

## FIELD EFFICACY OF FOUR INSECT REPELLENT PRODUCTS AGAINST VECTOR MOSQUITOES IN A TROPICAL ENVIRONMENT

H. H. YAP, K. JAHANGIR AND J. ZAIRI

Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

**ABSTRACT.** Four insect repellent products (RPs) (RP 1, Experimental Repellent Lotion [Bayrepel® 12%]; RP 2, Experimental Repellent Cream [Bayrepel® 5%]; RP 3, Off! Insect Repellent II® Aerosol [deet 15%]; and RP 4, Off! Skintastic II® Cream [deet 7.5%]) were evaluated simultaneously for their efficacy against vector and nuisance mosquitoes. The aim of this study was to compare the relative efficacy of RPs based on a new repellent compound, Bayrepel® (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester), with deet (*N,N*-diethyl-*m*-toluamide)-based RPs. An 8-h field efficacy of above repellents was evaluated against the day-biting mosquito (*Aedes albopictus*) and night-biting mosquitoes (*Culex quinquefasciatus* and *Anopheles* spp.). Evaluation was carried out by exposing humans with repellent-treated bare limbs to mosquitoes landing and to mosquitoes landing and biting. Repellent product 1 or 2 was applied on the left arm and leg, whereas RP 3 or 4 was applied on the right arm and leg, respectively. Application of these 4 RPs significantly reduced ( $P < 0.05$ ) the landing and the landing and biting of day-biting and night-biting mosquitoes. All 4 RPs were found to be equally effective ( $P < 0.05$ ) against *Ae. albopictus* and *Cx. quinquefasciatus*. However, for protection against *Anopheles* spp., RPs 1 and 3 exhibited significantly ( $P < 0.05$ ) better repellency effect than RPs 2 and 4.

**KEY WORDS** Mosquito, repellents, deet, Bayrepel®, field studies

### INTRODUCTION

The use of repellents to protect humans from mosquito bites already has been accepted as part of an overall integrated mosquito-borne disease control program (Schreck and McGovern 1989, Alias 1995, Frances et al. 1996, Chavasse and Yap 1997). The compound deet has been described as the most preferred compound used in insect repellent products (RPs), and as being effective against a broad spectrum of insects, since its introduction in 1956 (Smith 1957, Garson and Wennikie 1968, Bar-Zeev and Ben-Tamar 1971, Rutledge et al. 1978, Gupta and Rutledge 1994). Despite its worldwide usage, concerns have existed over the safety of this chemical. The compound deet has a possibility of causing a burning sensation on the skin, and it irritates the eyes when applied on the face. Furthermore, heavy application of deet on young children was suspected to precede encephalopathies (Lipscomb et al. 1992, Osimitz and Grothaus 1995, Hongchun et al. 1998). Other discouraging effects of deet are its capability to act as a solvent of paints, varnishes, some polyethylene materials, and synthetic fabrics (Trigg 1996).

Because of these undesirable effects of deet, research was actively carried out to find an alternative compound that is safer to use and is equally or more effective than deet (Robert et al. 1991, Schreck and Leonhardt 1991, Sukumar et al. 1991, Dua et al. 1996, Walker et al. 1996). Recently, Bayer AG (Leverkusen, Germany) developed and registered a new active compound named Bayrepel® (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester, CAS No. 119515-38-7), which is an insect repellent. This compound was previously known as KBR 3023. As reported earlier (Yap et al. 1998), this new repellent compound was

investigated according to toxicologic standards for skin repellents under U.S. Environmental Protection Agency requirements. The median lethal doses for oral and dermal acute toxicity of this compound on rats were 4,743 and 2,000 mg/kg, respectively. This product also was found to be nonneurotoxic and it did not bioaccumulate when tested on rats (Yap et al. 1998).

The present study is a continuation of a previous study, which used only active ingredients (Yap et al. 1998). The aim of this present study is to compare the relative field efficacy of Bayrepel-based RPs with deet-based RPs on volunteers against the day-biting mosquito *Aedes albopictus* (Skuse) and the night-biting mosquitoes *Culex quinquefasciatus* Say and *Anopheles* spp.

### MATERIALS AND METHODS

**Test sites:** Field studies on the RPs were carried out at 3 separate sites with different compositions of mosquito species in the northwestern coastal area of Peninsular Malaysia. A night study was conducted outdoors in Pasir Gebu village, Penaga, Butterworth. This village is a nonmalaria rural residential area on mainland Peninsular Malaysia. In this area, *Anopheles* species, in particular *Anopheles sinensis* Wied., are abundant.

Another night study was carried out inside living premises in a squatter area at Ujung Batu, Butterworth, an urban area on mainland Peninsular Malaysia. Pretreatment trials carried out in this area indicated that more than 90% of indoor mosquitoes collected were *Cx. quinquefasciatus*.

A daytime study was carried out in a forest reserve at the Minden campus, Universiti Sains Malaysia, on Penang Island, adjacent to the mainland Peninsular Malaysia, where *Ae. albopictus* was the

Table 1. Treatment regimes for testing of repellent products (RPs).

| Treatment regime | Left/right limbs <sup>1</sup>                        | Right/left limbs <sup>1</sup>                       |
|------------------|--|---|
| A                | Experimental Repellent Lotion (Bayrepel® 12%) = RP 1 | Off! Insect Repellent II® Aerosol (deet 15%) = RP 3 |
| B                | Experimental Repellent Cream (Bayrepel® 5%) = RP 2   | Off! Skintastic II® Cream (deet 7.5%) = RP 4        |
| Control          | No treatment   | No treatment  |

<sup>1</sup> Limbs were alternated at each trial.

predominant species biting throughout the day. All 3 sites are situated in a tropical area with an average daily outdoor temperature of  $29 \pm 3^\circ\text{C}$  and a relative humidity of  $70 \pm 20\%$  year-round.

**Test procedure:** Four RPs with their stated active ingredients supplied by Bayer AG (Germany) were tested. These were Experimental Repellent Lotion (Bayrepel 12%, RP 1), Experimental Repellent Cream (Bayrepel 5%, RP 2), Off! Insect Repellent II® Aerosol (deet 15%, RP 3), and Off! Skintastic II® Cream (deet 7.5%, RP 4).

Human volunteers with bare arms (from wrist to elbow) and legs (from knee to ankle) were used as baits to assess the effectiveness of these RPs. The exposed surface of the arms and legs of volunteers were treated with RPs following treatment regimes stated in Table 1.

Repellent product 1 or 2 was applied on the left arm and leg, whereas RP 3 or 4 was applied on the right arm and leg, respectively. A total of 0.75 ml or 0.63 g of an RP was applied on each arm, whereas each leg was treated with a total of 1.5 ml or 1.25 g of an RP. For RP 3, the aerosol was sprayed into a beaker and left 30 min for the propellant to evaporate. The remaining mixture was then applied to the limbs. For the control experiment, the arms and legs were not treated with any RP. In order to minimize the effect of individual human variation, treatment regimes were alternated among the volunteers and between left and right limbs. Human volunteers were directed to wear cotton gloves, a long-sleeved shirt (folded up to the elbow), socks, and long pants (folded up to the knee) to prevent unwanted bites on the other parts of the body.

Field assessments for the study against *Aedes* spp. and *Culex* spp. have been described by Yap et al. (1998). However, for the present study, fewer volunteers were used. For the study against *Ae. albopictus* at the forest reserve, a total of 9 volunteers were involved (3 persons  $\times$  2 treatment regimes and 3 others for the control) per day trial. Volunteers were each positioned at least 5 m away from each other. Assessments were carried out between 0900 and 1700 h to coincide with the daytime biting activity of *Ae. albopictus*.

As for the night study against *Cx. quinquefasciatus* at the squatter houses, a total of 15 volunteers participated (3 persons  $\times$  2 treatment regimes  $\times$  2 timings and 3 others for the control) per night trial. Only one volunteer was seated in the living room inside each selected house. A total of 15 houses, predetermined to have a high mosquito population, was used in this study. A house was considered to have a high mosquito population when pretreatment catch of a single volunteer was above 25 mosquitoes per hour catch for a period of 3 h using the bare leg catch technique. To coincide with the biting peak of *Cx. quinquefasciatus*, the night study was conducted between 2100 and 0100 h. In order to determine an 8-h efficacy of the repellent formulations, 2 teams of volunteers consisting of 8 volunteers per team were formed. Volunteers in the 1st team were treated with repellent formulations 4 h before the initiation of the study, whereas the other volunteers in the 2nd team were treated at 2100 h. For the study against *Anopheles* spp., the assessment procedure was the same as was conducted against *Cx. quinquefasciatus*, but the volunteers

Table 2. Efficacy of 4 insect repellents against *Anopheles* spp. in a rural residential area on the mainland of Peninsular Malaysia. Values are the number of mosquitoes landing or landing and biting on exposed limbs during a total of 3 night trials.

| Assessment hour | Treatment <sup>1</sup> |           |           |           |           |           |
|-----------------|------------------------|-----------|-----------|-----------|-----------|-----------|
|                 | Untreated              | Untreated | Product 1 | Product 2 | Product 3 | Product 4 |
| 1               | 42a                    | 48a       | 0b        | 5c        | 0b        | 2c        |
| 2               | 90a                    | 87a       | 0b        | 4c        | 2d        | 1d        |
| 4               | 129a                   | 171a      | 2b        | 7c        | 2b        | 8c        |
| 6               | 171a                   | 147a      | 2b        | 9c        | 2b        | 15c       |
| 8               | 150a                   | 153a      | 6b        | 18c       | 9b        | 34c       |
| Mean            | 116.4a                 | 121.2a    | 2.0b      | 8.6c      | 3.0b      | 12.0c     |
| SE              | 22.9                   | 23.1      | 1.1       | 2.5       | 1.5       | 6.0       |

<sup>1</sup> Figures in the same row followed by same letter are not significantly different ( $P > 0.05$ ).

Table 3. Efficacy of 4 insect repellents against *Culex quinquefasciatus* in an urban squatter area on the mainland of Peninsular Malaysia. Values are the number of mosquitoes landing or landing and biting on exposed limbs during a total of 3 night trials.

| Assessment hour | Treatment <sup>1</sup> |           |           |           |           |           |
|-----------------|------------------------|-----------|-----------|-----------|-----------|-----------|
|                 | Untreated              | Untreated | Product 1 | Product 2 | Product 3 | Product 4 |
| 1               | 60a                    | 66a       | 0b        | 0b        | 0b        | 1c        |
| 2               | 54a                    | 48a       | 0b        | 0b        | 0b        | 1c        |
| 4               | 108a                   | 54a       | 0b        | 1c        | 0b        | 3d        |
| 6               | 69a                    | 78a       | 1b        | 2b        | 2b        | 4b        |
| 8               | 63a                    | 78a       | 4b        | 3b        | 6b        | 4b        |
| Mean            | 70.8a                  | 64.8a     | 1.0b      | 1.2bc     | 1.6bc     | 2.6d      |
| SE              | 9.6                    | 6.1       | 0.8       | 0.6       | 1.2       | 0.7       |

<sup>1</sup> Figures in the same row followed by same letter are not significantly different ( $P > 0.05$ ).

were seated outdoors in an open space with a distance of 5 m between one another. For each study, a total of 3 replicated trials (3 days/nights) was conducted.

The effectiveness of the RP was assessed based on the actual number of mosquitoes collected while landing or landing and biting on the treated arms and legs at the 1, 2, 4, 6, and 8 h posttreatment. All mosquitoes collected by volunteers in all trials were positively identified. The total number of mosquitoes collected at each respective hour was transformed to  $\log(n + 1)$ . The transformed data were analyzed with Statistical Graphic System (Statgraphics Version 5.0; STSC Inc. 1991) for analysis of variance and mean comparisons.

**RESULTS AND DISCUSSION**

Table 2 shows the efficacy of the 4 RPs against the predominant *Anopheles* spp. mosquitoes in Penaga, Butterworth. Throughout the 3 night trials, a total of 1,316 mosquitoes was collected, and 90.27% of the total collection was caught by the control volunteers. From the total control collection, 62.0% mosquitoes were *Anopheles* spp., followed by *Cx. quinquefasciatus* (22.6%) and *Mansonia uniformis* Theobald (15.4%). Repellent products 1 and 3 provided complete protection against all mosquitoes with no landing or landing and biting during the 1st hour posttreatment. Throughout the next 7 h of test period, all 4 RPs

provided high repellency against all mosquitoes. The average number of mosquitoes caught per hour on the limbs treated with RP 1 or 3 and RP 2 or 4 were less than 2 and 4, respectively. These values are low compared to the collection on control limbs with an average catch of 15 mosquitoes per hour per person. Repellent products 1 and 3 and RPs 2 and 4 were found to be equally effective ( $P < 0.05$ ) against each of the predominant mosquitoes, respectively. Also, the efficacy of RPs 1 and 3 was significantly ( $P < 0.05$ ) higher than that of RPs 2 and 4 in repelling the 3 predominant mosquitoes.

In the studies against the predominant *Cx. quinquefasciatus* in the living rooms of urban squatters, a total of 824 female mosquitoes (*Cx. quinquefasciatus*, 90.45%; *Ae. albopictus*, 9.1%; and *Ae. aegypti*, 0.45%) were collected throughout the 3 night trials. Of the total collection, only 0.61, 0.73, 0.97, and 1.58%, were collected on the limbs treated with RPs 1, 2, 3, and 4, respectively. Results of the analysis as shown in Table 3 indicated that RPs 1 and 3 provided superior protection against all 3 species of mosquito with no landing or landing and biting of mosquitoes up to 4 h posttreatment. Repellent products 1, 2, and 3 were equally effective ( $P < 0.05$ ) and all were more effective than RP 4 in repelling mosquitoes throughout 8 h posttreatment.

For the day study in the forest reserve, a total of 852 female mosquitoes (*Ae. albopictus*, 76.8%; *Armigeres subalbatus* Coq., 20.3%; and *Cx. quinquefasciatus*, 2.9%) were collected during the 3 day-

Table 4. Efficacy of 4 insect repellents against *Aedes albopictus* in a forest reserve on Penang Island, Malaysia. Values are the number of mosquitoes landing or landing and biting on exposed limbs during a total of 3 night trials.

| Assessment hour | Treatment <sup>1</sup> |           |           |           |           |           |
|-----------------|------------------------|-----------|-----------|-----------|-----------|-----------|
|                 | Untreated              | Untreated | Product 1 | Product 2 | Product 3 | Product 4 |
| 1               | 69a                    | 78a       | 0b        | 0b        | 0b        | 0b        |
| 2               | 31a                    | 56a       | 0b        | 0b        | 0b        | 0b        |
| 4               | 63a                    | 75a       | 1b        | 2b        | 2b        | 5b        |
| 6               | 81a                    | 108a      | 2b        | 12c       | 3b        | 25c       |
| 8               | 84a                    | 78a       | 8b        | 14b       | 12b       | 43c       |
| Mean            | 65.6a                  | 79.0a     | 2.2b      | 5.6bc     | 3.4bcd    | 14.6bcd   |
| SE              | 9.5                    | 8.3       | 1.5       | 3.1       | 2.2       | 8.5       |

<sup>1</sup> Figures in the same row followed by same letter are not significantly different ( $P > 0.05$ ).

time trials. From the total catch, 84.85% of the collection was caught on control limbs. All 4 RPs provided complete protection against the predominant mosquitoes for the 1st 2 h of posttreatment. During the following 6 h posttreatment, the total collection of mosquitoes landing or landing and biting on limbs treated with RPs 1, 2, 3, and 4 were only 1.29, 3.29, 2.00, and 8.57% of the total collection, respectively. As exhibited in Table 4, all 4 RPs were effective ( $P < 0.05$ ) against the predominant mosquitoes, with at least 5 times reduction in landing or landing and biting of mosquitoes.

Comparisons of Bayrepel-based RPs (RPs 1 and 2) with deet-based RPs (RPs 3 and 4) indicated insignificant differences ( $P > 0.05$ ) of high efficacy against *Cx. quinquefasciatus* (Table 3) and *Ae. albopictus* (Table 4). However, in the study against *Anopheles* spp. (Table 2), the liquid formulations of Bayrepel (RP 1) and deet (RP 3) exhibited significantly ( $P < 0.05$ ) better repellency than the cream formulations (RPs 2 and 4). Such differences are probably due to the high concentration of active ingredients in the liquid formulations. Overall, the present field studies concluded that all 4 RPs significantly ( $P < 0.05$ ) reduced the number of mosquitoes landing or landing and biting on treated limbs compared to control limbs, throughout the 8 h of the test period.

Results obtained from the present study were concordant with those of the previous study, which used only active ingredients (Yap et al. 1998). As reported by Yap et al. (1998), both KBR 3023 (Bayrepel) and deet formulations were found to provide effective protection against day-biting (*Ae. albopictus*) and night-biting (*Cx. quinquefasciatus*) mosquitoes, with percentage reductions of more than 65% and 90%, respectively, throughout an 8-h period. Furthermore, the present study demonstrated the effectiveness of Bayrepel-based and deet-based formulated products as reliable mosquito repellents in the field against all major vector mosquitoes in the genera *Aedes*, *Anopheles*, and *Culex*.

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