Evaluation of the in vitro efficacy of permethrin and deltamethrin on Aedes albopictus

C. SENMARTIN1, E. BOUHSIRA1, E. LIENARD1, P. JACQUIET1, P. DELAUNAY2, M. FRANC*

1Laboratoire de Parasiotologie, Ecole Nationale Vétérinaire de Toulouse, BP 87614, 23 chemin des capelles, 31076 Toulouse cedex, France
2Laboratoire de Parasitologie-Mycologie, Hôpital de l’Arche, Centre Hospitalier Universitaire de Nice-Université de Nice-Sophia Antipolis/Inserm U1065, Nice, France

* Corresponding author: m.franc@envt.fr

SUMMARY

*Aedes albopictus*, commonly called the "tiger mosquito" is now present in many European countries. It is the vector of several pathogens of human and veterinary importance such as viruses causing Dengue fever, Chikungunya and West Nile and also filarial nematodes. This experimental study aimed to determine the lethal doses LD_{50} and LD_{90} of permethrin and deltamethrin on *Aedes albopictus in vitro* using filter papers impregnated with each of the insecticide. Permethrin was tested in six different concentrations (0 to 200 mg/m²), while nine concentrations of deltamethrin (0 to 56 mg/m²) were tested. For each concentration, four replicates of 25 female mosquitoes were performed. Mosquitoes were exposed for one hour to the filter paper, after which dead insects were counted. The LD_{50} and LD_{90} were respectively 41.8 and 66.4 mg/m² for permethrin, and 3.3 and 8.5 mg/m² for deltamethrin. The LD_{50} determined in this study are higher than the concentrations of permethrin and deltamethrin found in commercialized products labelled for protection of dogs against other mosquito species, *Culex pipiens* or *Aedes aegypti*. Finally these data could be used to assess resistance levels and to monitor potential resistances of these molecules towards *Aedes albopictus*.

Keywords: *Aedes albopictus*, permethrin, deltamethrin, *in vitro* test, filter paper.

RESUME

Evaluation de l’efficacité de la perméthrine et de la deltaméthrine in vitro sur *Aedes albopictus*

*Aedes albopictus*, appelé communément le « moustique tigre », est désormais présent dans de nombreux pays européens. Il est le vecteur de nombreux agents pathogènes ayant une importance en médecine humaine et vétérinaire, tels que les virus de la Dengue, du Chikungunya et du West Nile ainsi que des filaries.

Cette étude expérimentale avait pour but de déterminer in vitro les doses létales 50 et 90 (DL_{50} et DL_{90}) de la perméthrine et de la deltaméthrine sur *Aedes albopictus* en utilisant des papiers filtrés imprégnés de chacun de ces insecticides. La perméthrine a été testée à six différentes concentrations (0 à 200 mg/m²), tandis que neuf concentrations de deltaméthrine ont été testées (0 à 56 mg/m²). Pour chacune des concentrations testées, quatre répétitions utilisant 25 femelles moustiques ont été réalisées. Les moustiques ont été exposés pendant une heure aux papiers filtrés imprégnés, puis les insectes morts ont été comptés. Les DL_{50} et DL_{90} sur *A. albopictus* ont été respectivement de 41.8 et 66.4 mg/m² pour la perméthrine, et de 3.3 et 8.5 mg/m² pour la deltaméthrine. Les DL_{50} calculées dans cette étude sont plus élevées que les concentrations de perméthrine et de deltaméthrine utilisées dans les produits commercialisés ayant pour indication la protection des chiens contre d’autres espèces de moustiques comme *Culex pipiens* ou *Aedes aegypti*. Les données de cette étude pourraient aider au suivi de la détection de potentielles résistances de ces molécules sur *A. albopictus*.

Mots-clés : *Aedes albopictus*, perméthrine, deltaméthrine, test in vitro, papier filtre.

Introduction

*Aedes albopictus*, commonly called the « Tiger mosquito » is native from South-East Asia [11]. In the last 30-40 years, this mosquito species has expanded to North and South America [4, 12, 19, 23], Africa [6, 14, 24], North of Australia [21] and Europe [1] [16]. It is now present in a lot of European countries such as Albania [1], Italy [20], Croatia [13], Spain [3], Greece, France [8] [25], Belgium [26], Germany, and the Netherlands [27].

*Aedes albopictus* mosquitoes are very aggressive towards their hosts and can feed on a multitude of animals such as mammals, birds, reptiles or amphibians [12]. They transmit several important pathogens to humans such as Dengue fever, Chikungunya and West Nile viruses [10, 16, 17]. They are also involved in the transmission of filarial nematodes of veterinary importance such as *Dirofilaria immitis*, *Dirofilaria repens* and *Setaria labiatopapillosa* [5, 18].

Several products available on the market are labelled to protect dogs against sandfly or mosquito (*Culex pipens, Aedes aegypti*) bites. However, to the authors’ knowledge, none of these products are labelled against *Aedes albopictus* yet. Given the geographical expansion of this mosquito species in Europe, it is highly important to protect dogs and humans against their bites to prevent the transmission of pathogens.

The aim of this study was to determine the lethal doses LD_{50} and LD_{90} of both widely used pyrethroids, permethrin and deltamethrin, on *A. albopictus* and to compare these values to the recommended doses applied to dogs. The test was performed by exposing *A. albopictus* mosquitoes for one hour to insecticide-impregnated papers. It would be of interest to determine whether or not the LD_{50} and LD_{90} are in the range of recommended doses.
Materials and methods

MOSQUITOES

The Aedes albopictus strain was obtained from eggs collected in a municipality called “Le Bar Saint Loup” in the French Alpes-Maritimes department, South East of France, and was provided by Dr Delaunay (Hôpital l’Archet, Nice, France) in 2011. Since then, it has been maintained under laboratory conditions at the Ecole Nationale Vétérinaire de Toulouse (ENVVT). For the study, adult mosquitoes were maintained in cages (30 x 30 x 30 cm) supplied with honey and water at 23°C (±3°C) of temperature and at 45% (±15%) of humidity under a 12h light /12h dark cycle. Unengorged female mosquitoes aged between 5 and 9 days were used in this study.

INSECTICIDES

Two pyrethroids were used: deltamethrin (deltamethrin Pestanal®, Sigma Aldrich, Saint-Quentin Fallavier, France) and permethrin (permethrin Pestanal®, Sigma Aldrich, Saint-Quentin Fallavier, France) that were diluted in pure acetone until required concentrations were reached. Acetone was also used as negative control. All doses were expressed in mg of active ingredient per m². In order to establish the 50% lethal dose (LD50) and the 90% lethal dose (LD90), insects were exposed to gradually increasing doses of deltamethrin and permethrin. Permethrin was tested at 25, 37.5, 50, 75, 100 and 200 mg/m². Deltamethrin was tested at 1.75, 2.625, 3.5, 5.25, 7, 10.5, 14, 28 and 56 mg/m². The body surface area of dogs was calculated as follows [22]:

\[ S(m^2) = 0.1 \times [\text{BodyWeight}]_{100} \times 0.685 \]

For example, the dose of permethrin in a spot-on of Advantix® (Bayer, Puteaux, France) is 500 mg, which corresponds to a dose of 730 mg/m² for a dog with a bodyweight of 10 kg (0.685 m²).

Exposure of mosquitoes to insecticide using filter papers

Filter paper discs of 185 mm diameter (Whatman®, 90 g/m² N°5, Healthcare UL limited, Little Chalfont, United Kingdom) were impregnated with 2 mL of acetone for the control replicates or with an insecticide-acetone solution. The solution was placed homogeneously on filter papers using a micropipette. Once dry, the filter papers were cut in two and were properly folded to form a cone. Whatman® filter paper sealing discs of 90 mm diameter were impregnated with 0.940 mL of solution and let to dry. The cones were placed in a cylindrical plastic container (5 cm diameter x 10 cm height) covered with glass featuring a 1-cm-diameter hole (Figure 1). Then, 25 A. albopictus females were aspirated from the colony cages and blown into the cone through the hole. The cone was carefully sealed with the impregnated filter paper disc of 90 mm diameter. The glass was removed and replaced by the container cap (Figure 1). The mosquitoes were not anesthetized during the manipulation. Following one-hour exposure, the cone was opened in a cage and the mosquitoes were counted. The mortality criteria were based on the WHO criteria [29]: mosquitoes were considered “dead” when they were dead, moribund or showing uncoordinated movements.

Results

All 100 mosquitoes (4 control groups of 25 mosquitoes each) were alive after one-hour contact with pure acetone-impregnated papers. Each drug, deltamethrin and permethrin, reached a maximum effect of 100% mortality. The dose-response curves are represented for the deltamethrin and permethrin in Figure 2. The first concentrations leading to mortality were 1.75 mg/m² for deltamethrin (8% mortality), and 25 mg/m² for permethrin (6% mortality). Then, the effect became proportional to the dose. The maximal mortality of 100 % was reached at a concentration of 100 mg/m² for permethrin and 14 mg/m² for deltamethrin. The lethal doses are the maximal effect, LD50, Sigma Aldrich, Saint-Quentin Fallavier, France) is 500 mg, which corresponds to a dose of 730 mg/m² for a dog with a bodyweight of 10 kg (0.685 m²). The body surface area of dogs was calculated as follows [22]:

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For each concentration, the test was repeated on four replicates of 25 mosquito females. Mortality was recorded for each concentration after one hour exposure with the insecticide for a total of 100 mosquitoes. In parallel, four control replicates using filter papers impregnated with acetone only were realized for each insecticide.

Data analysis

The results were expressed in percentage of dead mosquitoes. The bioassay data were analyzed by Probit regression using SPSS v 16.0 (IBM, New York, United States) to obtain the LD values.

The dose-effect relationships were analyzed using a sigmoid E_max model:

\[ \text{Effect} = \frac{E_{\text{max}} \times D^y}{(LD50^y + D^y)} \]

E_max is the maximal effect, LD50 is the lethal dose for 50% mortality, and y is the slope of the dose response curve. For each drug, the LD50 was calculated using the estimated values of E_max, LD50 and y.

Results

All 100 mosquitoes (4 control groups of 25 mosquitoes each) were alive after one-hour contact with pure acetone-impregnated papers. Each drug, deltamethrin and permethrin, reached a maximum effect of 100% mortality. The dose-response curves are represented for the deltamethrin and permethrin in Figure 2. The first concentrations leading to mortality were 1.75 mg/m² for deltamethrin (8% mortality), and 25 mg/m² for permethrin (6% mortality). Then, the effect became proportional to the dose. The maximal mortality of 100 % was reached at a concentration of 100 mg/m² for permethrin and 14 mg/m² for deltamethrin. The lethal doses are the maximal effect, LD50, Sigma Aldrich, Saint-Quentin Fallavier, France) is 500 mg, which corresponds to a dose of 730 mg/m² for a dog with a bodyweight of 10 kg (0.685 m²). The body surface area of dogs was calculated as follows [22]:

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For example, the dose of permethrin in a spot-on of Advantix® (Bayer, Puteaux, France) is 500 mg, which corresponds to a dose of 730 mg/m² for a dog with a bodyweight of 10 kg (0.685 m²).
LD$_{50}$ and LD$_{90}$ of permethrin were 41.8 mg/m$^2$ (with an interval of confidence of 95% between: 39.2 and 44.5) and 66.4 mg/m$^2$ (with an interval of confidence of 95% between: 60.8 and 74.3) respectively. For deltamethrin, the LD$_{50}$ and LD$_{90}$ were 3.3 mg/m$^2$ (with an interval of confidence of 95% between: 2.9 and 3.7) and 8.5 mg/m$^2$ (with an interval of confidence of 95% between: 7.3 and 10.2) respectively (Table I).

The same method was used to determine the LD$_{50}$ and LD$_{90}$ of permethrin and deltamethrin on another mosquito species: Culex pipiens [28]. The LD$_{50}$ of permethrin and deltamethrin were 39.05 mg/m$^2$ (± 2.5) and 5.75 mg/m$^2$ (± 0.63) respectively. A. albopictus and C. pipiens are similarly sensitive to permethrin, whereas A. albopictus seems slightly more sensitive to deltamethrin with a LD$_{50}$ of 3.3 mg/m$^2$. The LD$_{50}$ of permethrin and deltamethrin on C. pipiens were 99.60 mg/m$^2$ (± 10.08) and 58.92 mg/m$^2$ (± 13.61) respectively.

The ratio LD permethrin / LD deltamethrin was different in A. albopictus from C. pipiens. Deltamethrin was about 8 to 13 times more toxic than permethrin for A. albopictus (Table I), but was 1.4 to 8 times more toxic than permethrin for C. pipiens.

Lin et al. (2013) determined the sensitivity of four strains of Aedes aegypti (a "sensitive" strain, the NS strain, and three resistant strains, GM, LY and LYPR strains) to permethrin, after two hours contact with insecticide-soaked paper using the WHO standard method [15]. The LD$_{50}$ of the "resistant" strains ranged from 215 to 1603 mg/m$^2$, while the LD$_{50}$ of the "sensitive" strain was 22 mg/m$^2$. Thus, after two hours contact with permethrin, it turned out that the LD$_{50}$ was approximately half of the LD$_{50}$ obtained in the current study (41.8 mg/m$^2$) on A. albopictus, suggesting that these two species of mosquitoes have the same sensitivity to permethrin.

The doses of permethrin and deltamethrin recommended by WHO for the soaked mosquito nets are 500 mg/m$^2$ and between 15 and 25 mg/m$^2$ respectively [7], which is much higher than the LD$_{50}$ obtained in the current study. Among insecticide products containing permethrin that are commercialized for use in dogs in France, only three are labelled against mosquitoes. Advantix$^\text{®}$ (Bayer, Puteaux, France) is labelled against C. pipiens, Effitix$^\text{®}$ (Virbac, Carros, France) and Vectra 3D$^\text{®}$ (Ceva, Libourne, France) are labelled against C. pipiens and A. aegypti. Recommended doses of permethrin in these products are between 730

### Table I: Lethal Doses 50 (LD$_{50}$) and 90 (LD$_{90}$) of permethrin and deltamethrin on Aedes albopictus obtained after one hour exposure to insecticide-impregnated filter papers.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>LD$_{50}$ (mg/m$^2$) (95% Confidence Interval)</th>
<th>LD$_{90}$ (mg/m$^2$) (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permethrin</td>
<td>41.8 (39.2-44.5)</td>
<td>66.4 (60.8-74.3)</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>3.3 (2.9-3.7)</td>
<td>8.5 (7.3-10.2)</td>
</tr>
<tr>
<td>Ratio permethrin/deltamethrin</td>
<td>12.6</td>
<td>7.8</td>
</tr>
</tbody>
</table>

**Discussion**

In this trial, we implemented the method of filter paper impregnated with insecticides to determine the lethal dosage (LD) of the two pyrethroids, permethrin and deltamethrin, leading to 50% and 90% mortality (LD$_{50}$ and LD$_{90}$) in Aedes albopictus. To the author’s knowledge, there are no studies available in the literature estimating the lethal doses of permethrin and deltamethrin in A. albopictus adult females.

The filter paper technique is easy to realize and considered safe for mosquitoes as it did not cause any increase in mortality among control mosquitoes. This technique is different from the WHO test tubes, where mosquitoes are introduced into a cylinder tube where only the sides are recovered with treated filter papers [29]. Therefore, in this method, the lid and the base are not treated with insecticides. Our technique, on the contrary, ensures permanent contact between mosquitoes and the insecticide impregnated papers during the whole hour of challenge. As a consequence, the lethal doses could be overestimated with the WHO method compared with the current technique.
In conclusion, these commercialized products are likely to provide protection against A. albopictus bites in dogs. In vivo studies are however necessary to verify that such concentrations are efficient against this mosquito species.

Acknowledgement

The authors are grateful to Sonia Gounaud, Martine Roques and Solange Vermot (Service de Parasitologie-Maladies Parasitaires, ENVT, France) for the maintenance of the mosquito colony.

References


