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Laboratory study to investigate the efficacy of cyfluthrin EW treated nets against phlebotomine sandflies

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Summary. - Toxic and feeding inhibition effect of cyfluthrin treated nets was assessed and compared with the activity of deltamethrin impregnated nets, before and after 2 and 4 washes against *Phlebotomus papatasi*. Impregnated cyfluthrin nets (50, 25, 12.5 mg active ingredient (a.i.)/m^2) caused knock down and mortality rates higher than 90%, being of 100% at the highest dose, while the nets impregnated with deltamethrin (25, 12.5, 6.25 mg a.i./m^2) showed low mortality rates (13.3-43.5%). Also after washing cyfluthrin at the dose of 50 mg a.i./m^2 provided higher activity than deltamethrin (25 mg a.i./m^2), being mortality rates within 24 hs of 66.7 and 48.9% for cyfluthrin and 38.0 and 2.1% for deltamethrin, respectively after 2 and 4 washes. Before washing, cyfluthrin (50 mg a.i./m^2) significantly prevented blood feeding, with respect to both untreated (88.6%) and deltamethrin impregnated nets (56.8%). Deltamethrin (25 mg a.i./m^2) showed low feeding inhibition (48.4%), although it was significantly higher than control. The washing of cyfluthrin impregnated nets (50 mg a.i./m^2) produced a decrease of the feeding inhibition, being the reduction of 26.6% and 41.3%, respectively after 2 and 4 washes. Two washes of deltamethrin impregnated nets (25 mg a.i./m^2) yielded a very high reduction of antifeeding inibition (68.8%). These laboratory results if confirmed by field evaluation indicate cyfluthrin as good candidate for treatment of mosquito nets to be used against phlebotomine sandflies.

Key words: bioassay test, control, cyfluthrin EW, deltametrin SC, mosquito nets, phlebotomine sandflies.

Riassunto *(Studi di laboratorio per la valutazione dell'efficacia di reti impregnate con ciflutrina contro i flebotomi)*. - Sono stati condotti studi di laboratorio per valutare l'effetto tossico e l'inibizione alla puntura di reti impregnate con la ciflutrina contro una specie di flebotomo, *Phlebotomus papatasi*. La ciflutrina (50; 25; 12,5 mg di ingrediente attivo (a.i.)/m^2) ha mostrato una tossicità > del 90%, raggiungendo il 100% alla concentrazione più alta, mentre la deltametrina (25; 12,5; 6,25 mg i.a./m^2) ha mostrato una tossicità più bassa compresa tra un minimo del 13,3% ed un massimo del 43,5%. Dopo il lavaggio, la ciflutrina (50 mg i.a./m^2) ha conservato un'attività più elevata rispetto alla deltametrina (25 mg i.a./m^2). Entro le 24 ore la ciflutrina ha fatto registrare una mortalità del 66,7% e 48,9% mentre la deltametrina del 38,0% e 2,1%, rispettivamente dopo 2 e 4 lavaggi. La ciflutrina (50 mg i.a./m^2) ha mostrato di prevenire in modo significativo la puntura, a confronto sia con reti non trattate (88,6%) che con reti trattate con deltametrina (56,8%). La deltametrina (25 mg i.a./m^2) ha mostrato una minore protezione contro la puntura (48,4%), anche se sempre significativa rispetto al controllo. Il lavaggio delle reti impregnate con ciflutrina (50 mg i.a./m^2) ha provocato una diminuzione della protezione dalla puntura, con una riduzione del 26,6 e 41,3%, rispettivamente dopo 2 e 4 lavaggi. Questi risultati di laboratorio se confermati da valutazioni sul campo indicano la ciflutrina come un buon candidato per il trattamento di reti contro la puntura dei flebotomi.

Parole chiave: saggi biologici, controllo, ciflutrina EW, deltametrina SC, zanzariere, flebotomi.

Introduction

For malaria control, the use of insecticide treated mosquito nets (ITNs) is considered to be an effective, relatively cheap and sustainable method [1-3]. It has been also shown that ITNs may reduce morbidity and mortality from malaria in hyper-holoendemic areas of sub-Saharan Africa. This protection could partially depend on the transitory imbalance between the anti-malaria immunity acquired by the population before the intervention and the lowered sporozoite load resulting from the anti-vector measure [4]. The pyrethroids used for the treatment of the nets show low to moderate mammalian toxicity [5], low volatility and high insecticidal activity.

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ITNs have been evaluated also against *Leishmania* vectors in Venezuela and Colombia [6, 7] and in some countries in the Old World, namely Italy [8], Burkina Faso [9], Kenia [10-12], Sudan [13, 14] and Syria [15, 16]. From the experimental studies it appears that the use of ITNs may represent also one of the most sustainable method of reducing intradomiciliary transmission of *Leishmania* in endemic areas and particularly in communities surrounded by forest, where the diurnal resting sites of vectors are unknown or inaccessible [17]. Nevertheless, bioassay comparisons between nets impregnated with different pyrethroids are needed to ascertain which are the most efficacious against sandfly vectors.

The present note reports the results observed in *Phlebotomus papatasi* exposed to nets impregnated with cyfluthrin EW compared to those impregnated with deltamethrin SC, before and after 2 and 4 washes.

Materials and methods

Impregnated nets

Insecticide-treated and untreated nets tested in the present study were provided by the Institute de Recherche pour le Dévelopment, Laboratoire de Lutte contre les Insectes Nuisibles (LIN), Montpellier, France. Mosquito nets (mesh=156), 12x13 holes per sq. inch (= 25 mm), were impregnated in Montpellier (LIN) according to the standard procedures approved by WHO. Target concentrations of 50, 25, 12.5 and 25, 12.5, 6.25 mg active ingredient (a.i.)/m^2 were used for cyfluthrin EW (Cyf-EW) (Bayer, Leverkusen, Germany) and deltamethrin SC (Del-SC) (AgrEvo, Marseille Cedex, France), respectively. ITNs washes were carried out by using the standard protocol employed at WHO Collaborating centre on laboratory testing of insecticides, LIN, Montpellier. Cyfluthrin and deltamethrin content in washed ITNs was determined at Agricultural Research Centre, Pesticides Research Department, B-5030 Gembloux, Belgium, by using Capillary Gas Chromatography with ^{63}Ni Electron Capture Detection (GC-ECD). Table 1 reports cyfluthrin and deltamethrin content in ITNs (50 and 25 mg a.i./m^2, respectively), before and after 2 and 4 washes.

Sandflies

Sandflies used in the bioassay were from a laboratory colony of *P. papatasi* maintained according to the rearing methods previously described [18, 19]. The *P. papatasi* colony originated from fed females collected in a bedroom of a flat located in the historical centre of Rome [20]. For the colony establishment, each gravid female collected in the field was retained individually in a glass tube (20 ml) lined with a triangle of filter paper for oviposition. From the second generation the mass-rearing technique of Modi & Tesh [21] was used. The criteria to select *P. papatasi* for evaluating the efficacy of ITNs was because: (i) the species is the well known vector of the zoonotic cutaneous leishmaniasis due to *Leishmania major* [22, 23]; (ii) the efficacy of ITNs against this highly endophilic and endophagic species has been already reported in Sudan [13].

Bioassay

Toxic effect

To assess, by contact, the toxic effect of ITNs at the target insecticide dose, before and after washing, female sandflies 3-day-old sugar fed were made to walk on the nets for 3 min by using WHO [24; 25] recommended procedures to assess insecticide deposits on mosquito nets. Female sandflies were exposed to Cyf-EW and Del-SC treated nets in the transparent plastic bioassay cones. Each chamber was fastened on a piece of net, placed horizontally on a filter paper Test batches of fifteen sandflies were introduced by an aspirator into the cones. Bioassay was carried out in dark at 25-26 °C and 70-80% of relative humidity. After the exposure the sandflies were removed with a bent-end aspirator, through the opening of the chamber, and immediately transferred to holding pots closed with a fine mesh and provided with glucose solution for nourishment. They were kept at 26 °C and 17 hours light/7 hours dark in a plastic box lined with moistened filter paper for scoring the number knocked down during 1 hour post-exposure and the mortality rates after 24 hours. Three replicates were performed for each of the target dose of Cyf-EW and Del-SC as well as for each wash of the impregnated nets. The controls were processed in the same way. To assess the effectiveness of cyfluthrin impregnated nets an over total of 948 *P. papatasi* females was needed, of which 641were tested on ITNs, and 307 were used as control of the paired tests, by exposing the flies to untreated nets. The results have been expressed and compared as percentages of mortality at each concentration. Abbott's formula was used to correct mortality rates [24].

Antifeeding effect

To evaluate feeding inhibition of holed ITNs at the target dose of 50 and 25 mg a.i./m^2, respectively for Cyf-EW and Del-SC, before and after 2 and 4 washes,

Table 1. - Cyfluthrin and deltamethrin content in mosquito treated nets (50 and 25 mg a.i./m^2, respectively) before and after 2 and 4 washes

Identification	Samples analysed		Content (*)			
	Weight (mg)	Area (cm^2)	mg/kg	Mean	mg/m^2	Mean
Cyfluthrin 50 mg a.i./m^2						
Before washing	281.8	97.0	1952		56.7	
	305.9	103.0	1993	1972	59.2	57.9
2 washes	303.9	100.0	233		7.1	
	302.6	97.0	226	229	7.1	7.1
4 washes	290.8	98.9	267		7.9	
	280.8	95.1	206	237	6.1	7.0
Deltamethrin 25 mg a.i./m^2						
Before washing	295.1	101.0	786		23.0	
	290.2	99.0	723	754	21.2	22.1
2 washes	292.5	101.0	186		6.9	
	288.5	97.0	232	209	6.9	6.1
4 washes	294.0	100.7	95		2.8	
	288.3	99.0	80	88	2.3	2.6

Each result is the mean of 2 chromatographic injections; a.i. active ingredient.

flies were exposed into a tunnel [26]. The type of the tunnel and the diagram of the two tunnels used simultaneously to compare the anti-feeding effect of ITNs are shown in Fig. 1. Test batches of 50 female sandflies (some males have been introduced in the cages in order to stimulate the blood feeding behaviours of females) 3-day-old, sugar fed were used for each tunnel test. For each target dose two replicates were carried out by using about 1200 *P. papatasi* females. Flies were introduced into Cage A and C, and when released they had the possibility to fly respectively towards the window (16.5 x 18 cm), fitted with ITNs or with control net, attracted by the host (hamster) kept sedated into the tunnel (Fig. 1). To allow for any possible difference in attractiveness, the hamster position in the cages was inverted from one test to another. Insecticide-treated and control nets fitted in the ports were deliberately damaged by cutting 9 holes (Ø 1 cm). After 1 hour, flies passed through the nets were collected in cage B and D. Dead, fed and unfed females in the cages and those remaining into the tunnel have been scored. Tunnel test was carried out in dark condition, at 25-26 °C and 70-80% of relative humidity. Overall mortality rate was scored at 60 min and after 24 hours. The results are expressed and compared as protection of ITNs from the sandfly bites.

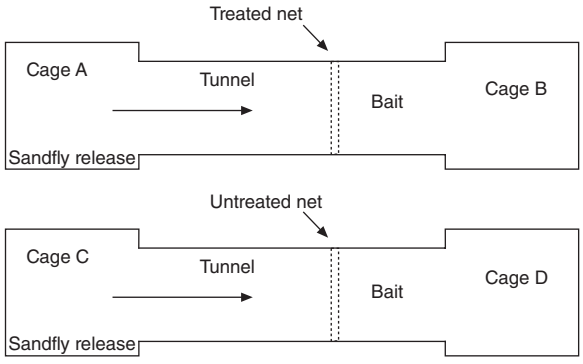

Fig. 1. - Diagram of the two tunnels used simultaneously for the paired test to evaluate blood-feeding rate of sandflies exposed to an animal source and separated by holed insecticide treated mosquito nets.

Results and discussion

Bioassay tests (Cones)

Mortality rate at 24 h is adjusted only for the net, impregnated at the dose Cyf-EW 25 mg a.i./m^2, being the control mortality rate higher than 5%. In general, toxic effect of Cyf-EW-ITNs was always higher than that showed by Del-SC-ITNs. In particular, the three tested doses of Cyf-EW caused knock down and mortality rates higher than 90%; the exposure to ITNs 50 mg a.i./m^2 killed all the insects within 24 hours. Del-SC-ITNs showed toxic values between 13.3 and 43.5%. Also after washing Cyf-EW treated net (50 mg a.i./m^2) provided higher activity than Del-SC (25 mg a.i./m^2), being mortality rates of 66.7 and 48.9% for Cyf-EW and 38.0 and 2.1% for Del-SC, respectively after 2 and 4 washes.

Table 2 shows knock down within 1 hour and mortality rates at 24 hours of *P. papatasi* females following 3 min exposure to each tested dose of Cyf-EW, before and after 2 and 4 washes compared with that of Del-SC. The toxic effect of Del-EW treated nets resulted significantly lower (p<0.01), both before and after washing, than that showed by Cyf-EW impregnated nets.

Sandflies exposed in the bioassay cones to the ITNs lost some of their legs before dying. Surviving sandflies able to fly with several legs missing, sometimes up to 4, were also commonly observed. This phenomenon of leg auto-section upon exposure to pyrethroids is likely to occur also in the field and the mutilated flies are unlikely to survive [17].

Feeding inhibition (Tunnels)

Table 3 shows feeding inhibition effect of Cyf-EW-ITNs at the dose of 50 mg a.i./m^2, before and after washing, with respect to both untreated net and Del-SC-ITNs (25 mg a.i./m^2), and knock down and mortality rates, in *P. papatasi* females exposed into a tunnel for a period of one hour.

Before washing. Nets treated with Cyf-EW performed effectively against blood feeding, compared to both untreated net (88.6%,) and Del-SC-ITNs (56.8%), while Del-SC showed low feeding inhibition (48.4%), although it was significant higher than that of untreated net.

After 2-4 washes. The content determination of Cyf-EW (50 mg a.i./m^2) and Del-SC (25 mg a.i./m^2) treated nets, before and after 2 and 4 washes, is presented in Table 1. The washing of Cyf-EW-ITNs produced a decrease of the feeding inhibition with a the reduction of 26.6% and 41.3% after 2 and 4 washes, respectively. Two washes of Del-SC-ITNs produced a significant decrease of anti-feeding (68.8%). Mortality rates within 24 hours of *P. papatasi* exposed to the target doses for 1 hour into the tunnels were slightly lower of that recorded in the bioassay tests with cones. The data show that efficacy of Del-SC (25 mg a.i./m^2) was less resistant to washing than Cyf-EW (50 mg a.i./m^2), suggesting that at lower doses also 2 washes would almost remove the insecticidal effect.

In conclusion, comparing the two pirethroids, cifluthrin was more effective than deltamethrin. These laboratory results if confirmed by field evaluation indicate cyfluthrin as good candidate for treatment of mosquito nets to be used against phlebotomine sandflies.

Table 2. - Comparative insecticidal activity of cyfluthrin (Cyf) *vs* deltamethrin (Del) treated nets against *P. papatasi*

Paired test (a.i./m^2)	Females tested ±		Knock down 1 h post-exposure			Mortality rates at 24 h		
	Cyf	Del	Cyf (%)	Del (%)	p*	Cyf (%)	Del (%)	p*
Before washing								
Cyf (12.5) *vs* Del (6.25)	45	45	71.1	8.9	<0.01	91.1	13.3	<0.01
Cyf (25) *vs* Del (12.5)	46	46	76.1	28.3	<0.01	95.3	30.4	<0.01
Cyf (50) *vs* Del (25)	45	46	100.0	30.4	<0.01	100.0	43.5	<0.01
After 2 washes								
Cyf (50) *vs* Del (25)	93	92	65.6	23.9	<0.01	66.7	38.0	<0.01
After 4 washes								
Cyf (50) *vs* Del (25)	90	93	37.8	1.1	<0.01	48.9	2.1	<0.01

±: An over total of 307 *P. papatasi* were used as control of the paired tests, by exposing the flies to untreated nets; *: knock down and mortality rates of Cyf-EW *vs* Del-SC were tested for significance by one-tailed Fisher's exact test; a.i.: active ingredient.

Table 3. - Feeding inhibition, knock down 1h post-exposure, and mortality rates within 24 h of holed ITNs (Cyf-EW 50 mg a.i./m^2), before and after washing, in *P. papatasi* females exposed into a tunnel for a period of one hour

Antifeeding and toxic effect of Cyf-EW 50 mg a.i./m^2	Cyf-EW 50 mg a.i./m^2				Del-SC 25 mg a.i./m^2			
	Tested	Control	(%)	*p**	Tested	Control	(%)	*p**
Before washing								
Feeding inhibition *vs* control	113	127	88.6	<0.01	81	82	48.4	<0.01
Feeding inhibition *vs* Del			56.8	<0.01			-	
Knock down			92.9				9.9	
Mortality			97.3				36.2	
After 2 washes								
Feeding inhibition *vs* control	78	100	65.7	<0.01	70	102	15.6	<0.01
Feeding inhibition *vs* Del			32.5	<0.01			-	
Knock down			44.9				15.7	
Mortality			44.9				24.3	
After 4 washes								
Feeding inhibition *vs* control	111	113	52.0	<0.01	124	77	9.8	n.s.
Feeding inhibition *vs* Del			60.8	<0.01			-	
Knock down			25.2				6.5	
Mortality			30.6				17.7	

* Values were tested for significance by one-tailed Fisher's exact test; ITNS: insecticide treated mosquito nets.
Cyf: cyfluthrin; Del: deltamethrin; a.i.: active ingredient.

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