Comparative performances, under laboratory conditions, of seven pyrethroid insecticides used for impregnation of mosquito nets*

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Objective To compare the efficacy of seven pyrethroid insecticides for impregnation of mosquito nets, six currently recommended by WHO and one candidate (bifenthrin), under laboratory conditions.

Methods Tests were conducted using pyrethroid-susceptible and pyrethroid-resistant strains of *Anopheles gambiae* and *Culex quinquefasciatus*. Knock-down effect, irritancy and mortality were measured using standard WHO cone tests. Mortality and blood-feeding inhibition were also measured using a baited tunnel device.

Findings For susceptible *A. gambiae*, alpha-cypermethrin had the fastest knock-down effect. For resistant *A. gambiae*, the knock-down effect was slightly slower with alpha-cypermethrin and much reduced following exposure to the other insecticides, particularly bifenthrin and permethrin. For susceptible *C. quinquefasciatus*, the knock-down effect was significantly slower than in *A. gambiae*, particularly with bifenthrin, and no knock-down effect was observed with any of the pyrethroids against the resistant strain. Bifenthrin was significantly less irritant than the other pyrethroids to susceptible and resistant *A. gambiae* but there was no clear ranking of pyrethroid irritancy against *C. quinquefasciatus*. In tunnels, all insecticides were less toxic against *C. quinquefasciatus* than against *A. gambiae* for susceptible strains. For resistant strains, mortality was significant with all the pyrethroids with *A. gambiae* but not with *C. quinquefasciatus*. Inhibition of blood-feeding was also high for susceptible strains of both species and for resistant *A. gambiae* but lower for resistant *C. quinquefasciatus*; bifenthrin had the greatest impact.

Conclusions Efficacy for impregnation of mosquito nets against *A. gambiae* was greatest with alpha-cypermethrin. Bifenthrin is likely to have a significant comparative advantage over other pyrethroids in areas with pyrethroid resistance because of its much stronger impact on the nuisance mosquito, *C. quinquefasciatus*, despite its slower knock-down effect and irritancy. Selection of pyrethroids for mosquito vector control and personal protection should take into account the different effects of these insecticides, the status of pyrethroid resistance in the target area, and the importance of nuisance mosquitoes, such as *C. quinquefasciatus*.

Keywords Pyrethrins; Insecticides, Botanical/toxicity; Anopheles; Culex; Bedding and linens; Insecticide resistance; Comparative study (*source: MeSH, NLM*).

Mots clés Pyréthrine; Insecticides phytogènes/toxicité; Anophèles; Culex; Literie et linge; Résistance aux insecticides; Etude comparative (*source: MeSH, INSERM*).

Palabras clave Piretrinas; Insecticidas botánicos/toxicidad; Anopheles; Culex; Ropa de cama y ropa blanca; Resistencia a insecticida; Estudio comparativo (*fuente: DeCS, BIREME*).

الكلمات المفتاحية: المركبات البيرثرينية، مبيدات الحشرات، مبيدات الحشرات النباتية، سمية مبيدات الحشرات، الأنوفيلات، الباعضة، الأغطية والفرش، المقاومة لمبيدات الحشرات، دراسة مقارنة (*المصدر: رؤوس الموضوعات الطبية، المكتب الإقليمي لإقليم شرق المتوسط*).

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Voir page 331 le résumé en français. En la página 331 figura un resumen en español.

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Introduction

The widespread distribution of insecticide-impregnated mosquito nets is a major component of the WHO global strategy for malaria control, especially in sub-Saharan Africa, where more than 90% of malaria cases are reported annually (*1*). To date, six pyrethroid insecticides — the only group of insecticides currently considered suitable for impregnation of mosquito nets — have been evaluated by the WHO Pesticide Evaluation Scheme (WHOPES) and recommended for this purpose: alpha-cypermethrin, cyfluthrin, deltamethrin and lambdacyhalothrin (alpha-cyano pyrethroids), and etofenprox and permethrin (non-cyano pyrethroids) (*2–4*).

Pyrethroid resistance of malaria vectors has already developed in several malarious countries (*5–7*), and the absence of a suitable alternative insecticide class for impregnation of mosquito nets may undermine the gains in malaria control and personal protection being made through improved coverage

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with treated nets. Recent field studies in pyrethroid-resistant areas of Côte d'Ivoire, in experimental huts (*8, 9*) and on a larger scale (*10, 11*), indicated that pyrethroid-impregnated mosquito nets reduce malaria transmission despite a high frequency of the knock-down resistance (*kdr*) gene. A WHO consultation recommended that this should be confirmed in other studies, especially where pyrethroid-resistance mechanisms other than the *kdr* gene may be involved (*12*).

Protection against nuisance insects, especially *Culex quinquefasciatus*, which keep people awake at night, is the main motivation for the use of mosquito nets. However, pyrethroid resistance in this species is already widespread in the tropical world, including Africa (*13*).

Bifenthrin, a non-alpha-cyano pyrethroid, is used against a broad range of agricultural pests and has emerged as a promising candidate for malaria vector control in WHOPES-supervised trials in India, Mexico, Thailand, and United Republic of Tanzania (*14*), and in field studies in Côte d'Ivoire (*15*). Bifenthrin has been suggested for treatment of mosquito nets in view of its high efficacy against *Anopheles gambiae*, the major malaria vector in Africa, and *C. quinquefasciatus* (*16*). However, further testing and evaluation of the compound for such applications is needed, given its particular attributes, i.e. slower knock-down effect, lower irritability and higher toxicity to *C. quinquefasciatus* than other pyrethroids recommended for bednet impregnation.

Other studies on the efficacy, under laboratory conditions, of pyrethroid insecticides for impregnation of mosquito nets have been conducted in situations that did not permit direct comparison in terms of impact on mosquito mortality, knock-down effect, irritancy, and blood-feeding inhibition (*17–19*). The present study was undertaken, among other objectives, to gain a better understanding of how differences in these effects may translate in terms of efficacy of impregnated bednets, and to determine whether bifenthrin has any advantage over other pyrethroids in this regard.

Methods

Tests

The efficacy of a pyrethroid used for impregnation of mosquito nets is the result of the insecticide's intrinsic activity and the behaviour of the target mosquito in response to it. This is of particular relevance for fast-acting insecticides, such as pyrethroids and DDT, with knock-down and irritant properties. The intrinsic activity can be tested with adult mosquitoes using WHO cones (*20*), a device which forces tarsal contact with the impregnated netting material. This test does not indicate overall insecticide efficacy under field conditions, however, because the forced contact does not permit natural avoidance behaviour. The tunnel test provides a better simulation of field conditions. It has given results comparable with those obtained in the field in experimental huts (*21*), particularly for mortality and blood-feeding inhibition. In this study both these tests were used.

Insecticides

Formulations and concentrations for alpha-cypermethrin, cyfluthrin, deltamethrin, lambdacyhalothrin, etofenprox and permethrin were selected in accordance with WHO recommendations (Table 1 (available at: www.who.int/bulletin)). Bifenthrin was added because of encouraging results obtained previously. Tests were carried out at the WHO-recommended concentrations for impregnation of bednets against malaria vectors and at one-quarter of the recommended concentrations, as it was thought that the lower concentration might be a more sensitive indicator for detecting differences among products.

Mosquitoes

Two laboratory strains of *A. gambiae* and two of *C. quinquefasciatus* were used. The reference susceptible strains of *A. gambiae* (Kisumu), originating from Kenya, and *C. quinquefasciatus* (S-Lab), originating from California (*22*), have been colonized for many years and are free from any detectable insecticide-resistance mechanism. The resistant strain of *A. gambiae* (VKPR), originating from Burkina Faso, was already strongly resistant to permethrin when collected in the field and has been maintained under constant permethrin selection at each generation (*23*). The resistant strain of *C. quinquefasciatus* (BKPER) was collected in Côte d'Ivoire and has also been maintained under continuous selection with permethrin (*24*). Both are homozygous for the *kdr* gene (*25, 26*) with a 40-fold resistance factor (by topical application) (*20*). The *C. quinquefasciatus*-resistant strain also has a monooxygenase-resistance mechanism (*13*). Resistant and susceptible strains were checked every 3 months for resistance status and R-genotype.

Substrates and treatment

Tarsal contact tests were conducted using netting material (warp-knitted multifilament polyester 100 denier, mesh 156 (Siamdutch, Thailand)) treated with formulated product as recommended by WHO (*27*). Pieces of netting (25 cm × 25 cm) were treated with insecticide at the WHO-recommended concentration and at one-quarter of this dose, using the formulated product diluted with deionized water. The pieces were folded into three equal parts one way, then into three equal parts the other way to give nine layers and each piece placed in a disposable Petri dish. A quantity of formulation corresponding to the specific absorbency of the netting and prepared immediately prior to the treatment was dropped evenly onto the surface of each piece. The pieces were then carefully squeezed by hand (hands protected by plastic gloves) to ensure an even distribution of the solution and that no solution remained, and left in the dishes to dry. Tests were made 5–10 days after impregnation to ensure that deposits were of similar ages.

Tarsal contact with treated netting material

Knock-down effect and mortality resulting from tarsal contact with netting material were measured using standard WHO plastic cones and a 3-minute exposure time (*20*). During exposure, mosquitoes did not stay long on the cone wall and cones were closed with a polyethylene plug, which does not provide an attractive resting site. Five non-blood-fed females aged 2–5 days were introduced per cone. On each piece of netting 2–4 cones were attached. Tests were conducted at 25 ± 2 °C under subdued lighting. After exposure, the insects were grouped in batches of 20 in 150-ml plastic cups and held for 24 hours at 27 ± 2 °C and 80 ± 10% relative humidity, with honey solution provided. Each piece of netting was tested using a total of 50 mosquitoes consisting of ten replicates of 5 mosquitoes each to allow for inter-batch

variability. The number of knocked-down mosquitoes was recorded at fixed intervals (every 2–10 minutes depending on knock-down rates) for 60 minutes. The observed times to 50% knock-down (median knock-down time, KDT_{50}), and 25–75% knockdown ($KDT_{25–75}$) of mosquitoes were recorded. Separate Kaplan–Meier estimates of the proportion of knocked-down mosquitoes were plotted for each insecticide. The equality of risk of knock-down between insecticides was tested using Cox's proportional hazards models (*28*). Tied knock-down times were treated by an exact partial method considering time as discrete. The proportional hazard assumption was assessed graphically by plotting survival curves for each insecticide and using a test based on Schoenfeld residuals (*29*). These tests were conducted in parallel with a control with no insecticide. Mortality rates observed after 24 hours were corrected using the Abbott formula (*30*) and binomial exact 95% confidence intervals (CIs) were calculated for the corrected values. The insecticides were compared using Fisher's exact test.

Irritancy tests

Female, non-blood-fed mosquitoes, aged 2–5 days, were introduced individually into plastic cones applied to treated netting material. After an adaptation time of exactly 60 sec, the time elapsed between the first landing and the following take-off of the mosquito was recorded as the "time to first take-off" (FT). The observation was not continued for the very few mosquitoes that did not take off at least once after 256 sec. For each test, 50 mosquitoes were used. A simple program using the internal clock of a laptop computer was developed to run this test and to analyse the results by grouping mosquitoes by classes of first take-off time. The times taken for 50% and 25–75% of mosquitoes to leave the treated surface (median time to first take-off, FT_{50}, and $FT_{25–75}$, respectively) were also recorded. Separate Kaplan–Meier estimates of the proportion of mosquitoes that had left the treated surface were plotted for each insecticide. The equality of risk of take-off between insecticides was tested using Cox's proportional hazards models (*28*). Tied FT times were analysed using Breslow's method (*31*). The proportional hazard assumption was assessed graphically by plotting survival curves for each insecticide and by using a test based on Schoenfeld residuals (*29*). When the proportional hazard assumption was rejected, the equality of risk of take-off between insecticides was tested using the Peto–Peto–Prentice test (*32*). Fairly constant conditions of lighting (subdued) and air temperature (25 $\pm$ 2 °C) were maintained during the test. The number of take-offs has also been proposed as a measure of irritancy, but this is not a reliable indicator, especially for fast-acting insecticides (*21*).

Tunnel tests

The basic equipment consisted of a section of square glass tunnel (25 cm × 25 cm), 60 cm in length, similar to that used by Elissa & Curtis (*33*) and described in detail by Chandre et al. (*21*). A disposable cardboard frame mounted with a treated netting sample was placed across the tunnel 25 cm from one end. The surface area of netting accessible to mosquitoes was 400 cm^2 (20 cm × 20 cm) with nine holes, each 1 cm in diameter: one hole was located at the centre of the square, the eight others were equidistant and located at 5 cm from the border. In the shorter section of the tunnel, a bait (guinea pig for *A. gambiae*, quail for *C. quinquefasciatus*) was placed, unable to move. Animals used as baits were selected at random. At each end of the tunnel, a 30-cm square cage was fitted and covered with polyester netting. In the cage at the end of the longer section of the tunnel, 100 female, non-blood-fed mosquitoes, aged 5–8 days, were introduced at 18:00 hours. Females were free to fly in the tunnel but had to make contact with the treated piece of netting and locate the holes in it before passing through to reach the bait. After a blood meal, they usually flew to the cage at the end of the short section of the tunnel and rested. The following morning, at 09:00 hours, the mosquitoes were removed and counted separately from each section of the tunnel and the immediate mortality was recorded. Live females were placed in plastic cups with honey solution provided; delayed mortality was recorded after 24 hours. During tests, cages were maintained in a climatic chamber at 27 $\pm$ 2 °C and 80 $\pm$ 10% relative humidity under subdued light. Five tunnels were used simultaneously in the same climatic chamber, one tunnel, with untreated netting always being used as a control. Each net sample was used no more than twice within the same week and was then discarded. Blood-feeding inhibition was assessed by comparing the proportion of blood-fed females (alive or dead) in treated and control tunnels. For each experiment with insecticide-treated net, the expected number of blood-fed females was calculated by multiplying the total number of females tested by the proportion of blood-fed females observed among the total of tested females in the control tunnel. Percentage blood-feeding inhibition (BFI) was calculated by dividing the number of non-fed females by the expected number of blood-fed females; 95% CIs were estimated according to the binomial distribution, and insecticides were compared using Fisher's exact test. Overall mortality was measured by pooling the immediate and delayed (24-hour) mortalities of mosquitoes from the two sections of the tunnel. Mortality rates in treated conditions were corrected using the Abbott formula (*30*) and binomial exact 95% CIs were calculated for the corrected values. Insecticides were compared using Fisher's exact test.

Statistical analysis

The differences in outcome variables (mortality, blood-feeding inhibition and irritancy) between the insecticides were analysed separately for the two insecticide concentrations with Stata 7.0 statistical software (*34*), using the Bonferroni correction to take into account the multiplicity of tests (comparisons of 21 pairs of insecticides). The effects of two insecticides were considered to be significantly different when the *P*-value was less than $0.05/21 = 0.00238$.

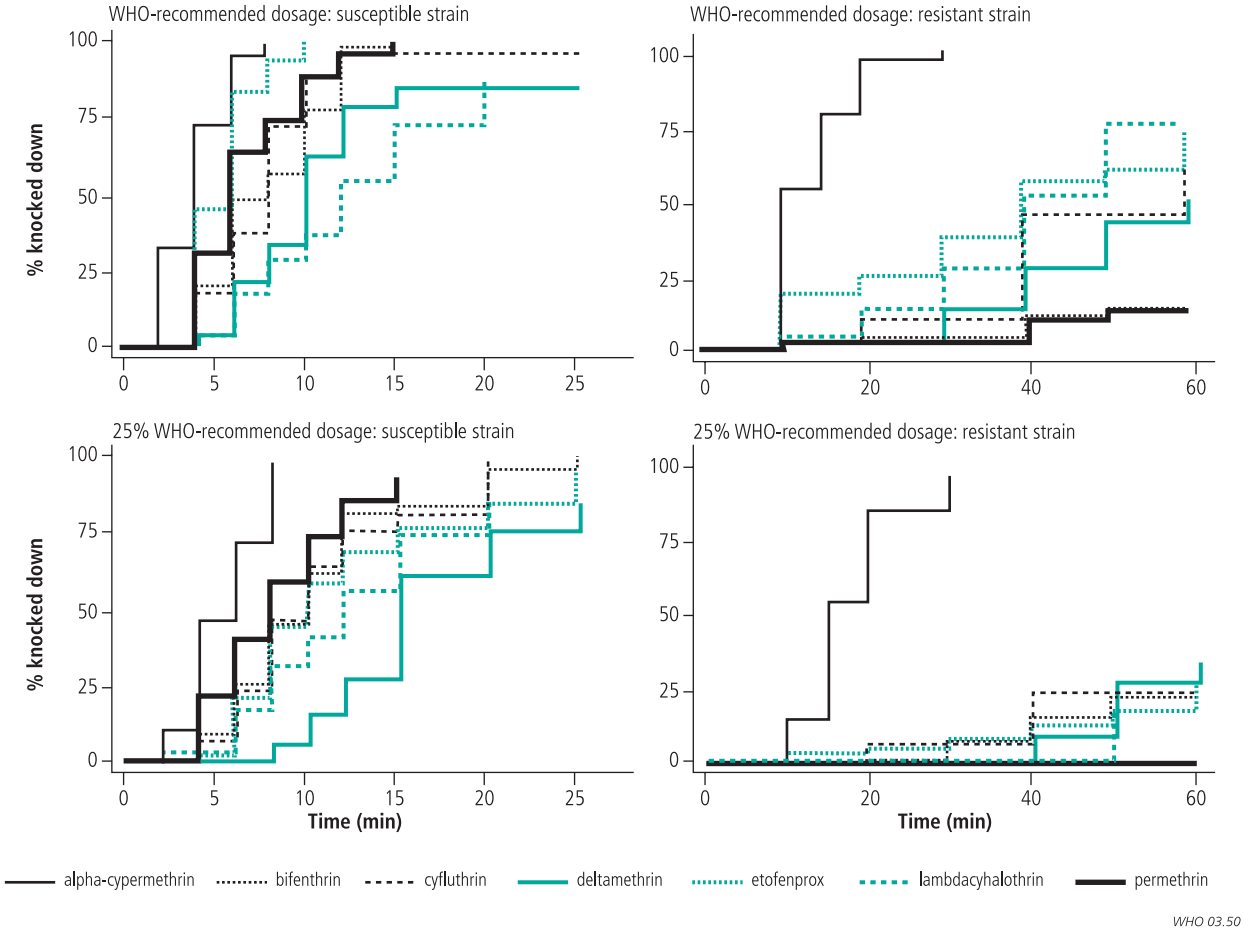
Results

Knock-down effect

Anopheles gambia

For susceptible *A. gambiae*, all tested pyrethroids were fast-acting at the WHO-recommended concentration, with KDT_{50} values of 4–12 min (Fig.1, Table 2 (available at: www.who.int/bulletin)). For four pyrethroids, including bifenthrin, the values of the $KDT_{25–75}$ were in the range 4–10 min; for three they were outside this range, one (alpha-cypermethrin) with lower $KDT_{25–75}$ values and the two others (deltamethrin and lambdacyhalothrin) with higher $KDT_{25–75}$ values. At one-quarter of the WHO-recommended concentration, differ-

Fig. 1. **Kaplan–Meier estimates of the proportion of knocked down *Anopheles gambiae*, by time (min) and insecticide**



ences were greater; alpha-cypermethrin and deltamethrin had the shortest and longest KDT_{50}, respectively; the value for bifenthrin was similar to those for the other five insecticides.

With the resistant strain, the KDT_{50} values at the higher concentration were six-to-eight times longer for cyfluthrin, deltamethrin and etofenprox, and two-to-three times longer for alpha-cypermethrin and lambdacyhalothrin, but more than 10 times longer for bifenthrin and permethrin. At the lower concentration, alpha-cypermethrin still had the shortest KDT_{50}; almost no knock-down was observed with etofenprox, lambdacyhalothrin and permethrin.

Culex quinquefasciatus

At the WHO-recommended concentration, the KDT_{50} values for susceptible *C. quinquefasciatus* (Fig. 2, Table 2) were more than twice those recorded with susceptible *A. gambiae*, except for etofenprox, lambdacyhalothrin and permethrin. The KDT_{50} for permethrin was the shortest, and not significantly different from the value observed with susceptible *A. gambiae*. The KDT_{50} for bifenthrin was three-to-15 times longer than the values observed with the other insecticides; this difference was also observed with the lower concentration. With the resistant strain, only alpha-cypermethrin retained some knock-down effect at the WHO recommended concentration. For the other insecticides there was no knock-down or a very long KDT_{50}. At the lower concentration, none of the insecticides had any knock-down effect.

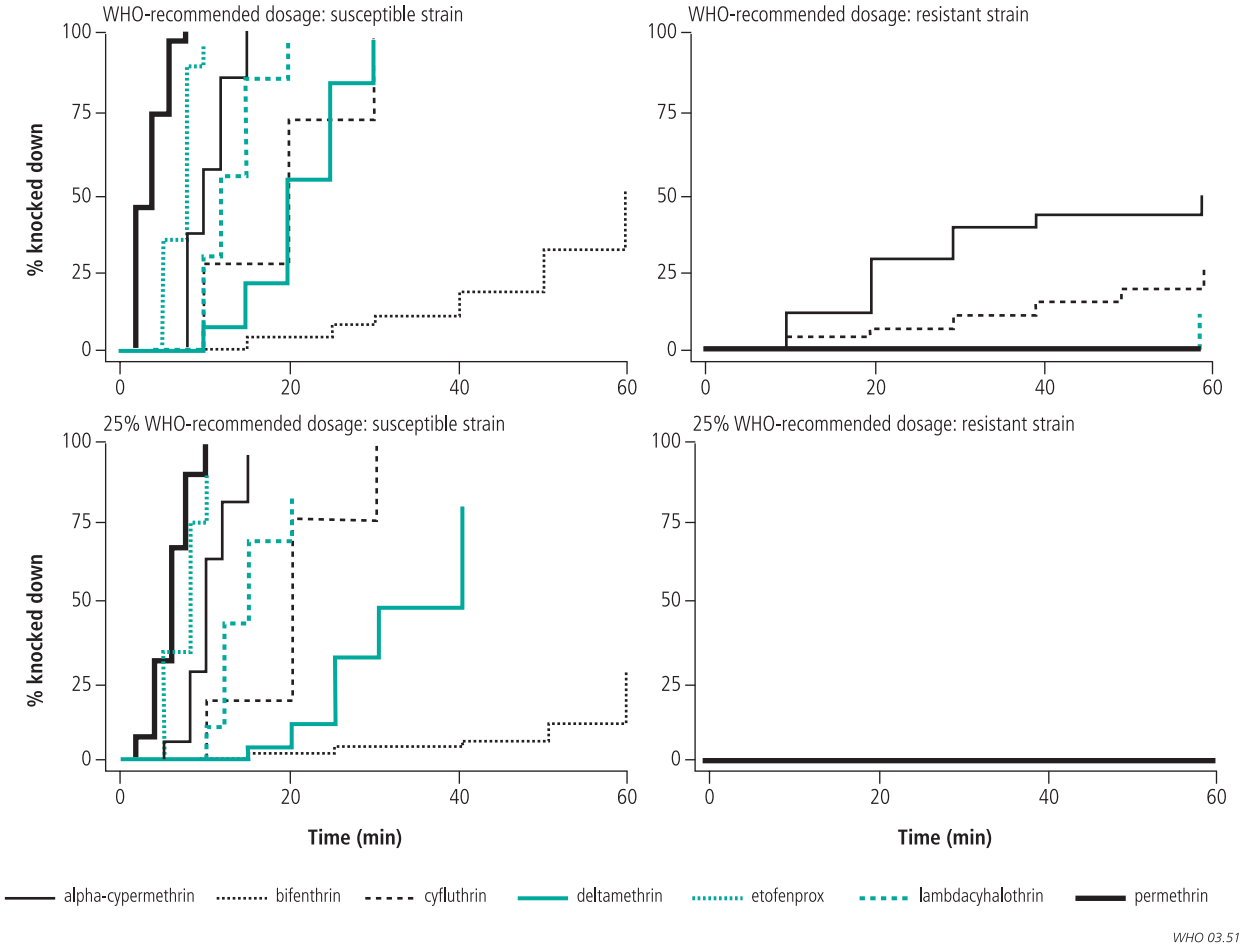
Mortality

The percentage mortalities observed in WHO cones and tunnels are summarized in Table 3 and Table 4, respectively (available at: www.who.int/bulletin). As expected, there was a strong difference in efficacy between the susceptible and resistant strains, particularly for *C. quinquefasciatus*, where resistance almost or completely prevented mortality with all compounds, except permethrin under WHO cones. With resistant *A. gambiae*, permethrin showed remarkable efficacy in the tunnel test, which was greater at the lower than the higher concentration, as earlier reported by Hodjati & Curtis (*35*). At the WHO-recommended concentration under WHO cones, alpha-cypermethrin was as effective as deltamethrin against susceptible *A. gambiae*, slightly more effective than etofenprox, and significantly more effective than the other insecticides. The results were similar at the lower concentration. Alpha-cypermethrin was clearly the most effective insecticide in terms of mortality.

Irritancy

With susceptible *A. gambiae* (Fig. 3, Table 5 (available at: www.who.int/bulletin)), the FT_{50} values for alpha-cypermethrin, cyfluthrin, deltamethrin, and lambdacyhalothrin were comparable at both concentrations. The most irritating treatments were the two etofenprox concentrations and the lower concentration of permethrin. Bifenthrin was by far the least irritant. With resistant *A. gambiae*, the FT_{50} values for

Fig. 2. **Kaplan–Meier estimates of the proportion of knocked down *Culex quinquefasciatus*, by time (min) and insecticide**



alpha-cypermethrin, cyfluthrin, lambdacyhalothrin, and permethrin at the higher concentration were similar to those with the susceptible strain; for etofenprox, deltamethrin and bifenthrin, they were 1.8–3.7 times higher. At the lower concentration, the irritant effect remained almost unchanged, except for bifenthrin, for which a 1.5-fold increase was noted.

For susceptible *C. quinquefasciatus* (Fig. 4, Table 5), the irritant effects did not greatly differ among the insecticides, including bifenthrin, except that a significantly longer FT_{50} was observed for deltamethrin at the higher concentration. With the resistant strain, the FT_{50} values were higher especially for bifenthrin and deltamethrin at the higher concentration, and bifenthrin, etofenprox and permethrin at the lower concentration.

Blood-feeding inhibition

Blood-feeding inhibition values are summarized in Table 6 (available at: www.who.int/bulletin). With susceptible *A. gambiae* and *C. quinquefasciatus*, there were no significant differences between insecticides at either concentration. With *C. quinquefasciatus*, significant differences in blood-feeding inhibition between the susceptible and resistant strains were observed at both concentrations. The differences were less pronounced for susceptible and resistant *A. gambiae*. With resistant *C. quinquefasciatus*, bifenthrin performed best at both concentrations. At the higher concentration, cyfluthrin, etofenprox, lambdacyhalothrin and permethrin had similar activities; deltamethrin was significantly less effective.

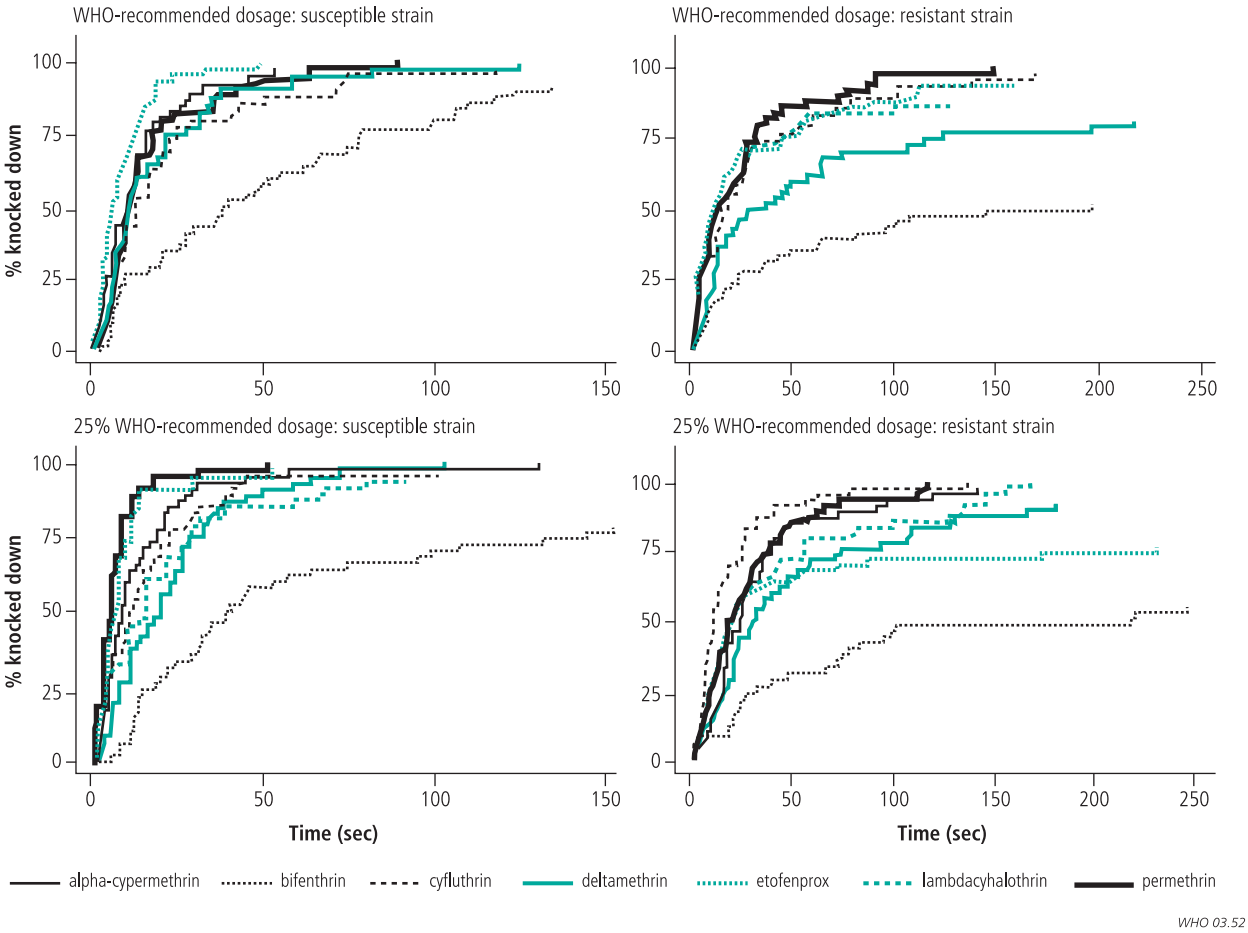
Overall insecticidal activity

The results on knock-down, mortality, irritancy and blood-feeding inhibition were graded into six categories (0–5; Table 7 and Annexes 1–4 (available at www.who.int/bulletin)). Rankings were established for each concentration on the basis of cumulative scores derived by combining the scores for the two mosquito species, susceptible or resistant, as indicated in Table 8 and Table 9 (available at: www.who.int/bulletin). The results for knock-down effect, irritancy and mortality obtained in the cone test were grouped as shown in Table 8. Alpha-cypermethrin performed significantly better than the other products regardless of mosquito species and strain; bifenthrin was significantly less efficient. The results for mortality and blood-feeding inhibition obtained in the tunnel test were grouped as shown in Table 9. Bifenthrin and, to a lesser extent, alpha-cypermethrin performed best regardless of mosquito species and resistance status.

Discussion

To our knowledge, this laboratory evaluation is the most comprehensive comparative study yet undertaken on the efficacy of pyrethroids for impregnation of mosquito nets. It involved the use of susceptible and resistant strains of two

Fig. 3. **Kaplan–Meier estimates of the proportion of *Anopheles gambiae* having left the test surface, by time (sec) and insecticide**



mosquito species of public health importance and permitted the assessment of three aspects of the insecticide activity (knock-down effect, irritancy, and mortality under WHO cones) and mortality and blood-feeding inhibition in free-flying mosquitoes in tunnels, where the result depends on various interacting factors, including irritancy and knock-down effect.

Insecticide activity under WHO cones

The activity under WHO cones varied significantly from insecticide to insecticide and between mosquito species and strains. The knock-down effect noted with the two susceptible mosquito species was rapid and related to concentration, whatever the insecticide tested (KDT_{50} was longer at the lower concentration). KDT_{50} increased dramatically with resistant *A. gambiae* and the knock-down effect almost completely disappeared with resistant *C. quinquefasciatus*. Mortality rates observed following forced tarsal contact in WHO cones or tunnels clearly showed the overall good performances of alpha-cypermethrin. Its efficacy at 20 mg/m^2 has been confirmed in Côte d'Ivoire by Koffi et al. (*36*) with laboratory and wild susceptible populations of *A. gambiae* but not with wild *kdr*-resistant populations. Mortality under cones was sometimes lower than expected, e.g. for cyfluthrin and lambdacyhalothrin. In other studies (*37, 38*), these insecticides have commonly shown 100% mortality when tested at the WHO-recommended concentration. However, mortality should not be considered alone, since a high irritant effect can considerably reduce tarsal contact with treated netting material, even with forced contact under WHO cones. For example, in the case of permethrin, which has a high irritant effect, mortality of less than 80% has commonly been observed with susceptible *A. gambiae* under WHO cones at the recommended concentration (62% in the present study).

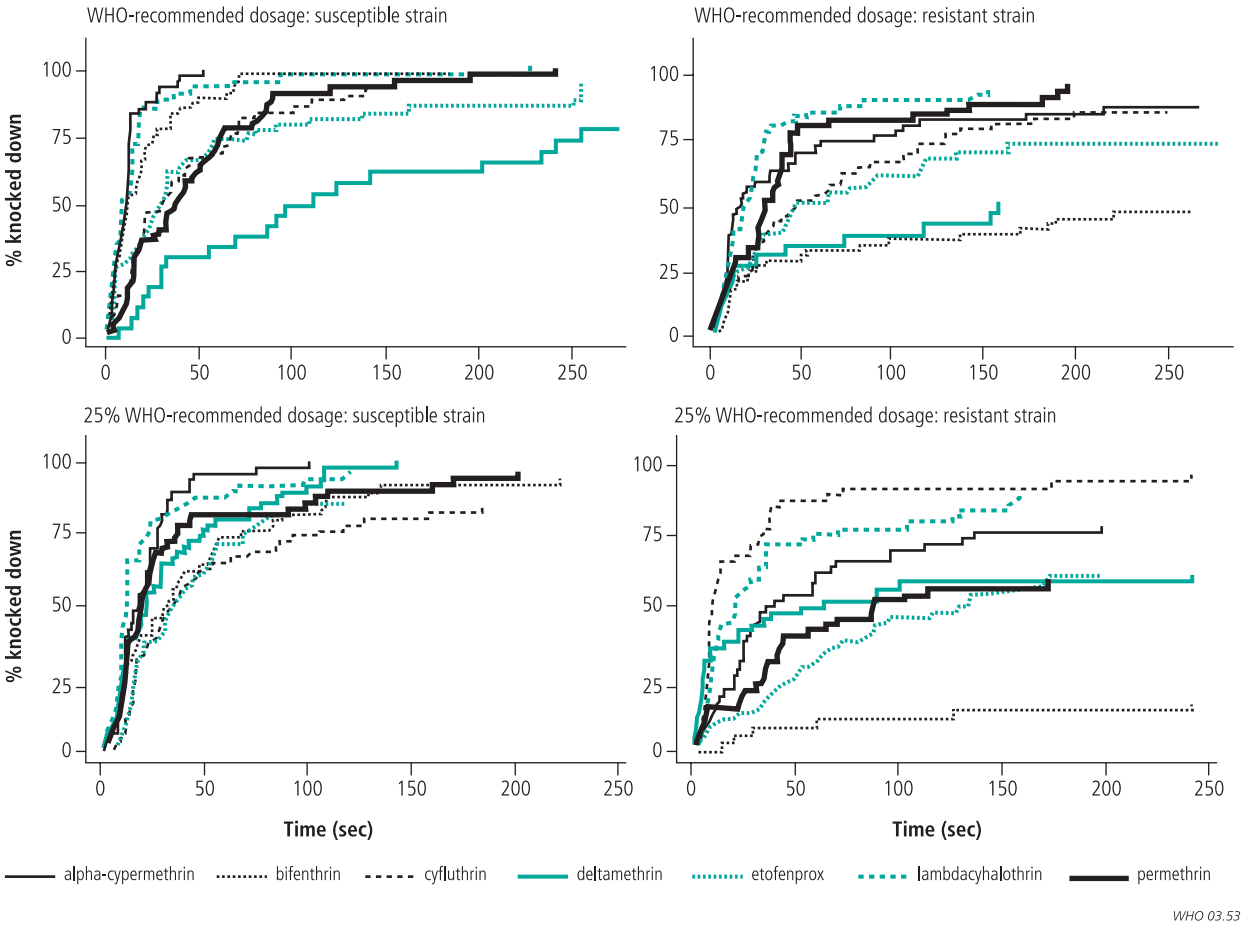
Irritant effect of insecticides

The irritant effect was not closely related to insecticide concentration, as observed previously for susceptible *A. gambiae* with DDT (*39*). Irritancy differed from insecticide to insecticide: bifenthrin was much less irritant to *A. gambiae* than the other pyrethroids, while etofenprox provided the highest irritancy, whatever the resistance status. The irritant effect was significantly reduced in resistant strains, as observed previously with permethrin versus resistant *A. gambiae* (*40*). The extent of this reduction differed between insecticides and mosquito species, however, being generally greater with *C. quinquefasciatus* than with *A. gambiae*.

Tunnel test

In the tunnel test, all the insecticides performed well against susceptible *A. gambiae* in terms of mortality and blood-feeding inhibition, even at the lower concentration. This last observation is of great importance, since the first wash of a treated mosquito net is expected to remove up to 50% of the insecticide, and every subsequent wash 25–30%, leaving about

Fig. 4. **Kaplan–Meier estimates of the proportion of *Culex quinquefasciatus* having left the test surface, by time (sec) and insecticide**



one-quarter of the original content after three-to-four washes (*41*). Our results were in line with unpublished field data that showed that mosquito nets were still effective after three-to-four washes. These results encouraged WHO to review the existing guidelines (*42*) in recommending in the next version the systematic re-treatment of nets after three washes or at least once a year. Pyrethroid resistance significantly decreased mortality of *A. gambiae* but did not dramatically interfere with blood-feeding inhibition. These results confirmed that mosquito nets treated with pyrethroids are still effective in reducing human–vector contact (*8, 9*) and malaria morbidity (*10, 11*) in resistant strains.

Conclusions

For susceptible *C. quinquefasciatus* in tunnels, only bifenthrin caused high mortality at the lower concentration and mortality with the resistant strain was extremely low or nil with all the insecticides. Contrary to Miller & Curtis, who observed a lower but not significant feeding rate with bifenthrin than with other treatments (*43*), we observed a significant inhibition of blood-feeding with this compound, even at the lower concentration. These results are of great importance since *C. quinquefasciatus* is responsible for most mosquito nuisance worldwide and is increasing in Africa and Asia because of the expansion of favourable habitats that usually accompanies urbanization. To gain better acceptance and compliance in the use of insecticide-impregnated mosquito nets, the nets should have a noticeable impact in reducing this pest nuisance. Alpha-cypermethrin performed best in both the cone and the tunnel test, whatever the mosquito species and resistance status. Bifenthrin also performed well in the tunnel test but showed the least effective performances in terms of knock-down effect, irritancy and mortality after short-term exposure. This demonstrates the fact that the impact of impregnated mosquito nets results from a complex interaction of factors, which cannot easily be dissociated from each other. Mortality and blood-feeding inhibition under tunnels should be considered as among the most important attributes of insecticides for use in impregnation of mosquito nets since these tests provide results comparable with those obtained in experimental hut studies (*21*).

Our results with bifenthrin and those obtained by others under laboratory (*16*) and field (*15*) conditions suggest that this insecticide is a promising pyrethroid for impregnation of mosquito nets because of its much stronger impact on *C. quinquefasciatus*.

When selecting pyrethroids for mosquito vector control and personal protection, specific attention should be given to the various properties of these insecticides, the behavioural response of the target mosquito species, the pyrethroid resistance status in the area and the importance of nuisance, especially that due to *C. quinquefasciatus*. ■

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Conflicts of interest: none declared.

Résumé

Performances comparées, dans des conditions de laboratoire, de sept insecticides de la classe des pyréthrinoïdes utilisés pour l'imprégnation des moustiquaires

Objectif Comparer l'efficacité de sept insecticides de la classe des pyréthrinoïdes pour l'imprégnation des moustiquaires, six recommandés par l'OMS et un produit candidat, la bifenthrine, dans des conditions de laboratoire.

Méthodes Les tests ont été réalisés sur des souches d'*Anopheles gambiae* et de *Culex quinquefasciatus* sensibles et résistantes aux pyréthrinoïdes. L'effet « knock-down », l'effet irritant et la mortalité ont été mesurés au moyen de tests standard de l'OMS avec pièges coniques. La mortalité et l'inhibition de la prise de repas de sang ont également été mesurées au moyen de tunnels contenant un appât.

Résultats Chez *A. gambiae* sensible aux pyréthrinoïdes, l'alpha-cyperméthrine avait l'effet « knock-down » le plus rapide. Chez les souches résistantes, cet effet était légèrement plus lent avec l'alpha-cyperméthrine et beaucoup plus réduit après exposition aux autres insecticides, notamment la bifenthrine et la perméthrine. Chez *C. quinquefasciatus* sensible, l'effet « knock-down » était significativement plus lent que chez *A. gambiae*, en particulier avec la bifenthrine, et chez les souches résistantes il était nul quel que soit le pyréthrinoïde utilisé. La bifenthrine était significativement moins irritante que les autres pyréthrinoïdes pour les *A. gambiae* sensibles et résistants, mais il n'y avait pas de différence marquée au niveau de l'effet irritant contre *C. quinquefasciatus*. Dans les tunnels, les insecticides étaient tous moins toxiques chez *C. quinquefasciatus* que chez *A. gambiae* en ce qui concerne les souches sensibles. Pour les souches résistantes, on a observé une mortalité importante avec tous les pyréthrinoïdes chez *A. gambiae* mais non chez *C. quinquefasciatus*. L'inhibition de la prise de repas de sang était forte chez les souches sensibles des deux espèces et chez les souches résistantes de *A. gambiae* mais plus faible chez les souches résistantes de *C. quinquefasciatus*; la bifenthrine avait l'impact le plus marqué.

Conclusion L'efficacité pour l'imprégnation des moustiquaires contre *A. gambiae* était maximale avec l'alpha-cyperméthrine. La bifenthrine possède probablement un avantage relatif important sur les autres pyréthrinoïdes dans les zones de résistance à cette classe d'insecticides en raison de son impact beaucoup plus marqué sur le moustique nuisant, *C. quinquefasciatus*, malgré un effet « knock-down » plus lent et un effet irritant plus faible. Le choix des pyréthrinoïdes destinés à la lutte contre les moustiques vecteurs et à la protection individuelle devra tenir compte des différents effets de ces insecticides, de l'état de la résistance aux pyréthrinoïdes dans la zone concernée et de l'importance des espèces nuisantes telles que *C. quinquefasciatus*.

Resumen

Resultados comparativos, en condiciones de laboratorio, de siete insecticidas piretroides utilizados para impregnar los mosquiteros

Objetivo Comparar la eficacia como tratamiento de impregnación de mosquiteros, en condiciones de laboratorio, de siete insecticidas piretroides: seis recomendados actualmente por la OMS, y un producto experimental, la bifentrina.

Métodos Se hicieron pruebas con cepas de *Anopheles gambiae* y *Culex quinquefasciatus* sensibles y resistentes a los piretroides. Los efectos de caída, irritación y mortalidad se midieron utilizando las pruebas ordinarias con conos de la OMS. También se midieron la mortalidad y la inhibición de la hemoingestión, empleando para ello un dispositivo tuneliforme con cebo.

Resultados En los ejemplares de *A. gambiae* sensibles, la alfa-cipermetrina tuvo el efecto de caída más rápido, mientras que en los resistentes dicho efecto fue ligeramente más lento con la alfa-cipermetrina y mucho menor tras la exposición a los otros insecticidas, particularmente la bifentrina y la permetrina. En *C. quinquefasciatus* sensible, el efecto de caída fue significativamente más lento que en *A. gambiae*, en particular con la bifentrina, y en el caso de la cepa resistente no se observó tal efecto con ninguno de los piretroides. La bifentrina fue significativamente menos irritante que los otros piretroides para *A. gambiae*, sensible y resistente, pero no se observaron diferencias claras en el poder de irritación entre los piretroides en el caso de *C. quinquefasciatus*. En los dispositivos tuneliformes, todos los insecticidas fueron menos tóxicos contra *C. quinquefasciatus* que contra *A. gambiae* para las cepas sensibles. Considerando las cepas resistentes, la mortalidad fue importante con todos los piretroides en el caso de *A. gambiae*, pero no en el de *C. quinquefasciatus*. La inhibición de la hemoingestión fue alta también en las cepas sensibles de ambas especies y en *A. gambiae* resistente, pero inferior en *C. quinquefasciatus* resistente; el máximo efecto fue el conseguido con la bifentrina.

Conclusión La alfa-cipermetrina fue el producto más eficaz como tratamiento de impregnación de los mosquiteros contra *A. gambiae*. La bifentrina puede presentar ventajas comparativas importantes frente a otros piretroides en las áreas con resistencia a estos productos, debido a su mucho mayor efecto en el mosquito causante de molestias *C. quinquefasciatus*, pese a la mayor lentitud de su efecto de caída y de irritación. A la hora de seleccionar los piretroides para combatir los mosquitos vectores y asegurar la protección personal, deberían tenerse en cuenta los diferentes efectos de estos insecticidas, la situación de resistencia a los piretroides en la zona en cuestión y la importancia de los mosquitos causantes de molestias, como *C. quinquefasciatus*.

ملخص

مقارنة كفاء سبعة من مبيدات الحشرات البيرثرونيدية التي استخدمت في تشريب الناموسيات، في ظروف المختبر

الغرض: مقارنة كفاءة سبعة من مبيدات الحشرات البيرثروئيدية التي استخدمت في تشريب الناموسيات؛ والتي توصي منظمة الصحة العالمية باستخدام ستة منها، وذلك في ظروف المختبر.

الطريقة: لقد أجريت الاختبارات باستخدام ذراري تتاثر بالبيريثروئيد وذراري مقاومة لتأثيره من الأنوفيلات الغامبية والباعضة الخماسية الخطوط، وتم قياس معدلات التأثير المميت، والمحدث للتهيج، والمسبب للسقوط باستخدام اختبار المخروط المعياري لمنظمة الصحة العالمية. كما تم قياس معدلات الموت وتثبيط التغذي على الدم باستخدام جهيزة نفقية مزودة بطعم جاذب للبعوض.

الموجودات: لقد كان لألفا-سيبرمثرين أسرع تأثير مسقط للأنوفيلات الغامبية التي تتأثر بمركبات البيريمرثروئيد، أما الأنوفيلات الغامبية المقاومة لمركبات البيريثروئيد فقد كان التأثير المسقط عليها للسيبرمثرين أبطأ قليلاً، وقد انخفض كثيراً بعد تعرضها لمبيدات الحشرات الأخرى ولاسيما البيفينثارين والبرمثرين. وكان التأثير المسقط على الباعضة الخماسية الخطوط أبطأ بشكل واضح مما كان عليه على الأنوفيلات الغامبية، ولاسيما بالنسبة للبيفينثرين، فيما لم يلاحظ أي تاثير مسقط لأي من مركبات البيرثيروئيد على الذراري المقاومة. وكان للبيفنثرين تأثير أقل بشكل ملحوظ عما كان للمركبات البيرثيروئيدية الأخرى من تأثير مهيج على الذراري السريعة التأثُّر وعلى الذراري المقاومة من الأنوفيلات الغامبية، ولم يكن هناك ترتيب سُلَّمي واضح للتأثير المسبب للتهيج على الباعضة الخماسية الخطوط. أما في الأنفاق فقد كانت جميع مبيدات الحشرات أقل سمية على الذراري المتأثرة من الباعضة الخماسية الخطوط منها على الأنوفيلات العامبية، أما بالنسبة للذراري المقاومة، فقد كانت معدلات الموت في الأنوفيلات الغامبية أعلى لكل المركبات البيروتروئيدية المستخدمة، ولم يكن الأمر كذلك في الباعضة الخماسية الخطوط. كما كان تثبيط التغذي على الدم أعلى لدى الذراري المتأثرة من كلا النوعين وللأنوفيلات الغامبية المقاومة، إلا أن ذلك التثبيط كان أقل بالنسبة للباعضة الخماسية الخطوط، وقد كان أكبر التأثير للبيفنثرين.

الاستنتاج: تكون كفاءة إشراب الناموسيات لصد الأنوفيلات الغامبية على أشدها باستخدام ألفاسيبيرمثرين. ويبدو أن للبيفنثرين فوائد موازية وهامة تزيد على ما للمركبات البيروثروئيدية في المناطق التي تنتشر فيها المقاومة لتلك المركبات، نظراً لتأثيره الأكثر قوة على صغار البعوض والباعضة الخماسية الخطوط، وذلك رغم بطء تأثيره المسقط والمهيج، ويجب الأخذ بالحسبان عند انتقاء المركبات البيروثروئيدية لمكافحة نواقل المرض وللحماية الشخصية كلاً من التأثيرات المختلفة لهذه المبيدات الحشرية وحالة المقاومة للبيروثيروئيد في المنطقة المستهدفة، وأهمية البعوض الصغير مثل الباعضة الخماسية الأرجل.

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Table 1. **List and characteristics of the seven test pyrethroid insecticides**

Insecticide	Formulation[a]	Trade name	Sample ref.	Tested concentration (mg/m^2)
Alpha-cypermethrin	SC 10%	Fendona	R1811-187	40 and 10
Bifenthrin	ME 0.3%	Talstar	PL99-0189	25 and 6.25
Cyfluthrin	EW 5%	Solfac	233-926-708	50 and 12.5
Deltamethrin	SC 1%	K-Othrin	LELH10169700	25 and 6.25
Etofenprox	EW 10%	Vectron	MN-106	200 and 50
Lambdacyhalothrin	CS 2.5%	Icon	BSNIC-1614	20 and 5
Permethrin	EC 10%	Peripel	LEEHI0189800	500 and 125

[a] CS = Capsule suspension; EC = emulsifiate concentrate; EW = emulsion, oil in water; ME = micro-emulsion; SC = suspension concentrate.

Table 2. **Median knock-down times (KDT_{50}) and times to knock-down of 25–75% ($KDT_{25–75}$) of mosquitoes in susceptible and resistant strains of *Anopheles gambiae* and *Culex quinquefasciatus* for seven pyrethroid insecticides used to impregnate mosquito nets**

Insecticide	Concentration (mg/m^2)	Time (min)							
		A. gambiae				*C. quinquefasciatus*			
		Susceptible		Resistant		Susceptible		Resistant	
		KDT_{50}[1]	$KDT_{25–75}$	KDT_{50}[1]	$KDT_{25–75}$	KDT_{50}[1]	$KDT_{25–75}$	KDT_{50}[1]	$KDT_{25–75}$
WHO-recommended concentration									
Alpha-cypermethrin	40	4[a]	2–6 (51)[2]	10[a]	10–15 (49)	10[a]	8–12 (50)	> 60[a]	20 to > 60 (54)
Bifenthrin	25	8[b]	6–10 (53)	> 60[b]	> 60 to > 60 (53)	60[b]	50–> 60 (51)	> 60[a, b]	> 60 to > 60 (51)
Cyfluthrin	50	8[b]	6–10 (50)	60[c, d]	40 to > 60 (50)	20[c]	10–30 (51)	> 60[a, b]	> 60 to > 60 (52)
Deltamethrin	25	10[c]	8–12 (50)	60[c]	40 to > 60 (50)	20[c]	20–25 (51)	> 60[b]	> 60 to > 60 (51)
Etofenprox	200	6[b]	4–6 (50)	40[c, d]	20 to > 60 (51)	8[d]	5–8 (50)	> 60[a, b]	> 60 to > 60 (52)
Lambdacyhalothrin	20	12[c]	8–20 (51)	40[d]	30–50 (50)	12[e]	10–15 (54)	> 60[b]	> 60 to > 60 (49)
Permethrin	500	6[b]	4–10 (50)	> 60[b]	> 60 to > 60 (50)	4[f]	2–6 (50)	> 60[a, b]	> 60 to > 60 (51)
25% WHO-recommended concentration									
Alpha-cypermethrin	10	6[a]	4–8 (51)	15[a]	15–20 (53)	10[a]	8–12 (49)	> 60[a]	> 60 to > 60 (51)
Bifenthrin	6.25	10[b, d]	6–12 (51)	> 60[b]	60 to > 60 (54)	> 60[b]	60–> 60 (50)	> 60[a]	> 60 to > 60 (50)
Cyfluthrin	12.5	10[b, d]	8–15 (51)	> 60[b]	60 to > 60 (51)	20[c]	20–20 (49)	> 60[a]	> 60 to > 60 (50)
Deltamethrin	6.25	15[c]	12–25 (50)	> 60[b]	50 to > 60 (49)	40[d]	25–40 (51)	> 60[a]	> 60 to > 60 (50)
Etofenprox	50	10[b, c]	8–15 (60)	> 60[b]	> 60 to > 60 (49)	8[e]	5–10 (51)	> 60[a]	> 60 to > 60 (51)
Lambdacyhalothrin	5	12[b, c]	8–20 (51)	> 60[b]	> 60 to > 60 (52)	15[f]	12–20 (50)	> 60[a]	> 60 to > 60 (49)
Permethrin	125	8[d]	6–12 (50)	> 60[a, b]	> 60 to > 60 (51)	6[g]	4–8 (51)	> 60[a]	> 60 to > 60 (50)

[1] KDT_{50} values with different superscripts (a – g) within the same group of concentrations and in the same column differ significantly (Cox's model).
[2] Figures in parentheses are number of insects tested.

Table 3. **Mortality rates in WHO cone tests for susceptible and resistant strains of *Anopheles gambiae* and *Culex quinquefasciatus* for seven pyrethroid insecticides used to impregnate mosquito nets**

Insecticide	Concentration (mg/m²)	Mortality rate											
		A. gambiae						*C. quinquefasciatus*					
		Susceptible			Resistant			Susceptible			Resistant		
		n[1]	%[2]	(95% CI)[3]	*n*	%[2]	(95% CI)	*n*	%[2]	(95% CI)	*n*	%[2]	(95% CI)
WHO-recommended concentration													
Alpha-cypermethrin	40	51	100[a]	(93–100)	49	94[a]	(83–99)	50	100[a]	(93–100)	54	4[a, b]	(0–13)
Bifenthrin	25	51	61[b]	(46–74)	53	8[b]	(2–18)	51	18[b]	(8–31)	51	2[a, b]	(0–10)
Cyfluthrin	50	50	74[b, c]	(60–85)	50	0[b]	(0–7)	51	33[b, c]	(21–48)	52	4[a, b]	(0–13)
Deltamethrin	25	50	100[a]	(93–100)	50	14[b]	(6–27)	51	98[a]	(90–100)	51	2[a, b]	(0–10)
Etofenprox	200	50	94[a, c]	(83–99)	51	6[b]	(1–16)	50	18[b]	(9–31)	52	0[a]	(0–7)
Lambdacyhalothrin	20	51	43[b]	(29–58)	50	0[b]	(0–7)	54	50[c]	(36–64)	49	0[a]	(0–7)
Permethrin	500	50	60[b]	(45–74)	50	2[b]	(0–11)	50	38[b, c]	(25–53)	51	22[b]	(11–35)
25% WHO-recommended concentration													
Alpha-cypermethrin	10	51	100[a]	(93–100)	53	70[a]	(56–82)	49	100[a]	(93–100)	51	0[a]	(0–7)
Bifenthrin	6.25	51	24[b]	(13–37)	54	7[b]	(2–18)	50	2[b]	(0–11)	50	0[a]	(0–7)
Cyfluthrin	12.5	51	47[b, c]	(33–62)	51	8[b]	(2–19)	49	16[b, c]	(7–30)	50	0[a]	(0–7)
Deltamethrin	6.25	50	92[a]	(81–98)	49	4[b]	(0–14)	51	86[a]	(74–94)	50	2[a, b]	(0–11)
Etofenprox	50	50	56[c]	(41–70)	49	4[b]	(0–14)	51	8[b, c]	(2–19)	51	0[a]	(0–7)
Lambdacyhalothrin	5	51	43[b, c]	(29–58)	52	2[b]	(0–10)	50	18[b, c]	(9–31)	49	0[a]	(0–7)
Permethrin	125	50	44[b, c]	(30–59)	51	2[b]	(0–10)	51	25[c]	(14–40)	50	20[b]	(10–34)

[1] *n* = No. of insects tested.
[2] % in the same column with different superscripts (a– c) within the same group of concentrations, differ significantly (Fisher's exact test).
[3] 95% CI = 95% confidence interval.

Table 4. **Mortality rates in tunnel tests with susceptible and resistant strains of *Anopheles gambiae* and *Culex quinquefasciatus* for seven pyrethroid insecticides used to impregnate mosquito nets**

Insecticide	Concentration (mg/m²)	Mortality rate											
		A. gambiae						*C. quinquefasciatus*					
		Susceptible			Resistant			Susceptible			Resistant		
		n[1]	%[2]	(95% CI)[3]	*n*	%[2]	(95% CI)	*n*	%[2]	(95% CI)	*n*	%[2]	(95% CI)
WHO-recommeded concentration													
Alpha-cypermethrin	40	92	100[a]	(96–100)	91	59[a]	(49–70)	89	87[a]	(78–93)	101	3[a]	(1–8)
Bifenthrin	25	94	100[a]	(96–100)	87	33[b, d]	(24–44)	97	91[a]	(83–96)	97	4[a]	(1–10)
Cyfluthrin	50	97	95[a]	(88–98)	98	21[b, c]	(14–31)	97	87[a]	(78–93)	83	0[a]	(0–4)
Deltamethrin	25	93	97[a]	(91–99)	99	38[a, d]	(29–49)	94	43[b]	(32–53)	99	0[a]	(0–4)
Etofenprox	200	88	98[a]	(92–100)	93	9[c]	(4–16)	106	21[c]	(13–30)	100	1[a]	(0–5)
Lambdacyhalothrin	20	92	99[a]	(94–100)	100	17[b, c]	(10–26)	97	78[a]	(69–86)	96	1[a]	(0–6)
Permethrin	500	95	95[a]	(88–98)	96	10[c]	(5–18)	96	77[a]	(67–85)	99	0[a]	(0–4)
25% WHO-recommended concentration													
Alpha-cypermethrin	10	92	95[a, b]	(88–98)	97	24[a, e]	(16–33)	96	65[a]	(54–74)	98	1[a]	(0–6)
Bifenthrin	6.25	84	99[a, b]	(94–100)	84	49[b, c, e]	(38–60)	96	99[b]	(94–100)	79	0[a]	(0–5)
Cyfluthrin	12.5	99	90[b]	(82–95)	99	5[d]	(2–11)	111	36[c]	(27–46)	100	0[a]	(0–4)
Deltamethrin	6.25	94	94[a, b]	(87–98)	92	39[e]	(29–50)	94	33[c]	(24–43)	111	0[a]	(0–3)
Etofenprox	50	81	100[a]	(96–100)	94	9[a, d]	(4–16)	93	28[c]	(19–38)	99	0[a]	(0–4)
Lambdacyhalothrin	5	92	99[a, b]	(94–100)	93	0[d]	(0–4)	91	79[a]	(69–87)	102	0[a]	(0–4)
Permethrin	125	97	96[a, b]	(90–99)	95	66[c]	(56–76)	95	35[c]	(25–45)	95	2[a]	(0–7)

[1] *n* = No. of insects tested.
[2] % in the same column with different superscripts (a–e) within the same group of concentrations, differ significantly (Fisher's exact test).
[3] 95% CI = 95% confidence interval.

Table 5. **Time to first take-off (FT_{50}) and time to take-off of 25–75% (FT_{25-75}) of mosquitoes in WHO cone tests with susceptible and resistant strains of *Anopheles gambiae* and *Culex quinquefasciatus* for seven pyrethroid insecticides used to impregnate mosquito nets**

Insecticide	Concentration (mg/m²)	Time (sec)							
		A. gambiae				*C. quinquefasciatus*			
		Susceptible		Resistant		Susceptible		Resistant	
		FT_{50}[1]	$FT_{25–75}$	FT_{50}[1]	$FT_{25–75}$	FT_{50}[1]	$FT_{25–75}$	FT_{50}[1]	$FT_{25–75}$
WHO-recommended concentration									
Alpha-cypermethrin	40	11[a, c]	5–18 (50)[2]	16[a, d]	6–47 (50)	11[a]	5–14 (50)	16[a, c, d*]	10–92 (50)
Bifenthrin	25	39[b]	10–79 (51)	145[b]	24 to > 256 (50)	12[a]	6–27 (50)	> 256 [b*]	23 to > 256 (50)
Cyfluthrin	50	13[a]	8–25 (50)	19[a, d]	7–45 (50)	31[b]	15–69 (51)	51[b, c*]	21–131 (51)
Deltamethrin	25	12[a]	7–22 (50)	29[d]	12–125 (51)	97[c]	31–256 (26)	159[a, b, c*]	15 to > 256 (25)
Etofenprox	200	7[c]	4–13 (50)	13[a]	5–46 (50)	29[b]	6–78 (50)	45[a, b, c, d*]	13 to > 256 (50)
Lambdacyhalothrin	20	11[a]	8–17 (50)	15[a, d]	6–41 (50)	9[a]	6–18 (50)	20[d*]	9–32 (50)
Permethrin	500	11[a]	7–20 (50)	13[a]	4–33 (50)	38[b]	15–64 (51)	32[a, c, d*]	12–47 (51)
25% WHO-recommended concentration									
Alpha-cypermethrin	10	9[a, c]	5–19 (50)	24[a*, d*]	16–38 (50)	18[a]	11–28 (50)	37[a, d*]	21–139 (50)
Bifenthrin	6.25	38[b]	14–140 (50)	219[b*]	27 to > 256 (50)	31[b]	12–68 (50)	> 256[b*]	> 256 to > 256 (31)
Cyfluthrin	12.5	11[a]	5–22 (50)	11[c*]	6–26 (50)	35[b]	15–106 (51)	10[c*]	7–34 (50)
Deltamethrin	6.25	19[a]	8–28 (50)	31[a*]	16–75 (50)	22[a, b]	13–50 (51)	54[a, c, d*]	6 to > 256 (50)
Etofenprox	50	6[c, d]	4–11 (50)	19[a, c*]	7–232 (50)	34[b]	16–71 (50)	131[d*]	44 to > 256 (50)
Lambdacyhalothrin	5	15[a]	8–28 (50)	20[a, c*]	8–56 (50)	12[a]	8–23 (50)	22[a, c*]	9–60 (50)
Permethrin	125	6[d]	3–9 (50)	19[a, c*]	9–40 (50)	20[a, b]	10–36 (51)	89[d*]	32 to > 256 (50)

[1] FT_{50} values with different superscripts (a–d) within the same group of concentrations and in the same column differ significantly (Cox's model).
[2] Figures in parentheses are the numbers of insects tested.
* Peto–Peto–Prentice test was applied to these entries.

Table 6. **Blood-feeding inhibition in tunnel tests with susceptible and resistant strains of *Anopheles gambiae* and *Culex quinquefasciatus* for seven pyrethroid insecticides used to impregnate mosquito nets**

Insecticide	Concentration (mg/m²)	*A. gambiae*						*C. quinquefasciatus*					
		Susceptible			Resistant			Susceptible			Resistant		
		n[1]	% inhibited[2]	(95% CI)	*n*[1]	% inhibited[2]	(95% CI)	*n*[1]	% inhibited[2]	(95% CI)	*n*[1]	% inhibited[2]	(95% CI)
WHO-recommended concentration													
Alpha-cypermethrin	40	23	100[a]	(85–100)	83	99[a]	(93–100)	66	98[a]	(92–100)	93	28[a, c, d]	(19–38)
Bifenthrin	25	34	97[a]	(85–100)	56	79[b]	(66–88)	49	100[a]	(93–100)	89	93[b]	(86–97)
Cyfluthrin	50	41	95[a]	(83–99)	89	87[a, b]	(78–93)	72	100[a]	(95–100)	59	49[c]	(36–63)
Deltamethrin	25	40	98[a]	(87–100)	71	94[a, b]	(86–98)	31	97[a]	(83–100)	70	16[d]	(8–26)
Etofenprox	200	32	100[a]	(89–100)	83	88[a, b]	(79–94)	80	96[a]	(89–99)	93	63[c]	(53–73)
Lambdacyhalothrin	20	23	100[a]	(85–100)	68	93[a, b]	(84–98)	73	100[a]	(95–100)	89	45[a, c]	(34–56)
Permethrin	500	41	100[a]	(91–100)	69	78[b]	(67–87)	49	100[a]	(93–100)	85	40[a, c]	(30–51)
25% WHO-recommended concentration													
Alpha-cypermethrin	10	23	91[a]	(72–99)	88	76[a]	(66–85)	71	100[a]	(95–100)	90	39[a]	(29–50)
Bifenthrin	6.25	30	97[a]	(83–100)	54	87[a, b]	(75–95)	49	100[a]	(93–100)	72	94[b]	(86–98)
Cyfluthrin	12.5	42	100[a]	(92–100)	90	93[b]	(86–98)	82	100[a]	(96–100)	74	0[c]	(0–5)
Deltamethrin	6.25	41	100[a]	(91–100)	66	80[a, b]	(69–89)	31	94[a]	(79–99)	79	1[c, d]	(0–7)
Etofenprox	50	29	100[a]	(88–100)	84	38[c]	(28–49)	70	94[a]	(86–98)	92	23[a, e]	(15–33)
Lambdacyhalothrin	5	23	100[a]	(85–100)	64	86[a, b]	(75–93)	69	100[a]	(95–100)	95	13[d, e]	(7–21)
Permethrin	125	42	93[a]	(81–99)	69	70[a]	(57–80)	48	98[a]	(89–100)	82	37[a]	(26–48)

[1] *n* = No. of insects tested.
[2] % in the same column with different superscripts (a to e) within the same group of concentrations differ significantly (Fisher's exact test).

Table 7. **Allocation of an insecticide score on the basis of the results for four main characteristics tested**

Score	KDT_{50}[a] (min)	Mortality (%)	FT_{50}[b] (sec)	Blood-feeding inhibition (%)
0	> 50	< 20%	>50	< 50%
1	30–49	20–39 %	30–49	50–69 %
2	15–29	40–59 %	20–29	70–79 %
3	10–14	60–79 %	15–19	80–89 %
4	5–9	80–94 %	10–14	90–94 %
5	< 5	>95 %	< 10	> 95 %

[a] Median knock-down time.
[b] Median time to first take-off.

Table 8. **Ranking of seven pyrethroid insecticides obtained by adding scores from cone test data (knock down, irritancy and mortality) for *Anopheles gambiae* and *Culex quinquefasciatus***

	A. gambiae SS[a] + *C. quinquefasciatus* SS		*A. gambiae* SS + *C. quinquefasciatus* RR[b]		*A. gambiae* RR + *C. quinquefasciatus* SS		*A. gambiae* RR + *C. quinquefasciatus* RR		All strains	
Insecticide	**Score**	**Rank[c]**	**Score**	**Rank**	**Score**	**Rank**	**Score**	**Rank**	**Score**	**Rank**
WHO-recommended concentration										
Alpha-cypermethrin	26	1	17	1	22	1	13	1	78	1
Bifenthrin	12	7	8	7	4	7	0	7	24	7
Cyfluthrin	15	6	11	6	7	6	3	5	36	6
Deltamethrin	19	2	12	4	9	5	2	6	42	5
Etofenprox	19	2	14	2	11	3	6	2	50	2
Lambdacyhalothrin	19	2	11	5	14	2	6	2	50	2
Permethrin	18	5	13	3	11	3	6	2	48	4
25% WHO-recommended concentration										
Alpha-cypermethrin	25	1	15	1	18	1	8	1	66	1
Bifenthrin	6	7	5	7	1	7	0	7	12	7
Cyfluthrin	12	6	13	2	7	6	8	1	40	3
Deltamethrin	16	3	9	6	8	3	1	6	34	6
Etofenprox	15	4	10	4	8	3	3	5	36	4
Lambdacyhalothrin	14	5	10	4	8	3	4	3	36	4
Permethrin	18	2	12	3	10	2	4	3	44	2

[a] SS = susceptible strain.
[b] RR = resistant strain.
[c] Rank = 1 is the highest, indicating best insecticide performance.

Table 9. **Ranking of seven pyrethroid insecticides obtained by adding scores from tunnel test data (mortality and blood-feeding inhibition) for *Anopheles gambiae* and *Culex quinquefasciatus***

Insecticide	*A. gambiae* SS[a] + *C. quinque-fasciatus* SS		*A. gambiae* SS + *C. quinque-fasciatus* RR[b]		*A. gambiae* RR + *C. quinque-fasciatus* SS		*A. gambiae* RR + *C. quinque-fasciatus* RR		All strains	
	Score	Rank[c]	Score	Rank	Score	Rank	Score	Rank	Score	Rank
WHO recommended concentration										
Alpha-cypermethrin	19	1	10	3	16	1	7	1	52	1
Bifenthrin	19	1	14	1	12	3	7	1	52	1
Cyfluthrin	19	1	10	3	13	2	4	4	46	3
Deltamethrin	17	6	10	3	12	3	5	3	44	4
Etofenprox	16	7	11	2	9	7	4	4	40	6
Lambdacyhalothrin	18	4	10	3	12	3	4	4	44	4
Permethrin	18	4	10	3	10	6	2	7	40	6
25% WHO-recommended concentration										
Alpha-cypermethrin	17	3	9	4	11	2	3	5	40	3
Bifenthrin	20	1	14	1	15	1	9	1	58	1
Cyfluthrin	15	4	9	4	10	5	4	3	38	5
Deltamethrin	14	7	9	4	9	6	4	3	36	6
Etofenprox	15	4	10	2	5	7	0	7	30	7
Lambdacyhalothrin	18	2	10	2	11	2	3	5	42	2
Permethrin	15	4	9	4	11	2	5	2	40	3

[a] SS = susceptible strain.
[b] RR = resistant strain.
[c] Rank = 1 is the highest, indicating best insecticide performance.

Annex 1. **Insecticide scores for performance against susceptible *Anopheles gambiae* for seven pyrethroid insecticides in five tests**

Insecticide	Knock-down effect		Irritancy		Mortality in WHO cones		Mortality in tunnels		Blood-feeding inhibition	
	Dose[a]	25% dose[b]	Dose	25% dose	Dose	25% dose	Dose	25% dose	Dose	25% dose
Alpha-cypermethrin	5	5	5	5	5	5	5	4	5	4
Bifenthrin	4	4	2	1	3	1	5	5	5	5
Cyfluthrin	4	4	4	5	3	2	5	4	4	5
Deltamethrin	4	2	5	4	5	4	5	4	5	5
Etofenprox	5	4	5	5	4	2	5	5	5	5
Lambdacyhalothrin	4	3	4	4	3	2	5	5	5	5
Permethrin	4	4	4	5	3	2	4	5	5	4

[a] WHO-recommended concentration for use in impregnating mosquito nets.
[b] One-quarter of WHO-recommended concentration.

Annex 2. **Insecticide scores for performance against resistant *Anopheles gambiae* for seven pyrethroid insecticides in five tests**

Insecticide	Knock-down effect		Irritancy		Mortality in WHO cones		Mortality in tunnels		Blood-feeding inhibition	
	Dose[a]	25% dose[b]	Dose	25% dose	Dose	25% dose	Dose	25% dose	Dose	25% dose
Alpha-cypermethrin	4	3	4	3	4	3	4	3	5	3
Bifenthrin	0	0	0	0	0	0	0	0	3	4
Cyfluthrin	1	0	4	5	0	0	0	0	4	4
Deltamethrin	0	0	1	2	1	0	1	0	4	4
Etofenprox	1	0	3	2	0	0	0	0	3	4
Lambdacyhalothrin	1	0	4	3	0	0	0	0	4	4
Permethrin	0	0	4	4	0	0	0	0	3	3

[a] WHO-recommended concentration for use in impregnating mosquito nets.
[b] One-quarter of WHO-recommended concentration.

Annex 3. **Insecticide scores for performance against susceptible *Culex quinquefasciatus* for seven pyrethroid insecticides in five tests**

Insecticide	Knock-down effect		Irritancy		Mortality in WHO cones		Mortality in tunnels		Blood-feeding inhibition	
	Dose[a]	25% dose[b]	Dose	25% dose	Dose	25% dose	Dose	25% dose	Dose	25% dose
Alpha-cypermethrin	4	4	5	4	5	5	4	3	5	5
Bifenthrin	0	0	4	2	1	0	4	5	5	5
Cyfluthrin	3	3	2	1	1	1	4	1	5	5
Deltamethrin	2	2	0	3	5	5	2	1	5	4
Etofenprox	4	4	2	2	1	0	1	1	5	4
Lambdacyhalothrin	3	3	5	2	2	0	3	3	5	5
Permethrin	5	5	2	3	1	1	3	1	5	5

[a] WHO-recommended concentration for use in impregnating mosquito nets.
[b] One-quarter of WHO-recommended concentration.

Annex 4. **Insecticide scores for performance against resistant *Culex quinquefasciatus* for seven pyrethroid insecticides in five tests**

Insecticide	Knock-down effect		Irritancy		Mortality in WHO cones		Mortality in tunnels		Blood-feeding inhibition	
	Dose[a]	25% dose[b]	Dose	25% dose	Dose	25% dose	Dose	25% dose	Dose	25% dose
Alpha-cypermethrin	0	0	2	1	0	0	0	0	1	1
Bifenthrin	0	0	0	0	0	0	0	0	4	4
Cyfluthrin	0	0	1	4	0	0	0	0	2	0
Deltamethrin	0	0	0	0	0	0	0	0	0	0
Etofenprox	0	0	1	0	0	0	0	0	3	1
Lambdacyhalothrin	0	0	3	2	0	0	0	0	2	0
Permethrin	0	0	2	0	1	1	0	0	2	1

[a] WHO-recommended concentration for use in impregnating mosquito nets.
[b] One-quarter of WHO-recommended concentration.