

The percentages of both toxic effects were analyzed by Kruskal–Wallis test [22]. The relationship between both toxic effects (knockdown and mortality) and physicochemical parameters (log *P* and viscosity) were analyzed by means of simple and multiple linear regressions [22].

Log *P* has been frequently used as a correlate of the toxic activity of essential oils against head lice [21]. Viscosity was selected for examination because in our preliminary screening results there appeared to be an optimal viscosity for penetration through the louse cuticle.

Results

Knockdown and mortality activities of the different hydroalcoholic solutions evaluated are shown in Fig. 1.

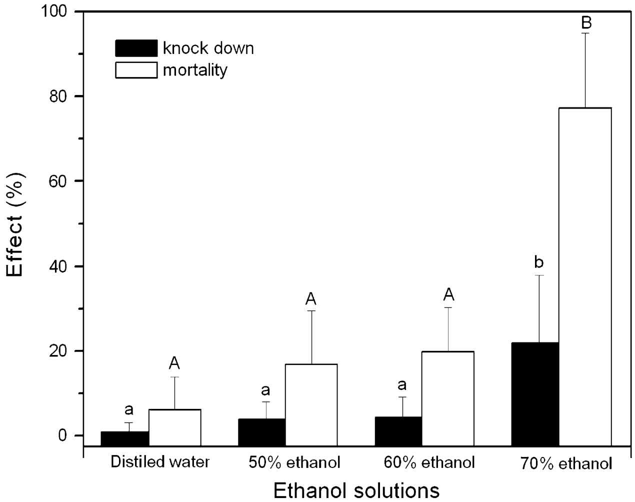

Fig. 1 Knockdown (*black bar*) and mortality (*white bar*) activities of hydroalcoholic lotions of different concentrations evaluated on head lice (10 adults of both sexes and third-stage nymphs). *Bars* of each effect with the *same letter above* are not significantly different (ANOVA) ($p < 0.05$)

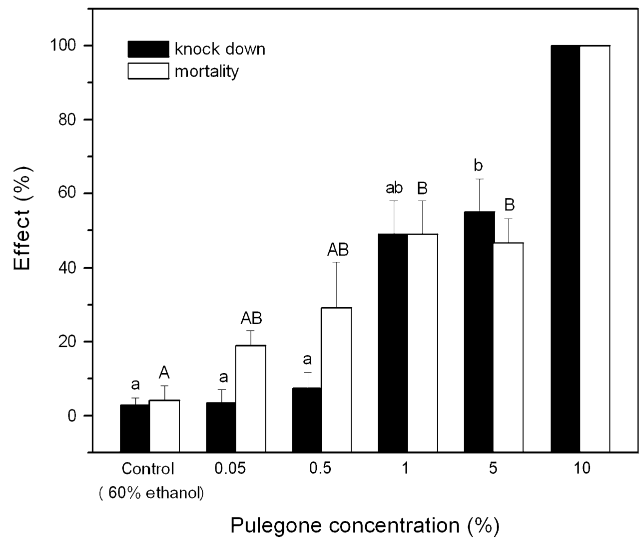

Fig. 2 Knockdown (*black bar*) and mortality (*white bar*) activities of the different concentrations of pulegone in hydroalcoholic solution evaluated on head lice (10 adults of both sexes and third-stage nymphs). *Bars* of each effect with the *same letter above* are not significantly different (ANOVA) ($p < 0.05$)

The solution containing 70% ethanol showed statistically higher activities in both knockdown (25.4 ± 8.1%; $p < 0.05$) and mortality (78.3 ± 12.4%; $p < 0.05$) than other ethanol solutions and controls. As the 70% ethanol–water solution is intrinsically toxic itself, it was not useful to evaluate the monoterpenoids. The toxic activities for the 50 and 60% ethanol–water solutions were statistically similar ($p > 0.05$) with knockdown percentages lower than 5% and mortality percentages lower that 20%. The test monoterpenoids were not completely soluble in the 50% ethanol–water solution. The 60% ethanol–water solution was a good diluent for all the test monoterpenoids and showed toxic activities that were not significantly different from controls. Because of these factors, it was chosen as the solvent for the test monoterpenoid solutions.

The toxic activities produced by various concentrations of pulegone in 60% ethanol–water solutions are given in Fig. 2. Typical dose–response relationships for both toxic activities were elicited, where the highest concentration evaluated (pulegone 10%) produced the maximum effect (100%) in both activities. The 5% pulegone solution resulted in a significant increase in knockdown (55 ± 4.5%, $p < 0.05$), whereas both the 1 and 5% solutions resulted in significantly increased mortality (49 ± 9 and 47 ± 3.4%, respectively, $p < 0.05$) compared to controls. Thus, the 5% pulegone solution dilute in 60% ethanol–water resulted in significantly increased knockdown and mortality, each reaching their approximate 50% levels. Additionally, the other test monoterpenoids were not completely soluble at their respective 10% concentrations in the 60% ethanol–water solution. Because of these factors, the 5% concentration was chosen to evaluate the toxic activities of the test monoterpenoids.

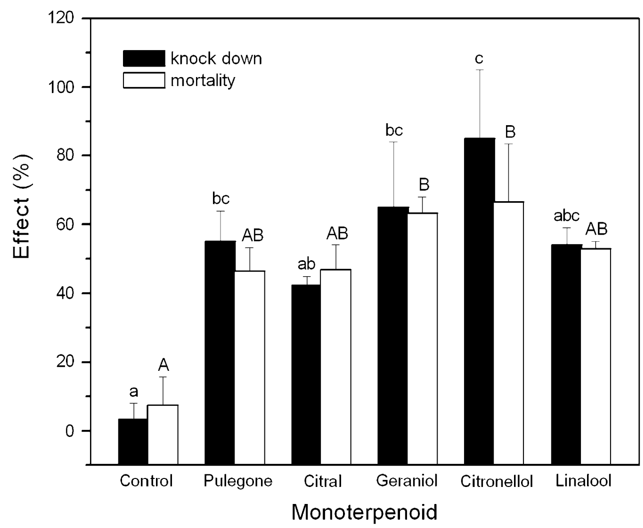

Fig. 3 Knockdown (*black bar*) and mortality (*white bar*) activities of the monoterpenoids in hydroalcoholic solution (5%) evaluated on head lice (10 adults of both sexes and third-stage nymphs). *Bars* of each effect with the *same letter above* are not significantly different (ANOVA) ($p < 0.05$)

The knockdown and mortality activities of all evaluated monoterpenoids are shown in Fig. 3. For both toxic effects, citronellol and geraniol showed the highest activities (>60%). However, there were not significant differences among the evaluated monoterpenoids for both toxic effects ($p > 0.05$) except between citronellol and citral for knockdown ($p < 0.05$).

Table 1 Log *P* and viscosities values of evaluated monoterpenoids

Monoterpenoid	Log *P*	Viscosity (millistokes)
Citral	3.76 ± 0.42	0.847 ± 0.01
Geraniol	2.27 ± 0.22	1.030 ± 0.03
Citronellol	3.38 ± 0.24	1.085 ± 0.03
Linalool	3.28 ± 0.26	0.94 ± 0.01
Pulegone	2.56 ± 0.24	0.887 ± 0.003

Viscosity was measured by Ostwald method and expressed in millistokes. Log *P* is the octanol/water partition coefficient

The log *P* and viscosity values of evaluated monoterpenoids are shown in Table 1. The simple linear regression analysis of our results showed that there is a close association between each observed toxic effect and the viscosity of the evaluated monoterpenoids ($p < 0.05$), but there are not significant relationship between those effects and log *P* of the compounds ($p > 0.05$) (Table 2). On the other side, the results of the multiple linear regression analysis did not justify the inclusion of both viscosity and log *P* as independent variables in the same model.

Discussion

Several studies evaluated the pediculicidal activity of essential oils and their constituents. Essentials oils from different plants showed high pediculicide activity when evaluated pure and were highly effective when evaluated as commercial formulation [2, 3, 6, 8]. Additionally, those commercial products were safe [3]. Respect to monoterpenoids, the main constituents of essential oils, Yang et al. [30] showed that 1.8-cineole and linalool were the most toxic compounds evaluated as fumigant, meanwhile (−)-camphor and linalool were the most effective evaluated by contact. Toloza et al. [24, 27] studied the fumigant activity of monoterpenoids on adult lice and found that linalool and pulegone have better knockdown times than citronellol, menthol, geraniol, and eugenol. Priestley et al. [19] tested lethality of essential oils constituents on human clothing lice adults by exposition to impregnated paper and found, among other components, that pulegone and linalool were more effective than geraniol, citral, and menthol. The results of these studies seem to be contradictory with our results, probably due to the different methodologies used for bioassays. It is well established that different methodologies represent different ways of exposure and biological availability of the active principle. The exposure methodology of the toxic determines the primary site and main mode of absorption. In the bioassays of fumigant activity, absorption occurs mainly through the tracheal system, and the toxic activity is produced by the vapor phase of the compound. Moreover, in the contact bioassays (topical application or immersion method), compounds penetrate through the cuticle, and the toxic activity is mainly caused by the toxic in solution. In the last case, viscosity and lipophilicity are involved in the rate of penetration through cuticle and travel to the target. Thus, the physicochemical parameters involved in the toxicity of the compounds depend on the bioassay used. As discussed below, the vapor pressure determines the toxicity in fumigant bioassays, while lipophilicity and viscosity determine the toxicity in contact bioassays. So, bioassays with different method of exposure determine differences in the absorption of the toxics and, consequently, different toxicity ranking for the same compounds evaluated. Therefore, it appears necessary to standardize bioassays [16].

Although there are previous reports on correlations between pediculicidal efficacy of essentials oils compounds and its physicochemical parameters [24, 28, 29], this study is the first that shows the association between toxic effect and viscosity. According to our results and in the range of viscosities studied, the more viscous the compound, the more effective it was as a pediculicide. This could be interpreted in terms of an optimal viscosity for penetration through the insect cuticle. Greater viscosities (not studied) are likely to inhibit penetration and thus diminish biological activity.

Yang et al. [28, 29] discussed anti-lice results with compounds from essential oils of *Eugenia caryophyllata*

Table 2 Description of the models fitted by simple and multiple linear regression analysis

Independent variable (*x*)	Dependent variable (*y*)	Equation	R^2	*p* value
Viscosity	Knockdown	$y = -86.81 + 153.6x$	0.83	0.015
Viscosity	Mortality	$y = -33.44 + 92.67x$	0.97	0.0025
Log *P*	Knockdown	$y = 74.41 + 4.63x$	0.03	0.77
Log *P*	Mortality	$y = 64.71 + 3.10x$	0.04	0.74
Viscosity (x_1) and log *P* (x_2)	Knockdown	$y = -93.96 + 156.08x_1 + 1.57x_2$	0.90	0.10
Viscosity (x_1) and log *P* (x_2)	Mortality	$y = -36.33 + 93.66x_1 + 0.64x_2$	0.96	0.03

Association between viscosity and/or log *P* of each of the evaluated monoterpenoids with their mortality and/or knockdown effect and *Eucalyptus globulus* in terms of the lipophilicity of the compounds and found no direct correlation between contact toxicity and lipophilicity. However, Toloza et al. [24] found a significant association between estimated KT_{50} values and corresponding vapor pressures of the monoterpenoids, and between KT_{50} and vapor pressure and octanol–water partition coefficients of the monoterpenoids when both predictors were included in the same model. Again, the differences in the used bioassay method can explain the different associations reported between pediculicide activity of monoterpenoids and its physicochemical parameters. Typically, compounds with greater activity in contact bioassay, which need to penetrate the outer lipophilic and inner lipophobic barriers of the cuticle, will be those with an optimal or intermediate lipophilicity, while compounds with better activity in fumigant assay would be those with higher vapor pressure [21]. The immersion bioassay and the polar diluents used in the present study probably decrease the influence of lipophilicity in the toxicity of monoterpenoids. However, this result must be considered as preliminary due to the low number of evaluated compounds.

In conclusion, the toxic activities of monoterpenoids against *P. h. capitis* reported in the present study suggest that they may have potential for development of pediculicidal products, taking into account that geraniol and linalool are patent covered. Anyway, for the practical use of these lotions containing essential oils to proceed, further research is necessary on several issues, including dermal and ocular irritation tests to ensure safety of these lotions for human health.

It should be emphasized that the immersion bioassay with alcoholic lotions used in this work is an adequate standardized laboratory test respect to potential commercials lotions [15]. In consequence, the present results represent a realistic alternative to synthetic insecticides for lice control.

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