Repellency of essential oils and their components to the human body louse, Pediculus humanus humanus

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Abstract

Five essential oils and nine of their components were compared to diethyl toluamide (DEET) for their repellent activity against the human body louse, Pediculus humanus humanus. The absolute or intrinsic repellency of the compounds was tested by applying the repellent to corduroy patches and comparing them with untreated patches. It was found that the most effective repellents were DEET and citronella, whose activity lasted at least 29 days. The activity of rosemary lasted at least 18 days and that of eucalyptus more than 8 days. The repellent activity of the oil components such as citronellal and geraniol lasted more than 15 and 8 days, respectively. DEET remained effective at a dilution of 1:32, geraniol at 1:8, citronella at 1:4 and rosemary and citronellal at 1:1. The comparative or standard repellency of the candidate repellents was examined with the aid of a new screening technique using hairs treated with ammonium bicarbonate which is attractive to lice. Using this technique it could be shown that the repellent activity of citronella and geraniol lasted 2 days and that of rosemary and citronellal for only one day. DEET was active for less than one day. Serial dilutions of these substances also revealed that citronella was the most potent repellent for lice, followed by citronellal, rosemary, geraniol and DEET. The differences however, were not significant.

Introduction

The number of cases of head and body lice infestation throughout the world is estimated to run into hundreds of millions (Taplin & Meinkeing, 1987). Infestation by head lice has increased worldwide since the mid-sixties (Gratz, 1977) and continues to be a major school and community health problem.

The conventional treatment of hair with chemical substances, does not solve the problem of reinfection which occurs frequently among children. Head lice are mainly transmitted among children during close social contacts. Therefore, a substance that repels the lice should also protect them from becoming infested.

The fact that lice quickly develop resistance to existing pediculicides makes the development of effective lice repellents even more urgent. These repellents must be nontoxic, nonirritating, long lasting, cosmetically acceptable, easy to apply and with a pleasant fragrance.

In the past lavender oil was used to protect children from head lice infestation (Burgess, 1993a) and impregnation of uniforms with benzyl benzoate, N,N-diethyl-m-toluamide (DEET) and pyrethrum was used to protect soldiers from body lice (Eldridge, 1973). Recently, the pediculicide, piperonal (1,3-benzodioxol-5-carboxaldehyde) was reported to have repellent activity to human body lice (Burgess, 1993b). Although the repellent activity of essential oils and their components against blood-sucking arthropods has been known for a long time (Painter, 1967), their efficacy has never been tested against human lice to the best of our knowledge.

The aim of this study was to test the efficacy of some essential oils and their ingredients as repellents
against the human body louse and to compare their activity with the standard repellent DEET.

**Materials and methods**

**Lice.** Human body lice, *Pediculus humanus humanus*, were reared in the laboratory by feeding them every 48 h on rabbits. Lice were placed on the shaved abdomen of a white New Zealand rabbit and left until they fed to satiety. Between feedings the lice were maintained at a temperature of 30 ± 1 °C and 70–80% relative humidity. For each experiment, female lice, which had been fed 24 h previously, were used.

**Essential oils.** The essential oils citronella (Tamar, Tel-Aviv), rosemary (Frutarom, Haifa), eucalyptus, lavender and D-limonene (Aromor, Givat Oz, Israel) and some of their ingredients such as citronella (Sigma, St. Louis, USA), geraniol (Sigma), linalool (Sigma), bornyl acetate (Sigma), camphor (Sigma), cineole (Aldrich, Milwaukee, USA), pinene (Sigma), camphene (Aldrich) and borneol (Sigma) were used. Different concentrations were prepared by dissolving them in ethanol. Camphor and borneol were dissolved 1:1 (w/v) in ethanol. DEET was used as a positive control and ethanol as a negative control.

**Bioassay.** The absolute or intrinsic repellency of a compound was tested by applying the substance to corduroy patches and comparing them with ethyl alcohol treated patches. For this purpose Whatman (No. 4) filter paper discs (9 cm in diameter) were placed on petri-dishes of the same size. A 100 μl drop of the test solution was placed on a corduroy patch (1.5 cm²). The material was allowed to dry overnight in an incubator at 35 °C and 85–90% relative humidity. Control patches were treated in the same manner. The treated and control patches were placed opposite each other at the periphery of the filter paper. Twenty lice were placed in the middle of the disk and the dish was covered with a dark box in order to avoid the influence of light on the lice. The number of lice found on each patch was recorded after 10 min. Each formulation was tested three times. The duration of repellency was examined by testing the treated patches every 1–2 days. In the absence of repellents no significant differences were observed in the number of lice found on the two control patches.

The comparative repellency of the compounds was tested using ammonium bicarbonate (0.05 M) treated hairs which were placed behind repellent-treated hairs. Lice are strongly attracted to ammonium bicarbonate which is present in their feces and cause them to aggregate around it (Mumcuoglu et al., 1986). In this experimental set-up, lice had to crawl over the repellent-treated hairs in order to arrive at ammonium-treated hairs.

Small batches of human hair were immersed in 5 ml of the test solution and left for 1 hr on a filter paper until they were dry. A batch of 15–20 treated hairs one cm long was glued to a Whatman filter disc (No. 4, 7 cm in diameter) using a double-sided sticky tape (Fig. 1). Another batch of hairs was treated with ammonium bicarbonate 1 hr before the experiment. Ten female lice were placed between the two control batches and the number of lice on each batch was recorded after 10 min. Lice found on the ammonium bicarbonate treated hairs were counted as being on the treated batch. Each experiment was repeated three times. In the absence of repellents, ammonium bicarbonate treated hairs attracted 92.5% of the lice.

**Analysis of data.** The repellency was calculated according to the following equation:

\[
\% \text{ Repellency } = \left(1 - \frac{n}{A}\right) \times 100
\]

where \(A\) = Total number of lice, \(n\) = Number of lice found on the patch treated with repellent

A compound was considered to be active if it repelled more than 50% of the lice when it was screened for its absolute repellency and 25% for its comparative repellency.

Data were analyzed by the SPSS (release 4.1 for VAX/VMS, The Hebrew University). The t-test for paired samples was used to compare the repellent activ-
Fig. 2. Percentage of the absolute repellency of three essential oils to *Pediculus humanus humanus* during 29 days as compared to DEET.

Fig. 3. Percentage of absolute repellency of citronellal and geraniol to *Pediculus humanus humanus* over a period of 15 days.

Results

The undiluted compounds tested by the petri-dish method revealed that the most effective repellents against lice were DEET and citronella, whose activ-
ity lasted at least 29 days. The activity of rosemary lasted at least 18 days and that of eucalyptus more than 8 days (Table 1A and Fig. 2). The repellent activity of DEET was significantly higher (P<0.05) than that of citronella, rosemary and eucalyptus. Citronella was more effective than rosemary and eucalyptus (P<0.05). The differences between rosemary and eucalyptus however, were not significant. Out of nine oil components examined citronellal was the most potent repellent, and its activity lasted for 15 days. This compound was significantly more effective that geraniol, whose activity lasted for 8 days (P<0.05). (Table 1B & Fig. 3). DEET remained active at a dilution of 1:32, geraniol at 1:8, citronella at 1:4 and rosemary and citronellal at 1:1.

The comparative repellency of citronella and geraniol lasted about 2 days, rosemary and citronellal were active for one day and DEET was active for less than one day (Fig. 4). Citronella and geraniol repelled significantly more (P<0.05) than DEET, rosemary and citronellal. The differences between citronella and geraniol, rosemary and citronellal, rosemary and DEET and citronellal and DEET were not significant. Serial dilutions of these substances tested one day after the impregnation of the batches, showed that citronellal had the highest activity, followed by citronellal, rosemary, geraniol and DEET (Fig. 5). However, the differences were not significant.

**Discussion**

In this study DEET and citronella showed the highest absolute repellency and were active for at least 29 days. However, when tested for their comparative repellency citronella and geraniol were active for only 2 days, rosemary and citronellal for one day and DEET for less than one day, showing that the latter lost its superiority over the essential oils.

Citronellal and geraniol are the ingredients that seem responsible for the repellent activity of citronella, eucalyptus and lavender. The repellent activity of rosemary is probably due to the combined effect of its ingredients: borneol, bornyl acetate, camphor, eucalyptol, pinene and camphene.

The long lasting activity of the repellents under laboratory conditions may be caused by a slow release of the repellent from the impregnated corduroy. Under field conditions the duration of their activity would be even shorter due to strong influence of host attraction, light exposure, sweat, grooming and shampooing.

Therefore, any candidate repellent should be tested in a placebo-controlled clinical trial.

DEET impregnated netting sleeves made from cotton were effective in repelling *Anopheles gambiae* for about 20 days. The repellent activity of citronella lasted only 5 days (Curtis *et al.*, 1987). When applied to the skin these compounds were active for only few hours. The high values with the sleeves are presumably because the cotton retains liquid repellents and reduces their rate of evaporation, as compared with repellents applied on the skin (Curtis *et al.*, 1987).

Some *in vitro* screening techniques for repellency use neutral surfaces and do not take into consideration the attractiveness of the host animal (Dethier, 1956; Bar-Zeev, 1962; Burgess, 1993a, b). When given the choice between treated and non-treated patches, a blood-sucking arthropod would obviously select the latter. In the absence of any attractant even small amounts of repellent would be active. However, in nature these parasites are also strongly influenced by host factors such as temperature, odor and CO₂ emission, and therefore infestation will still take place if the host is not properly protected by a repellent. Therefore, attractants such as heat, moisture and light were used in the past to test repellents (Dethier, 1956). In this study the attractiveness of the host animal was simulated by the presence of ammonium bicarbonate, which is a strong attractant for lice (Mumcuoglu *et al.*, 1986).

It is also known that the mosquito’s standard of tolerance to DEET is not absolute, but depends partly on a comparison of the options available to it. This was confirmed by presenting a treated and untreated arm to the mosquitoes simultaneously. With *Anopheles gambiae* and *Anopheles albimanus* the repellency was considerable lower when one treated and one untreated arm was presented to them (Curtis *et al.*, 1987).

DEET is the most widely used repellent against arthropods of medical importance such as mosquitoes and ticks. Since its discovery, it has completely replaced citronella and other essential oils. However, in recent years it was shown that DEET may cause side-effects such as cardiovascular disturbances, encephalopathies, allergic and psychotic reactions (Robbins & Cheniack, 1986, Edwards & Johnson, 1987; Leach *et al.*, 1988).

Studies to prolong the repellent activity of the above-mentioned substances and to decrease their odor by emulsification and micro-encapsulation techniques are in progress (Magdassi *et al.*, 1994).
Fig. 4. Percentage of comparative repellency of four essential oils and of DEET to Pediculus humanus humanus over 5 days.

Fig. 5. Comparative repellency to Pediculus humanus humanus (t = 1 day) of various dilutions of four essential oils and DEET.
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References


