

Arm in cage tests to compare skin repellents against bites of *Aedes albopictus*

Antonio MASETTI, Stefano MAINI

Dipartimento di Scienze e Tecnologie Agroambientali - Entomologia, Università di Bologna, Italy

Abstract

The efficacy of commercial insect repellents was evaluated against *Aedes albopictus* (Skuse) (Diptera Culicidae) by means of arm in cage assays. The tested products included a DEET-based repellent (Off Spray), a commercial preparation of Icaridin (Autan Active), three formulations containing DMP (Helpic, Sandokan Body Gel, and Sandokan Body Spray) and four plant-derived products (Orphea, Senzazzz, Tigreño Dermo, and Zanzoff Natura). Products were applied to volunteers' forearms and the time elapsed between the application and the first mosquito bite was considered as the time of protection. Off Spray and Autan Active provided the longest mean protection times (250 and 220 min, respectively). Helpic, Sandokan Body Gel, Sandokan Body Spray, Tigreño Dermo, and Orphea were less effective showing protection times between 85 and 34 min. Zanzoff Natura afforded protection from mosquito bites for 15 min after the application. Sticky plasters (Senzazzz) provided less than 1 min of mean protection time.

Key words: Mosquito repellents, Arm in cage test, *Aedes albopictus*, DEET, Icaridin, DMP.

Introduction

Applying mosquito repellents on the skin is one of the oldest and commonest tools for personal protection and in many circumstances it is the only way to avoid mosquito bites (Fradin and Day, 2002). The efficacy of DEET (N,N-diethyl-3-methylbenzamide, formerly known as N,N-diethyl-m-toluamide) in providing a long-lasting protection against many mosquito species has been documented in several studies (Schreck and McGovern, 1989; Fradin and Day, 2002; Barnard and Xue, 2004; Roberts and Reigart 2004; Trongtokit *et al.*, 2005a; Klun *et al.*, 2006). However, DEET is irritating for mucous membranes, and concentrated formulations dissolve plastic. Moreover, some toxic effects have been reported although very infrequently and generally associated with over application of the product (reviewed in Koren *et al.*, 2003). Because of the concerns raised on the safety of DEET, an array of alternative repellent products has been developed. Some formulations contain synthetic active ingredients such as dimethyl phthalate (DMP), ethyl hexanediol (EHD), ethyl-3-(N-butylacetamido) propionate (IR3535), and 2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methyl propyl ester (Bayrepel, Icaridin, or KBR 3023). Others products are entirely based on plant extracts. Most of plant-derived repellents contain one or more essential oils from citronella, eucalyptus, geranium, lemongrass, and soybean. Even though each product claims its efficacy as mosquito repellents, peer-reviewed studies demonstrating these statements are often lacking (Pollack *et al.*, 2002).

In the last two decades, *Aedes albopictus* (Skuse) (Diptera Culicidae), has been introduced in many countries, including USA (Knudsen *et al.*, 1996) and Italy (Sabatini *et al.*, 1990) with a new establishment in Sardinia (Cristo *et al.*, 2006). The spread of this species raised serious concerns because of its relevant nuisance and also for its possible role in the transmission of several diseases (Gratz, 2004).

In this study, the repellent efficacy against *Ae. albopictus* of nine products available in Italian market was compared. The study was carried out in controlled laboratory conditions according to arm in cage methods (Schreck, 1977).

Materials and methods

Mosquitoes

Ae. albopictus were taken from a diseases-free lab colony maintained for several years at Centro Agricoltura Ambiente "G. Nicoli", Crevalcore, Italy. The strain was originally established from individuals collected in Desenzano del Garda, northern Italy. Rearing was maintained in a photoperiod of 14:10 (L:D) at 28 ± 1 °C and $80 \pm 10\%$ RH. Larvae were reared at the density of 1 individual/ml and ground cat chows (Friskies, Vevey, Switzerland) were supplied as food.

Products

Table 1 reports a detailed list of the commercial products included in this study. All products are commonly available on Italian market and were purchased in different stores located in Bologna province, northern Italy.

Assays procedure

Tests were carried out in cubic cages with 30-cm sides. The cages were entirely built in transparent Plexiglas and had, on the front side, a cotton socket sleeve for forearm introduction. On the remainder sides except the bottom, four round openings ($\varnothing = 15$ cm) sealed with fine mosquito net provided air circulation. Thirty mated females (5 - 20 day old) were placed in each cage, so that the final density was about 1 individual per litre. Blood meal was never supplied to mosquitoes used in the tests, whereas paper pads moistened with 10% aqueous sucrose solution were constantly provided. Cages were held at 27 °C and 80% RH during all the

Table 1. Features of the tested products.

Product name	Composition ^a	Formulation
Autan Active	Icaridin 20%, Inert ingredients	Pump Spray
Helpic	Denatured Alcohol, Aqua, Dimethyl Phthalate, Eugenia caryophyllus, Pimpinella anisum, Lavanda hybrida, Hypericum perforatum, PEG 40, Hydrogenated Castor Oil, Propylene Glycol, Plantago lanceolata Achillea millefolium, Camomilla recutita	Pump Spray
Off Spray	DEET 15%, Inert ingredients	Spray
Orphea	Denatured Alcohol, Aqua, Propylene Glycol, PEG 40, Hydrogenated Castor Oil, Pelargonium capitatum, Cymbopogon nardus, Parfum, Citronellol, Coumarin; Limonene; Hydroxycitronellal, Citral, Geraniol, Linalool	Pump Spray
Sandokan Body Spray	Aqua, Isopropyl alcohol, Polysorbate 20, Dimethyl Phthalate, Aloe barbadensis, Pelargonium graveolens, Eucalyptus globulus, Cymbopogon winterianus, Salvia officinalis, Rosmarinus officinalis, Melaleuca alternifolia, Methylchloroisothiazolinone, Methylisothiazolinone Citral, Eugenol, Farnesol, Geraniol, Linalool	Pump Spray
Sandokan Body Gel	Aqua, Dimethyl Phthalate, Polysorbate 20, Aloe barbadensis, Pelargonium graveolens, Eucalyptus globulus, Cymbopogon winterianus, Salvia officinalis, Rosmarinus officinalis, Melaleuca alternifolia, Acrylates/C10-30 Alkyl acrylate crosspolymer, Disodium EDTA, Sodium hydroxide, Phenoxyethanol, Methylparaben, Propylparaben, Citral, Eugenol, Farnesol, Geraniol, Linalool	Skin gel
Senzazzz	Cymbopogon nardus, 2-undecanone, Eucalyptus citriodora	Sticky plaster
Tigreño Dermo	Aqua, Denatured Alcohol, Propylene Glycol, PEG 40, Hydrogenated Castor Oil, Eucalyptus globulus, Ocimum basilicum, Cymbopogon nardus, Limonene, Eugenol, Linalool, Verbena officinalis, Pelargonium capitatum, Salvia officinalis, Citral, Menthol, Camphor, Geraniol, Hydroxycitronellal, Sodium hyaluronate, Phenoxyethanol, Methylparaben, Ethylparaben, Propylparaben, Imidazolidinyl urea, Citronellal, Citronellol	Pump Spray
Zanzoff Natura	Aqua, Denatured Alcohol, Propylene Glycol, PEG 40, Hydrogenated Castor Oil, Eucalyptus globulus, Ocimum basilicum, Cymbopogon nardus, Limonene, Eugenol, Linalool, Verbena officinalis, Pelargonium capitatum, Salvia officinalis, Citral, Menthol, Camphor, Geraniol, Hydroxycitronellal, Sodium hyaluronate, Phenoxyethanol, Methylparaben, Ethylparaben, Propylparaben, Imidazolidinyl urea, Citronellal, Citronellol	Pump Spray

^a = as stated on product labels.

assays. The tests were started 2 h after the beginning of the diurnal cycle.

Products were uniformly applied to the skin from wrist to the elbow. One ml of product was used for spray formulations, whereas 1 g was used for gel formulations. The tests of sticky plasters were carried out by applying one plaster (1 cm²) in the middle of forearm. Hands were always protected by wearing nitrile gloves.

At the beginning of each product test, the readiness of the mosquito to bite was confirmed inserting the untreated forearm into the cage. Then the test was carried out inserting the treated forearm inside the cage for 1 min every 5 min. If no bite was observed within 30 min after product application, subsequent expositions were performed for 1 min at 30 min intervals. The deterrent effect of the product was considered over when two or more bites occurred in the same exposition period, or one bite occurred in two consecutive periods. Protection time was recorded as the time elapsed between the product application and the interval time immediately

previous to that in which a confirmed bite was obtained. The same mosquitoes were repeatedly exposed to treated forearm until a confirmed bite was observed. If no bites occurred within 6 h after product application, tests were suspended and a protection time of 6 h was recorded. Subjects were told to introduce the forearm without touching the sleeves with treated skin in order to prevent a treatment from being wiped off and contaminating the closure. The sleeves were warped up behind the elbow to avoid the escape of mosquitoes.

Arm in cage tests were performed by three volunteer subjects (two males and one female) on six different mosquito batches. Each product was tested twice by each subject on two randomly selected batches. By the end of the study, each batch of mosquitoes was exposed once to each product. Only one test per day was conducted.

Owing to the neurotoxic effect of DEET on insects (Licciardi *et al.*, 2006), slightly different protocol was carried out when testing DEET-based repellent in order

Table 2. Effectiveness of each product evaluated as mean time of protection against the bites of *Ae. albopictus*.

Product	Mean time of protection (min)	Standard deviation (min)	Homogeneous groups (Tukey's HSD)
Off Spray	250.00	94.23	a
Autan Active	220.00	121.00	a
Helpic	85.00	29.50	b
Sandokan Body Gel	75.00	41.35	b
Sandokan Body Spray	64.17	40.79	b
Tigreño Dermo	60.00	26.83	b
Orphea	34.17	29.73	bc
Zanzoff Natura	15.00	25.10	c
Senzazz	0.83	2.04	c

to prevent long lasting knock down or paralyzes of mosquitoes. The first insertion of treated forearm for DEET-based product was delayed to 1 h after application and subsequent 1 min exposures were performed at 30 min intervals. The efficacy of this method in avoiding intoxication of mosquitoes was verified by inserting untreated forearm in the cages and checking the readiness of the mosquitoes to bite.

Data Analysis

Times of protection were transformed in square root to prevent heteroscedasticity of variances and were analyzed by means of two way ANOVA (considering repellents and subjects as effects). Mean protection times were separated by Tukey's honestly significant difference (HSD) test. Because of the small number of replicates, interactions between repellents and subjects were not evaluated.

Results and discussion

The density of mosquito (about 1 individual per litre) used in this study can be considered quite low when compared to other similar studies: up to 200-250 mosquitoes had been used in cages of comparable volume (Barnard and Xue, 2004; Trongtokit *et al.*, 2005b). Although a low mosquito density is likely to cause an increased variability in protection time estimations (Schreck, 1977), Fradin and Day (2002) reported that a small number of individuals per cages more accurately represent the conditions in the environment.

Three synthetic active ingredients were formulated in the tested products. Off Spray contained 15% DEET. Icaridin (20%) was the active ingredients of Autan Active. DMP was included in Helpic, Sandokan Body Gel, and Sandokan Body Spray. None of these products reported DMP amounts on the label. The remainder four repellents did not declare any synthetic active ingredients on the label. On the basis of an empirical evaluation, all the tested products did not cause any evident skin irritation or other adverse effects during or after application.

Mean protection time for each repellent is reported in table 2. Off Spray and Autan Active were the most effective repellents and they provided 250 and 220 min protection, respectively. The two products did not differ significantly. Helpic, Sandokan Body Gel, Sandokan

Body Spray, Tigreño Dermo, and Orphea were statistically less effective. Although the mean protection times of these products ranged between 85 and 34 min, statistical analysis did not detected any significant difference between them. Zanzoff Natura protected from mosquito bites for 15 min after the application, whereas Senzazz sticky plasters provided less than 1 min of mean protection time. The high standard deviation relative to the mean protection time of Orphea, Zanzoff Natura, and Senzazz is a reliable explanation for the lack of statistical differences between them. The finding that sticky plasters do not provide any protection is consistent with the known inability of repellents to prevent bites beyond 4 cm from the site of application (Fradin and Day, 2002).

The three subjects did not show significant differences in means protection times [$F(2, 43) = 1.774, P = 0.182$]. Due to the limited number of tests of each product on each subject it is not possible to draw any conclusion on the interaction between repellents and subjects.

Several studies testing repellents in cages assay have been published (Fradin and Day, 2002; Girgenti and Süß, 2002; Badolo *et al.*, 2004; Barnard and Xue, 2004; Trongtokit *et al.*, 2005a). However, direct comparison of results between different studies may be confused by mosquito species used and by discrepancies in test protocol, which may lead to differences in the estimation of protection times. The mean protection times reported in table 2 cannot be considered as absolute estimations of protection provided by each repellent. The results indicate the relative ranking of protection afforded by the commercial products against *Ae. albopictus* tested under our controlled laboratory conditions. It is to be pointed out that relative effectiveness of the products could be different in the field.

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Authors' addresses: Antonio MASETTI (corresponding author: amasetti@entom.agrsci.unibo.it), Stefano MAINI, DiSTA - Entomologia, Alma Mater Studiorum Università di Bologna, viale G. Fanin, 42, 40127 Bologna, Italy.

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