Ovicidal and adulticidal effects of monoterpenoids against permethrin-resistant human head lice, Pediculus humanus capitis

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Ovicidal and adulticidal effects of monoterpenoids against permethrin-resistant human head lice, *Pediculus humanus capitis*

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Abstract. The improper use of pediculicides containing permethrin has led to the development of resistance. Thus, new alternatives for control are needed. Plant-derived insecticides are attractive alternatives to common chemical insecticides because most of them are environmentally friendly and non-toxic to mammals. The toxic activity of 23 monoterpenoids belonging to several chemical classes was tested against the eggs of permethrin-resistant head lice, *Pediculus humanus capitis* De Geer (Phthiraptera: Pediculidae). Significant differences in ovicidal action were observed among the tested substances. The most effective chemicals were hydrocarbons and ethers, followed by ketones, alcohols, phenols and esters. A linear relationship between egg mortality and knockdown time (KT_{50}) on adults by the tested components revealed that most of the components were effective on both egg and adult stages. The monoterpenoids described herein are good candidates as effective pediculicides.

Key words. *Pediculus humanus capitis*, adult head lice, fumigant activity, head lice eggs, monoterpenes, toxicity.

Introduction

The human head louse, *Pediculus humanus capitis* De Geer, is an important cosmopolitan pest mainly affecting school-aged children. Louse infestation is annoying and may cause itching, loss of sleep and social sanctioning (Gratz, 1997). The spread of lice occurs mainly through direct host-to-host contact (Roberts, 2002). Although head lice have not been incriminated in the transmission of deleterious pathogens, it has been suggested that they can be potential transmitters of *Rickettsia prowazekii* Da Rocha-Lima, a causative agent of epidemic typhus, and *Bartonella quintana* Brenner, a causative agent of trench fever (Robinson *et al.*, 2003; Sasaki *et al.*, 2006).

There are several treatment options for the control of head lice, which include over-the-counter products, prescription pediculicides, manual removal (wet combing), homeopathic remedies and oral therapy (West, 2004). Infestation with head lice is increasing every year in both developed and developing countries, as reflected in pediculicide failure through resistance, improper application and formulation changes (Burgess, 2004). Since the mid-1990s, levels of resistance to the synthetic pyrethroids permethrin and phenothrin have been widely reported in several countries, such as France (Coz *et al.*, 1993), the Czech Republic (Rupes *et al.*, 1995), the U.K. (Burgess *et al.*, 1995), Israel (Mumcuoglu *et al.*, 1995), the U.S.A. (Pollack *et al.*, 1999), Denmark (Kristensen *et al.*, 2006), Japan (Tomita *et al.*, 2003), Australia (Hunter & Barker, 2003) and Argentina (Picollo *et al.*, 1998, 2000; Vassena *et al.*, 2003). Therefore, the use of alternative plant-derived products has increased worldwide due to the tendency of consumers to prefer ‘natural’ products. Plant essential oils, the steam distillate of aromatic plants, constitute a rich source of bioactive chemicals with compounds generally consisting of low-molecular-weight monoterpenes and phenols (Guenther, 1972). Some of these components are effective against a wide variety of insect pests (Rice & Coats, 1994; Isman, 1999), including human lice (Burgess, 2004; Priestley *et al.*, 2006; Toloza *et al.*, 2006). Plant-derived products such as shampoos, sprays and lotions have previously been reported to be effective as pediculicides (Mumcuoglu *et al.*, 2002; Heukelbach *et al.*, 2006; Abdel-Ghaffar & Semmler, 2007; Gonzalez-Audino *et al.*, 2007). In general terms, adult lice are easier to kill than eggs. Effective control is to ensure that the dosage, exposure and formulation of the products are sufficient to kill them (Buxton, 1947; Veal, 1996). Concerning eggs, there are two

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types of mortality: the embryo in the egg is killed or the developing nymph dies when it comes into contact with the surface insecticide as it chews its way out of the chorion during ecolosion (Leonard *et al.*, 1991). Differences among the biological systems of eggs and hatched forms include the absence of a differentiated nervous system in early embryonic stages and certain processes for utilization of stored energy that are not common to hatched forms. On the other hand, mature embryos have some of the same vital systems found in newly emerged nymphs, such as the nervous, respiratory and circulatory systems. Thus, it may be possible to develop an ovicidal product that is effective against all stages, if it could target vital processes common to mature embryos and hatched forms, as well as those that are unique to embryos (Smith & Wagenknecht, 1959). The aim of this work was to study the pediculicidal activity of several monoterpenoids against eggs and adults of *P. h. capitis* populations reported to have permethrin resistance.

Materials and methods

Chemicals

The monoterpenoids tested were those previously evaluated against adult head lice (Toloza *et al.*, 2006). All compounds were of more than 98% purity (Fig. 1), and were supplied by Fluka, Munich, Germany and by Sigma, St. Louis, MO.

Head lice

Adults and eggs were collected in 2007 from infested children 6–12 years old, using a fine-toothed antilouse comb. Head lice were obtained from three elementary schools located in different parts of Buenos Aires city. Previously, a topical application method (Vassena *et al.*, 2003) had demonstrated high levels of

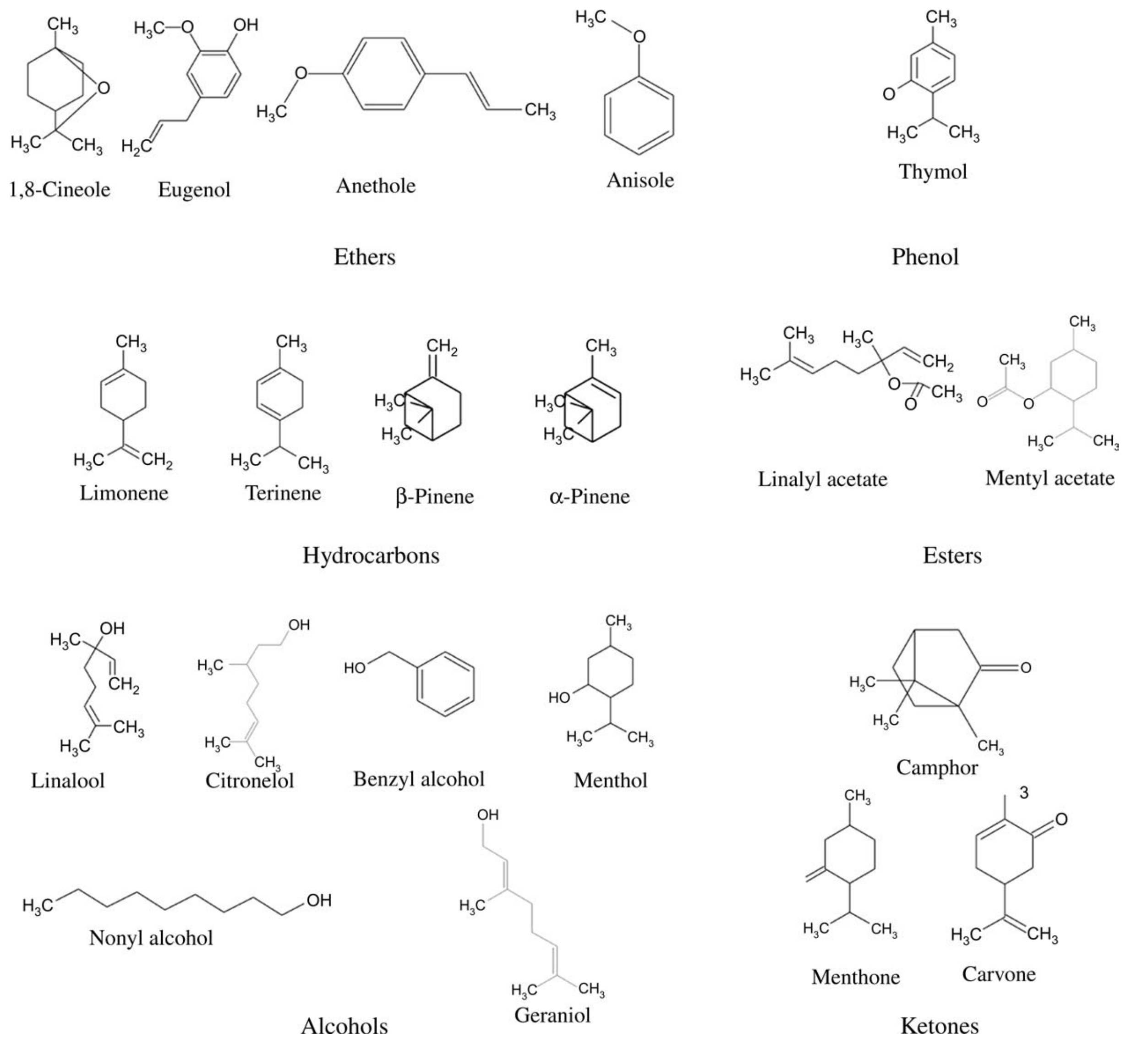

Fig. 1. Structures of the monoterpenoids selected for investigation.

resistance to permethrin in these schools (resistance ratios >150), which were confirmed to be at a similar level during the present study. The material was collected and transported to our laboratory as previously reported (Picollo *et al.*, 1998, 2000). The protocol for adult and egg collection was approved by the *ad hoc* committee at the Centro de Investigaciones de Plagas e Insecticidas (Research Centre of Pests and Insecticides, Buenos Aires, Argentina) and archived in our laboratory.

Ovicidal effects of monoterpenoids on Pediculus humanus capitis *eggs*

Each experimental unit consisted of a covered plastic Petri dish (55 mm diameter), a filter paper and 60 μL of a test substance on a small plastic container. The negative control consisted of the same experimental unit without the addition of any substance. Groups of 10–20 late development viable eggs were selected, as described previously by Mougabure Cueto *et al.* (2006), and transferred to the enclosed chamber. Late eggs showed black eye spots and clearly visible appendages of the embryo. Each set of eggs was exposed to the test monoterpenoid for 24 h at 28 ± 1°C and 75 ± 5% RH. After this time, the small container with the test substance was removed and the treated batches of eggs were incubated at 28 ± 1°C and 75 ± 5% RH (closed containers with saturated aqueous solutions of NaCl). Mortality data of treated eggs were recorded 5 days after the eclosion of controls. Treatments and controls were replicated three times. The criterion for mortality was eggs with a closed operculum or eggs with an opened operculum and the nymph inside (Mougabure Cueto *et al.*, 2006).

Fumigant activity against adult head lice

The method of Toloza *et al.* (2006) was employed to evaluate the fumigant activity of those monoterpenoids whose efficacy has not been previously reported. The compounds were evaluated in an enclosed chamber (9 cm diameter Petri dish) containing 60 μL of the test substance. A 5.5 cm diameter Petri dish with a filter paper and 15 adult head lice was placed within the chamber. The control test consisted of the same unit without the addition of any substance. Knockdown of exposed lice, defined here as the inability of the insects to walk on a filter paper, was recorded every 5 min for 1 h.

With the fumigant data reported here and those previously reported by us (Toloza *et al.*, 2006), a linear regression analysis was performed between knockdown time (KT_{50}) values on adults and the percentage mortality of the eggs.

Statistical analysis

All mortality data were corrected for control mortality by using Abbott's equation (Abbott, 1925). The percentage mortality was determined and transformed to arcsine square-root values for ANOVA. Treatment means were compared and separated by the Duncan test at $P < 0.05$ (StatSoft Inc., 2001). The relationship between KT_{50}s on adults and egg mortality was estimated by regression analysis. All statistical tests were performed with $\alpha = 0.05$ for significance of statistical tests.

Results

The ovicidal activity of monoterpenoids, the negative control and permethrin are summarized in Fig. 2. Significant differences in ovicidal action were observed among the tested substances ($F = 14.88$, d.f. = 23, 58, $P < 0.0001$). Ten of the compounds gave louse egg mortality above 80% (as compared with the negative control). The most effective chemicals were hydrocarbons and ethers, followed by ketones, alcohols, phenols and esters. Assays of individual monoterpenoids indicated that the most effective were anisole (100%), α-pinene (96.7 ± 4.7%), β-pinene (96.4 ± 6.2%), (+)-α-pinene (96.1 ± 1.4%), 1S-(-)-α-pinene (94.21 ± 0.3%), anethole (92.5 ± 7.5%), carvone (91.6 ± 11.7%), limonene (89.88 ± 11.3%), linalool (88.17 ± 10.9%) and 1,8-cineole (84.47 ± 8.0%) at the 95% confidence level.

The toxicity of six monoterpenoids against adult *P. h. capitis* are shown in Table 1. Of the tested compounds, only (+)-α-pinene and S-(-)-α-pinene were effective on head lice. Additionally, the fumigant activity of monoterpenoids previously reported by Toloza *et al.* (2006) is shown in comparison (Table 1).

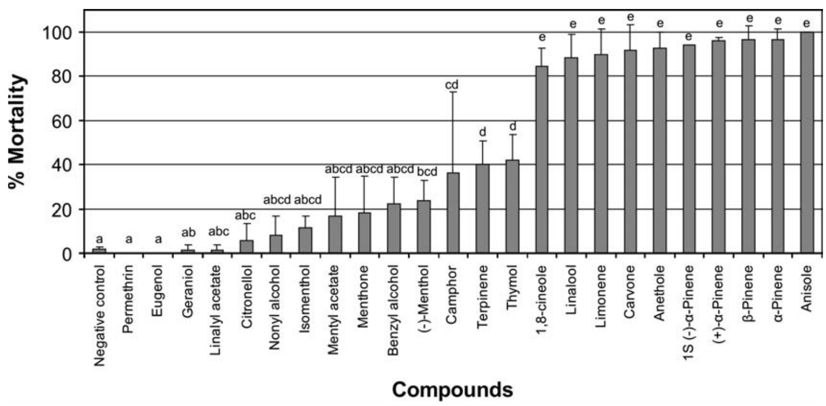

Fig. 2. Ovicidal effects of compounds against *Pediculus humanus capitis*. Different letters are significantly different by Duncan's test ($P < 0.05$).

Table 1. Fumigant activity of monoterpenoids against adult head lice.

Chemical class	Monoterpenoid	KT_{50} (95% confidence level)
Ether	1,8-cineole	11.10 (8.32–13.11)*
	Eugenol	>60*
	Anethole	>60*
	Anisole	12.7 (10.80–14.40)*
Hydrocarbon	Limonene	27.20 (22–32.41)*
	Terpinene	>60*
	β-Pinene	33.86 (28–39.61)*
	α-Pinene	42.70 (31.50–62.20)*
	(+)-α-Pinene	34.48 (25.31–53.97)
	1S-(-)-α-Pinene	28.54 (19.89–43.35)
Alcohol	Linalool	37.73 (34.79–40.49)*
	Citronellol	>60*
	Benzyl alcohol	59.72 (55–68.90)*
	Nonyl alcohol	>60
	Isomenthol	>60*
	(-)-Menthol	>60*
	Geraniol	>60
Ketone	Menthone	39.70 (35.60–43.60)*
	Carvone	>60*
	Camphor	>60*
Phenol	Thymol	>60*
Ester	Mentyl acetate	>60
	Linalyl acetate	>60
Negative control		>60
Permethrin		>60

*Data from Toloza *et al.* (2006).
KT_{50}, knockdown time (min).

When adult *P. h. capitis* fumigant KT_{50}s were compared with ovicidal mortality percentages, we detected a strong relationship between the pediculicidal activity of the monoterpenoids in both adult and egg stages ($KT_{50} = 62.9234–0.290902$ egg mortality, $r^2 = 0.47$, $F = 17.61$, d.f. = 1, 20, $P < 0.001$) (Fig. 3).

Discussion

The present work reports that some monoterpenoids are potentially useful for head louse control because many of them were effective against both adults and eggs. These results suggest that the process involved in killing both later development eggs and adults may be similar. Previous studies have also reported the efficacy of some essential oil components on *P. humanus* eggs (Lahlou & Berrada, 2003; Yang *et al.*, 2004; Priestley *et al.*, 2006), however, different bioassay methodologies were employed. In comparison with the results of Yang *et al.* (2004), who used a filter paper contact bioassay and observed that 1,8-cineole was ineffective, our results indicate that it was highly effective. In addition, the most effective components reported by Priestley *et al.* (2006), who employed an immersion methodology on clothing lice, ranged from ineffective to moderately effective in our study. This difference may be related, at least in part, to the mode of application. The fumigant action of monoterpenoids, which have a high vapour pressure, indicates that the toxicity of the compounds offers an effective means of penetration via the

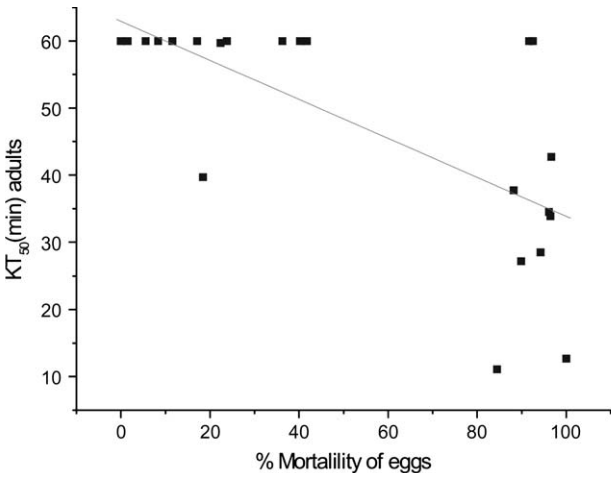

Fig. 3. The linear regression between the toxicity of monoterpenoids on adults (knockdown time: KT_{50}) and eggs (% mortality).

respiratory system (Smith & Salkeld, 1966). Monoterpenoids are lipophilic compounds, and Veal (1996) mentioned that penetration through the louse haemolymph may be slow and limited, and instead entry may occur via the tracheal system. In eggs, the exchange of gases between the atmosphere and the embryo depends upon diffusion through the micropyles of the operculum, and monoterpenoids may reach the embryo through these structures. Moreover, changes in the membranes surrounding the embryo affect the permeability of the shell, increasing the toxicity of the insecticide (Smith & Salked, 1966).

Differences in the evaluation of pediculicide efficacy could also be due to differences in the mortality criteria applied. According to Heukelbach *et al.* (2008), head lice can recover within 180 min of sham death after exposure to a pediculicide. Thus, a short post-treatment recording time could overestimate the mortality of lice and the efficacy of a tested substance or product.

Our study indicates that monoterpenoids could be incorporated into formulations as potential pediculicides against both eggs and adults. Moreover, the addition of excipients to increase the efficacy and stability of the products should also be taken into account (Burgess, 2004). High volatility might be remedied by formulating candidate monoterpenoids for sustained release, or by formulating them with other compounds in a vehicle base. Further complementary studies including human health, such as burning sensations and skin irritation, should also be considered before any of these compounds can be employed as commercial products.

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