ELSEVIER

Fitoterapia 77 (2006) 303 – 309

FITOTERAPIA

www.elsevier.com/locate/fitote

Lethality of essential oil constituents towards the human louse, *Pediculus humanus*, and its eggs

C.M. Priestley [a], I.F. Burgess [b], E.M. Williamson [c,*]

[a] *GlaxoSmithKline Nutritional Healthcare, Royal Forest Factory, Coleford, Gloucestershire, GL16 8JB, UK*
[b] *Medical Entomology Centre, Insect Research & Development Limited, Cambridge House, Barrington Road, Shepreth, Royston SG8 6QZ, UK*
[c] *The School of Pharmacy, University of Reading, Whiteknights, Reading, Berkshire, RG6 6AJ, UK*

Received 31 August 2005; accepted 3 April 2006

Abstract

Essential oils have been widely used in traditional medicine for the eradication of lice, including head lice, but due to the variability of their constitution the effects may not be reproducible. In an attempt to assess the contribution of their component monoterpenoids, a range of common individual compounds were tested in in vitro toxicity model against both human lice (*Pediculus humanus*, an accepted model of head lice lethality) and their eggs, at different concentrations. No detailed study into the relative potencies of their constituent terpenoids has so far been published. Adult lice were observed for lack of response to stimuli over 3 h and the LT_{50} calculated, and the percentage of eggs failing to hatch was used to generate ovicidal activity data. A ranking was compiled for adult lice and partially for eggs, enabling structure–activity relationships to be assessed for lethality to both, and showed that, for activity in both life-cycle stages, different structural criteria were required. (+)-Terpinen-4-ol was the most effective compound against adult lice, followed by other mono-oxygenated monocyclic compounds, whereas nerolidol was particularly lethal to eggs, but ineffective against adult lice.

Keywords: Monoterpenes; Head lice; Pediculosis; Essential oils; Pediculicidal; Ovicidal

1. Introduction

Essential oils have been widely used in traditional medicine for their insecticidal and repellent activity against many species of insect, including body and head lice. Although a widespread phenomenon, head louse infestations (pediculoses) usually cause comparatively minor problems such as pruritus and social embarrassment [1], especially in developed countries where the majority of infested scalps generally bear only a few lice. In the 20th century, the introduction of pediculicides and concomitant decrease in social tolerance of lice led to falling infestation rates in Western societies.

In the UK, the frequency of infestation with head lice (*Pediculus capitis*) was probably at its lowest in the late 1980s and early 1990s, but now the incidence throughout the West is increasing [2], with insecticide resistance the most likely cause.

* Corresponding author. Tel.: +44 118 378 7017; fax: +44 118 378 7017.
E-mail address: e.m.williamson@reading.ac.uk (E.M. Williamson).

0367-326X/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.fitote.2006.04.005

In developing countries, most conventional Western (synthetic) pediculicides are either unavailable or prohibitively expensive and lice infestations are therefore even more common. Cures for pediculosis centre mainly on social grooming and the use of an enormous number of plants. In recent years, interest in plant-derived medicines has increased in the West, due to the current trend for "natural" consumer items.

Plant essential oils have been used for centuries as medicines, fragrances and insect repellents. They consist of numerous different, mostly volatile, low molecular weight (LMW) terpenoids [3]. Constituents of plant volatile oils have long been known to affect the behavioural responses of pests, with the monoterpenoid components appearing most useful as insecticides or anti-feedants [4].

The composition of any one type of essential oil however varies according to the plant cultivar, growing conditions and extraction methods used to obtain it. Therefore, if an essential oil is to be used in the treatment of lice, a more predictable performance will be attained if the active component(s) are identified and standardised in the product to within set limits, by for example, selecting or blending the raw plant material and/or distilled oil. It is also possible that active single compounds may find a use in treatment.

Many modern pediculicides tend to fail because of low efficacy on lice eggs, whereas essential oil constituents are reputed to have good ovicidal capabilities [2]. An ovicidal activity screen was therefore used to produce a preliminary ranking for LMW terpenoids versus lice eggs and to investigate whether the terpenoids most lethal to adult lice were also the most active ovicides. It was also important to determine whether a single compound could suffice in a pediculicidal formulation, or whether a combination of agents would perform significantly better.

Essential oils (especially tea tree oil) have often been proposed as alternative pediculosis control agents in both scientific and lay media articles, and they and their constituents therefore provide a good starting point for an investigation into the development of novel pediculicides. The main aim of this study was to test a range of isolated monoterpenoids so that the structural features relating to pediculicidial and ovicidal activity could be determined, and perhaps used to predict the potency of an oil from its composition, to some extent.

2. Experimental

2.1. General

Lice and eggs used in this study were obtained from a laboratory-reared colony of human clothing lice maintained by Insect Research and Development Limited, Cambridge, UK. Terpenoids (Table 1) were obtained from Sigma-Aldrich, UK, Lancaster Synthesis, UK, Acros Organics, UK, Fisher Scientific, UK, or Avocado Research Chemicals, UK.

2.2. Testing for in vitro pediculicidal activity

The method used to assess the pediculicidal activity was adapted from a World Health Organization method for testing sensitivity or resistance to conventional insecticides [5]. Preliminary experiments were carried out to determine suitable experimental parameters, such as the dilution factors of test substances and the duration of exposure to lice. The finalised assay was carried out as follows: each test substance was diluted to the appropriate concentration as before. A volume of 600 μl of diluted sample was distributed evenly over a 9 cm diameter filter paper, held in the lower half of 9 cm glass Petri dish. After 5 min, the liquid had spread out, the filter paper was fully impregnated and no excess moisture was left in the dish. Impregnated papers were hung in a fume cupboard for 5 min to allow the solvent to evaporate. After this time, the papers were returned to their dishes and 10 clothing lice placed on top of each. The lice were selected at random from a mixture of adult males and females that had been fed 1–6 h previously.

Petri dishes containing impregnated filter papers and lice were covered with glass lids and incubated under normal maintenance conditions for lice and eggs of 28±2 °C, 60±20% relative humidity (RH). Two (or more) control tests were performed concurrently with each batch of assays: In the first, lice were placed on unimpregnated filter papers; in others, lice were exposed to solvent-impregnated filter papers that had been dried for 5 min. The Petri dish lids were kept in place during the tests but removed every 15 min so that the lice could be observed and the number of fatalities recorded. Death was defined as lack of movement of limbs and gut, and failure to respond when the legs were stroked with forceps.

Table 1
Order of the relative efficacy of essential oil constituents on human clothing lice adults and eggs

Versus adults lice		Versus eggs	
1	(+)-Terpinen-4-ol	1	Nerolidol
2	Pulegone	2	Thymol
3	(−)-Terpinen-4-ol		Geraniol
4	Thymol	4	Carveol
5	α-Terpineol	5	Menthol
6	Menthone		α-Terpineol
7	Carvacrol	7	Citral
8	Linalool		Citronellic acid
9	Perillaldehyde		Linalool
10	Geraniol		(+)-Terpinen-4-ol
11	Citral		(−)-Terpinen-4-ol
12	Carveol	12–19	Cineole
13	Menthol		α-Pinene
14	Thujone		α-Terpinene
15	Geranyl acetate		γ-Terpinene
16	Linalyl acetate		Limonene
			Menth-6-ene-2,8-diol
			β-Pinene
			Linalyl acetate
			Menthone
17–28	Camphene		
	Camphor		
	Cineole		
	Citronellic acid		
	Limonene		
	Menth-6-ene-2,8-diol		
	Methane-3,8-diol		
	Myrcene		
	Nerolidol		
	α-Pinene		
	β-Pinene		
	α-Terpinene		

Each substance was assayed five times and, for each time point, the mean percentage mortality ± 1 S.E.M was calculated. These data were expressed graphically and the relative pediculicidal efficacy (LT_{50}, i.e. the time taken to kill 50% of the insects) then compared graphically as shown in Fig. 1. To calculate the LT_{50}s, log-logit transformed raw data for each test agent were subject to linear regression analysis and the LT_{50} estimated from the intercept of the graphed line with the X-axis. Data transformation and regression analyses were performed using the in-built equations in Graph-Pad Prism Software (Graphpad, UK).

2.3. In vitro assessment of ovicidal assay

The ovicidal activity of terpenoids in solution was assessed according to Burgess [6,7]. Actively reproductive adult lice were provided with nylon gauze as a substrate on which to lay eggs over a 2-day period. After removal of the lice, the gauze, with eggs still attached, was incubated under normal maintenance conditions until the tests were carried out, 1 or 2 days later.

The sheets of gauze were cut into squares of convenient size (ca. 2 cm × 2 cm). Terpenoids were diluted as before and used to fill 20 ml glass bottles. Gauze bearing approximately 300 eggs (200 minimum) was immersed in each test substance for 10 min. After this time, the gauze was removed, blotted and dried of solvent on a medical wipe tissue laid flat in a fume cupboard, on low setting, for 5 min. A control batch of eggs exposed to solvent only was run concurrently with each batch of tests, and this enabled correction of test results for solvent activity. An untreated control batch was included periodically to ensure that solvent treatment continued to have no significant effect on the background mortality rate.

After treatment, treated batches of eggs were incubated in separate glass Petri dishes, under normal maintenance conditions, until all the nymphs in the control batches had hatched and died. For calculation of percent mortality, all

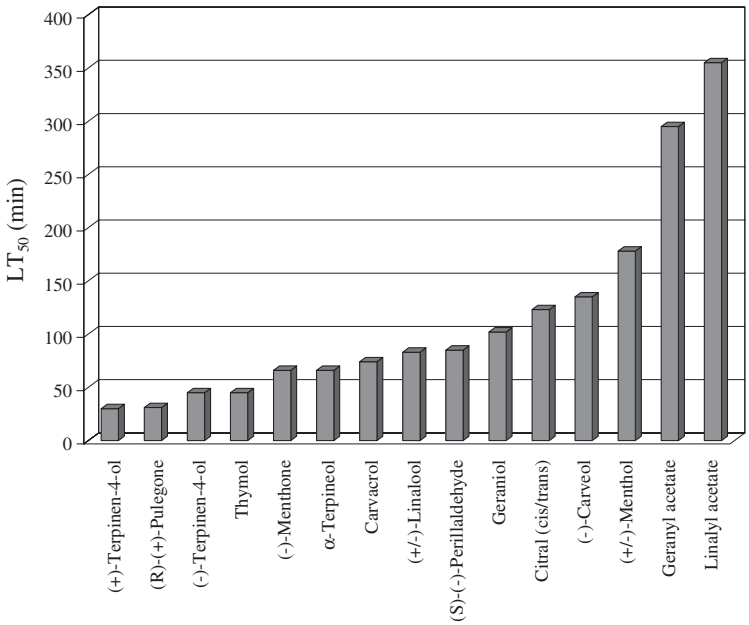

Fig. 1. Activity of essential oil constituents against the *P. humanus* louse. Inactive: camphene, camphor. cineole, citronellic acid, limonene, menth-6-ene-2,8-diol, methane-3,8-diol, myrcene, merolidol, α-pinene, β-pinene, α-terpinene.

hatched nymphs were classified as having survived the treatment and those failing to hatch or only partially hatching as having been killed by the treatment. A preliminary screen at 10% (w/v or v/v) allowed the test agents to be classified into two groups, one high activity, where the total mortality was 82% or above, and one low activity, giving mortalities of 34% and below. As there was a clear demarcation between the high activity and the low activity group i.e. none of the compounds gave a (mean) mortality result in the 35% to 81% range, the number of replicates were kept to a minimum (N 1–3, Fig. 2), which was necessary as the supply of eggs was limited. However, the low number of replicates means that these results can only be used as preliminary findings but not analysed statistically. The compounds from the high activity group at 10% were then screened at 5% and to increase the accuracy of the mean percentage mortality estimate at this concentration; the number of replicates was increased so that N 2—5. From this screen, six test agents with the highest mean mortalities (89% and above) were further tested; those rejected gave mean mortalities of 70% and below. The selected agents were then tested at two lower concentrations 2% and 1% w/v or v/v (Fig. 2i,ii).

Background mortality values (taken from the solvent treated control) were regularly above 5%; therefore, Abbott's Correction [8] was used to adjust the percent mortality caused by test agents. Where at least three replicates were obtained for one test agent at one concentration and each replicate had a corresponding control mortality of less than 20% (higher values render the data less reliable), the data were subjected to statistical analysis. The quantal response measure of % mortality is not directly amenable to ANOVA, so a common transformation for percent values, arcsin ($\sqrt{Y}/100$) (Y=percent mortality) [8], was used prior to one-way ANOVA. Tukey's post-test was employed subsequently to identify pairs of results with significantly different means. Both ANOVA and post-test were performed by GraphPad Prism computer package.

3. Results and discussion

The results demonstrate that essential oil constituents show a wide range of pediculicidal and ovicidal efficacies, as shown in Fig. 1. The LT_{50} of each was used to rank the compounds in order of activity towards adult clothing lice (Table 1).

Mono-oxygenated compounds, those structures with a single alcohol, phenol or ketone functional group, were the most active against adult lice. Non-oxygenated terpenoids (alkanes and alkenes: camphene, myrcene, α- and β-pinene, α-terpinene, γ-terpinene and limonene) were inactive throughout the test period. Di-oxygenated

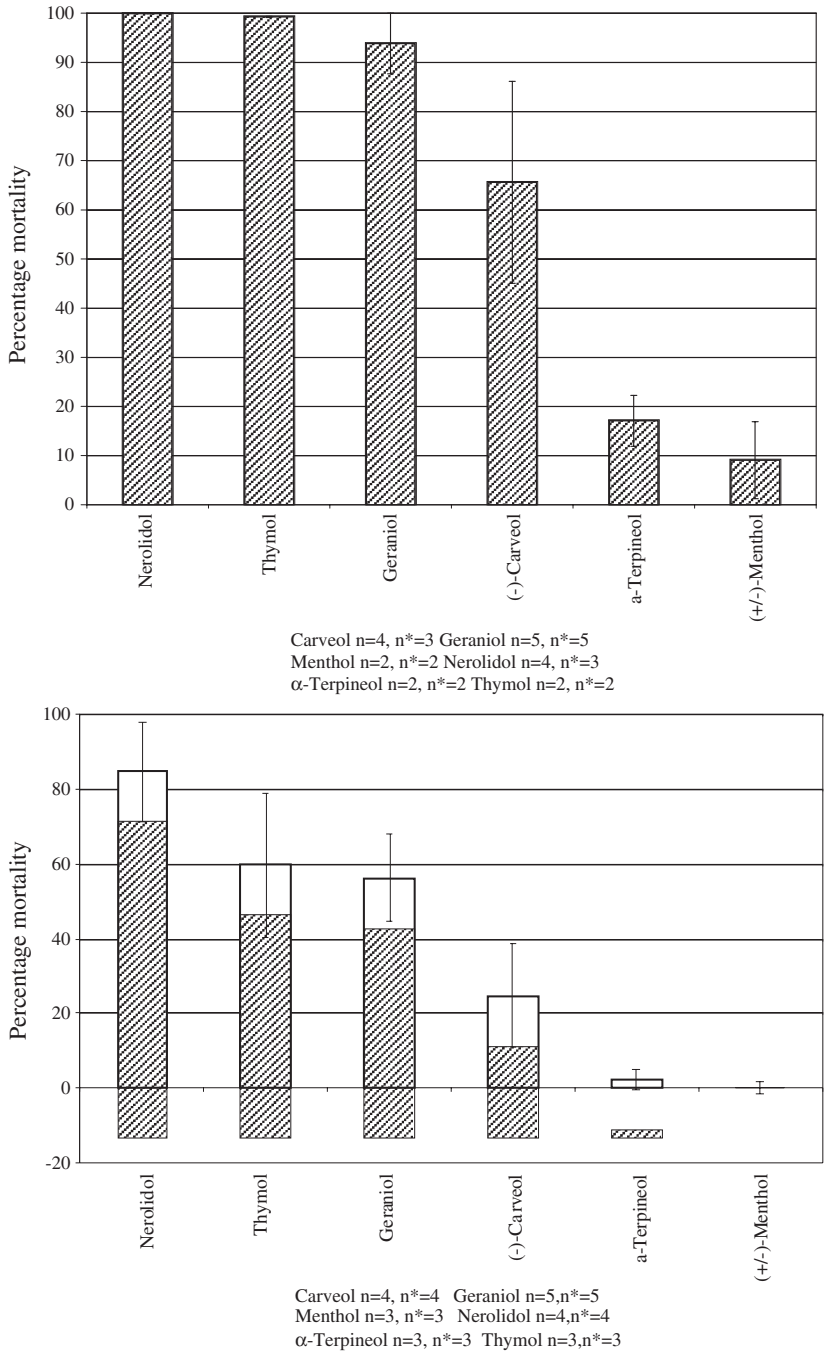

Fig. 2. Ovicidal activities of essential oil constituents towards *P. humanus* louse eggs. Data shown here have been adjusted for control mortality utilising Abbott's correction (see text). The number of replicates (*N*) for each test is shown alongside the number of these replicants where the control mortality was less than 20% (*N**). (i) The most potent compounds against *P. humans* louse eggs at a concentration of 2%. Carveol $n=4$, $n^*=3$; geraniol $n=5$, $n^*=5$; methanol $n=2$, $n^*=2$; nerolidol $n=4$, $n^*=3$; α-terpineol $n=2$, $n^*=2$; thymol $n=2$, $n^*=2$. (ii) The most potent compounds against lice eggs at a concentration of 1%. Carveol $n=4$, $n^*=4$; geraniol $n=5$, $n^*=5$; methanol $n=3$, $n^*=3$; nerolidol $n=4$, $n^*=4$; α-terpineol $n=3$; thymol $n=3$, $n^*=3$.

compounds were either inactive, as with those with two alcohol functional groups, menthane-3,8-diol, menth-6-ene-2,8-diol and the carboxylic acid, citronellic acid, or had low activity, such as the esters geranyl acetate and linalyl acetate.

Flat, compact terpenoids were more effective pediculicides than extended or bulky structures. For example linalool, geraniol and nerolidol are linear terpenoids with increasingly extended structures and decreasing efficacies. Bicyclic terpenoids are more bulky than linear or monocyclic types and, despite the bicyclics camphor, cineole and thujone being mono-oxygenated terpenoids, they had either low or absent efficacy in this test.

Although mono-oxygenation and compact shape appear to be general determinants of activity, the data in Fig. 1 and Table 1 indicate that the most effective terpenoids against adult lice have more specific structural features in common. At all time-points, the six most effective terpenoids were unsaturated monocyclic structures with a *p*-menthane skeleton [3] . At time points from 90 min to 210 min, the top four most effective compounds were consistently (+)-terpinen-4-ol, (−)-terpinen-4-ol, pulegone and thymol, in that order. These compounds additionally share: a methyl group at position 1; a carbon attached to the ring via either a double or single bond at position 4, to which are bonded two methyl groups; and an =O or –OH functional group at position 3 or 4. The (+)- and (−)-enantiomers of terpinen-4-ol, with the O-containing group uniquely located at position 4, were the first and third most active pediculicidal terpenoids, respectively, in the ranking. The methyl group arrangement seen in the top four ranking terpenoids may also be a key determinant of activity. This feature is present in the top seven ranking pediculicidal terpenoids (Table 1). Furthermore, although the phenols thymol and carvacrol have similar structures and correspondingly similar pediculicidal activities, carveol, identical to carvacrol except that it has a double bond between C7 and C8 that disrupts the methyl group arrangement, has relatively low activity.

The ovicidal activity of the mono-oxygenated monocyclic terpenoids was also high in comparison to other structures in the preliminary ovicidal screens. There was, similarly, either no activity, or low activity, from non-oxygenated terpenoids (limonene, β-pinene, α-pinene, α-terpinene and γ-terpinene), a mono-oxygenated bicyclic terpenoid (cineole), or a dioxygenated monocyclic terpenoid (menth-6-ene-2,8-diol). Linalyl acetate again showed low activity in comparison to the alcohols. Unlike in the pediculicidal assay, (+)- and (−)-terpinen-4-ol performed only moderately well.

4. Conclusion

The data, at all dilutions, suggest that some compounds may be relatively more effective ovicides than pediculicides. For example, citronellic acid and nerolidol were highly ovicidal, but completely inactive in the pediculicidal study. Menthone, by contrast, was inactive as an ovicide although highly pediculicidal. The molecular size and level of oxygenation are general physicochemical properties that may influence different determinants of activity from those affected by more specific structural characteristics. For example, the lipophilicity and/or compactness of a molecule may affect the bioavailability by determining its ability to cross the cuticle and enter the louse (many insecticides enter via a trans-cuticular route although Veal [9] postulated that LMW terpenoids may be too lipophilic to be soluble in the haemolymph after crossing the cuticle, and proposed a route of entry through the tracheae). When very small changes in structure dramatically affect activity, it is likely to indicate that the molecule is interacting with a receptor protein to exert its effects. Most insecticides bind to receptor proteins in the insect nervous system and, in doing so, they interrupt normal neurotransmission, which leads to paralysis and subsequently, death. Evidence accumulated recently suggests that LMW terpenoids may also bind to target sites on receptors that modulate nervous activity. Ionotropic GABA receptors, the targets of organochlorine insecticides lindane and dieldrin, are modulated by LMW terpenoids with vastly different structures. Hold et al. [10] found that α-thujone blocked mammalian GABA receptors and elicited symptoms characteristic of an ionotropic GABA receptor blocker in flies. However, Priestley et al. found that thymol had the opposite effect and enhanced the activity of both insect and mammalian ionotropic GABA receptors in vitro [11].

As far as ovicidal activity is concerned, the preliminary screening data suggest that there may be similarities in the structural features or physicochemical properties of pediculicidal and ovicidal terpenoids; as mono-oxygenated monocyclic or linear terpenoids were more ovicidal than non-oxygenated, dioxygenated or bicyclic terpenoids. However, the relative rank positions of the six most ovicidal terpenoids (determined statistically) alone show that there were also differences in the rankings obtained on lice and eggs. The clearest distinction was that some straight chain structures such as nerolidol and geraniol have high relative ovicidal activity, but lower pediculicidal activity relative to monocyclic compounds.

It is possible that the distinct rankings for lethality of LMW terpenoids towards lice and eggs are due to differences in target site between the developing nymph and the adult. However, they may also be due to differential

bioavailability. The louse egg cuticle is hydrophobic and almost impenetrable in contrast to the louse cuticle and gaseous exchange occurs via one small group of pores (aeropyles). Insecticides require careful formulation to enter the aeropyles; the narrow entrance creates surface tension, preventing penetration of aqueous solutions, and the hydrophilic inner structure of the pore lining is a barrier to organic solvents. Geraniol and nerolidol might perform better than phenolics and cyclic alcohols in the ovicidal assay due to possible weak surfactant physicochemical properties, by virtue of a single –OH group at or near one end of a long aliphatic portion.

The ovicidal results obtained here agree with those of Veal [9], who tested essential oils and mixtures against clothing lice eggs, and found that rosemary and pine were the only oils not to cause any ovicidal mortality when tested individually. Rosemary contains primarily hydrocarbons and bicyclic monoterpenoids and both types of compound were ranked in the least ovicidal group in our study. The composition of pine oil depends on the species, but hydrocarbons are sometimes the primary constituents [4]. It is therefore unsurprising that rosemary and pine oils were found to be inactive by Veal et al.

Any in vitro study necessarily invites criticism and comparisons with in vivo studies and the therapeutic potential of formulated products, for example those made by Burkhart and Burkhart [12]. In many cases, however, the effect of the excipients was not taken into account and has been discussed by Burgess [2].

This study also indicates the potential for some monoterpenoids to be included in formulations as ovicidal agents. The ‘Suleo’ brand of head lice treatments currently available in the UK uses standard active ingredients (Suleo C is based on carbaryl and Suleo M on malathion) but has unusually high ovicidal activity. It also contains limonene and terpineol as fragrant excipients. This study supports the notion that terpineol and limonene may be contributing to the ovicidal effect of Suleo brand products.

Acknowledgments

The authors wish to thank Prof. Tom Fearn, University College London, for verification of the statistical treatment of the data. C.M. Priestley thanks the School of Pharmacy, University of London, for a studentship.

References

[1] Dodd CS. Interventions for treating head lice (Cochrane review), Cochrane Database. Syst Rev 2001;2 (CD001165).
[2] Burgess IF. Annu Rev Entomol 2004;49:457.
[3] Dewick PM. Medicinal natural products. Chichester: John Wiley & Sons; 1997.
[4] Palevitch D, Craker LE. Volatile oils as potential insecticides. Herb Spice Med Plant Dig 1994;12:1.
[5] WHO/VBC. Instructions for determining the susceptibility or resistance of body lice and head lice to insecticides. Geneva: World Health Organisation; 1981. 81.808.5 pp.
[6] Burgess IF. Pharm J 1990;44:159.
[7] Burgess IF. Pharm J 1991;46:630.
[8] Abbott WS. J Econ Entomol 1925;18:267.
[9] Veal L. Complement Ther Nurs Midwifery 1996;2:97.
[10] Hold KM, Sirisoma NS, Ikeda T, Narahashi T, Casida JE. Proc Natl Acad Sci USA 2000;97:3826.
[11] Priestley CM, Williamson EM, Wafford KA, Sattelle DB. Br J Pharmacol 2003;140:1363.
[12] Burkhart CN, Burkhart CG. J Med Entomol 2001;38:127.