

Experimental hut evaluation of Fendona 6SC[®]-treated bednets and Interceptor[®] long-lasting nets against *Anopheles gambiae s.l.* in Burkina Faso

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ABSTRACT

Background & objectives: Malaria prevention relies heavily on insecticide-treated bednets. Even though the benefits of bednets have been proven that in most of the studies carried out in Africa, their efficacy remains dependent on local conditions. In this study, under field conditions, we evaluated the efficacy of two LLINs (PermaNet[®] vs Interceptor[®]) and two bednet treatment kits (K-O TAB[®] vs Fendona 6SC[®]) against *Anopheles gambiae s.l.*

Methods: Bednets were evaluated using experimental huts in the village of Pissy located in the Saponé health district of Burkina Faso. Treatments and sleepers were randomly rotated between huts. Results are expressed in terms of induced exophily, mortality after 24 h and blood-feeding inhibition.

Results: A total of 1392 *An. gambiae s.l.* mosquitoes were collected during 120 nights in the experimental huts. The overall mortality rates were 85.4% (CL: 79.7–91.4) and 77.5% (CL: 56.9–97.3) for PermaNet[®] and Interceptor[®], respectively. For the conventionally treated bednets, the mortality was 78.2% (CL: 63.13–96.7) with the Fendona 6SC[®]-treated nets and 75.5% (CL: 61.2–93) with the K-O TAB[®]-treated nets. The proportion of blood-fed mosquitoes was significantly higher in the untreated bednet arm than in the treated one, as well as for long-lasting nets than for conventionally treated nets. The entry rate did not vary significantly according to the bednet type, but the treated bednets increased the level of exophily by at least 43%.

Conclusion: In the field, the Fendona 6SC[®] kit and the Interceptor bednets showed comparable efficacy to the already used K-O TAB[®] kit and PermaNet[®] bednets. These results could help National Malaria Program managers to formulate appropriate policy for effective vector control.

Key words *Anopheles gambiae*; bednet efficacy; experimental hut; Fendona, Interceptor[®]; PermaNet[®]

INTRODUCTION

Malaria remains a serious public health problem, with most fatalities occurring in pregnant women and children under five years old. It is estimated that there were 225 million cases and 781,000 deaths due to malaria in 2009¹. Since no effective malarial vaccine exists, prevention remains the best means of protection. The effectiveness of insecticide-treated nets (ITNs) has been demonstrated in most of the studies carried out in sub-Saharan Africa^{2–5}. The use of ITNs has yielded interesting results, including a reduction in malaria morbidity and mortality correlated with a reduction in vector biting rates and parasite inoculation rates^{6–10}. These results have reinforced the WHO’s strategy of ITNs utilization for malaria prevention in the last 10 yr¹¹.

One of the objectives of the Summit of Heads of African States held at Abuja in April 2000 as part of the

“Roll Back Malaria” campaign was to achieve coverage of 60% of vulnerable populations at risk for malaria by the year 2005¹². In spite of some progress, with increasing ITNs distribution in more than 14 African countries, this objective has not been reached in most of sub-Saharan African countries¹³. The 58th World Health Assembly has recently set loftier targets, namely that 80% of those at risk of or suffering from malaria benefits from major preventive and curative interventions by the end of 2015¹⁴. Of 18 African countries surveyed, ITNs coverage was far below the 80% target: only 34% of households owned an ITN, and 23% of children <5 yr and 27% of pregnant women slept under an ITN¹⁵. Recent studies, however, have demonstrated increasing bednet ownership and utilization among children <5 yr and pregnant women^{13,16} and the increasing general coverage among populations¹⁷.

The lack of availability of bednets and insecticides,

their cost, and the inadequacy of bednet treatment and re-treatment to maintain their efficacy are the most critical constraints¹⁸.

In Burkina Faso, impregnation of mosquito nets has been restricted to health structures where impregnation sites are set up, which are sometimes far from where populations reside¹⁸. One potential way to overcome this limitation is to use long-lasting nets and “dip it yourself” insecticide kits to enable people to treat their own bed nets¹⁹. Some manufacturers have developed more durable insecticide-treated bednets, including the Olyset Net[®], with permethrin incorporated in to polyethylene²⁰, the PermaNet[®] (polyester with wash-proof deltamethrin treatment) and the Interceptor[®] (treated with alpha-cypermethrin). These long-lasting bednets are recommended for use by WHOPES²¹. Such long-lasting insecticidal wash-resistant mosquito nets should be biologically active throughout the average life expectancy of the net.

Tools to complement these approaches are impregnation of kits, like K-O TAB[®] by Bayer²² and the new kit Fendona 6SC[®] from BASF, to treat bednets at home. These impregnation kits could also increase the rate of bednet re-treatment in cases where untreated bednets are in use.

In this study, we investigated the effectiveness of the Fendona 6SC[®] impregnation kit compared with the K-O TAB[®] kit, and the long-lasting Interceptor[®] bednet compared with the PermaNet[®], in the field experimental huts. We evaluated immediate mortality, mortality after 24 h, deterrence effect and blood-feeding inhibition of the malaria vectors, using treated bednets and long-lasting bednets.

MATERIAL & METHODS

Substrates and treatments

The Interceptor[®] bednet is manufactured by Sunshine World Net 2003 Company Ltd. (Ratchaburi, Thailand), under license of BASF Agro B.V. Arnhem (NL) (Wädenswil Branch, BP 69, CH-8820 Wädenswil, Switzerland), and distributed by BASF²³. This bednet has the following characteristics: 100% polyester, multifilament yarn: 75 denier, mesh: 25 holes/cm²; density: 30 g/m²; and active ingredient: 200 mg/m² alpha-cypermethrin-coated polyester fibers. The nets used were 1.8 m long, 1.6 m wide and 1.5 m high, with a total surface area of 13.92 m².

The PermaNet[®] is manufactured by Vestergaard-Frandsen Company with deltamethrin 55 mg a.i./m²-coated polyester fibers²⁴. PermaNet[®] is made of 100%

polyester, multifilament yarn: 75 denier, mesh: 25 holes/cm². The nets used were 1.8 m long, 1.6 m wide and 1.5 m high, with a total surface area of 13.92 m².

The netting used in this study for the control treatment and hand insecticide-treated bednets was made by Siam Dutch Netting Company (Bangkok, Thailand), and is made of 100% polyester, 100 denier, with mesh of 156 holes/cm² (mesh size of 1.5 mm) and white in color. The nets used were 1.8 m long, 1.6 m wide and 2 m high, with a total surface area of 16.48 m².

The insecticides used for bednet dipping were alpha-cypermethrin ‘Fendona 6SC[®]’ provided by BASF and deltamethrin ‘K-O TAB[®]’ provided by the National Malaria Control Program of Burkina Faso. Bednets were treated at the target doses of 40 mg/m² with alpha-cypermethrin and 25 mg/m² with deltamethrin (according to WHO target doses), taking into account the uptake of liquid after dipping and wringing the net. Each net was dipped in an insecticide mixture and dried horizontally. Six holes (4 × 4 cm) were made on each bednet, two holes on the large side and one on each of the other sides, to assess the mosquito blood-feeding inhibition and to simulate the conditions under which the bednets are usually used in the community.

Six treatments used were: (i) Fendona 6SC[®]-treated bednet; (ii) K-O TAB[®]-treated bednet; (iii) Interceptor[®] bednet; (iv) PermaNet[®] bednet; (v) Untreated bednet; and (vi) Interceptor[®] net washed 20 times. Five replicate nets were tested for each type of treatment for a total of 30 nets.

Bednet washing procedure

Nets were washed in 10 litres of well-water using 20 g of soap (“Savon de Marseille”) and manual agitation for 10 min at approximately 20 rotations per min. Nets were thoroughly rinsed twice in fresh water and dried horizontally in the shade. The nets were stored at ambient temperature between washes and put in plastic sachets for the field.

Study area and mosquito populations

The trial was carried out in six experimental huts built in Pissy village, Saponé Health district. This area is located about 45 km from the City of Ouagadougou. This area is in the Sudano-Sahelian climate domain with annual rainfall of 500–900 mm. The main malaria vectors are *Anopheles gambiae* complex mosquitoes, namely *An. arabiensis* and *An. gambiae s.s.* (Diptera: Culicidae) which are in sympatry and ensure the transmission of *P. falciparum* mainly during the rainy season. *An. funestus*, a third vector, extends transmission to the end of the rainy

season, when populations of *Anopheles gambiae s.l.* are in decline.

Experimental station

The field station is made of six standardized experimental huts situated near an artificial water dam. Each hut is 2.5 m long, 1.75 m wide and 2 m high²⁵. The walls are made of cement bricks, the floor is made of cement and the roof is made of corrugated iron sheets. A plastic cover is stretched under the roofing sheets to facilitate hand catching of mosquitoes. A water-filled channel to prevent entry of ants surrounds each hut. Entry of mosquitoes is only allowed through four window slits (1 cm wide) located on three sides of the hut, the slits being designed to prevent mosquitoes from escaping once they have entered the hut. Each hut is equipped with a *verandah* trap located on the fourth side, made of plastic sheeting and screening mesh.

Sleepers

During the trial period for 120 nights (5 months, from July to November 2007), six adult men, 18 to 25 years old, from the village of Pissy, slept under the nets in the experimental huts every night from 2000 to 0500 hrs. To avoid any bias due to differences in the sleepers' attractiveness to mosquitoes, they were rotated between huts on successive nights.

Experimental protocol

The treatments were organized in Latin squares (huts × weeks). The sequence of the sleepers and the treatment rotation were randomized between the huts using Excel "randbetween" function.

Every morning, four types of collection were carried out using a mouth aspirator: (i) live and dead mosquitoes were collected under the bednet; (ii) resting (alive) mosquitoes were collected from the ceiling, wall and floor; (iii) dead mosquitoes (outside the bednet) were collected; and (iv) live and dead mosquitoes were collected from the *verandah* exit trap.

Live females were held in netted paper cups, supplied with sugar solution, and transferred to the insectary for delayed mortality recording and for specimen processing. Collected mosquitoes were identified morphologically using the keys of Edwards²⁶ for culicines, and Gillies & Coetzee²⁷ for anophelines. Mosquitoes were stored in test tubes containing a desiccant (silica gel), labeled according to the net treatment, mosquito species and gonotrophic stages. The huts were cleaned, the walls and the ground were washed with water and the huts were ventilated during the day after each week of experiments

to avoid any contamination.

Ethical clearance

Treatment product informational inserts indicated that these insecticides could be toxic to aquatic organisms, but the risk of contaminating study participants was nil because the quantity of treatment solution used equalled only the amount that a bednet could absorb, but was less than a toxic dose. We requested and obtained written and signed informed consent from sleepers prior to their inclusion in the study. The informed consent procedure provided all the information concerning the study and evaluation process in the local language. The participants voluntarily agreeing to take part in the study, received a vaccination against yellow fever and were followed clinically to detect any sign of fever during and for two weeks after the evaluation had concluded. The National Ethics Committee for Health Research in Burkina Faso approved the study protocol.

Statistical analysis

The collected data were managed with File Maker Pro and the analysis was carried out with XLStat. The effect of each treatment was assessed relative to the control by using the following parameters:

- The exophily rate (ER) is estimated as the ratio in percent of the number of mosquitoes collected in the *verandah* entry trap by the total number of mosquitoes collected in the hut, under bednet and *verandah*.

$$ER = N_v/N_t \times 100$$
(N_v is the number of mosquito collected in the *verandah* trap and N_t is the total number in the hut including *verandah* and under bednet).
- The induce exophily (IE) corresponds to the increasing of exophily in the treatment of hut compared to the control.

$$IER (\%) = (N_{vt} - N_{vc})/E_t \times 100$$
(N_{vt} and N_{vc} are respectively the number of mosquitoes collected in the *verandah* under treatment and control).
- Blood feed rate is the ratio (in %) of the number of blood-fed mosquitoes collected in the hut (under bednet and *verandah* included) out of the total number of mosquito collected in the hut (under bednet and *verandah* included).

$$BFR (\%) = (BF/N_t) \times 100$$
- Blood-feeding inhibition (BFI) corresponds to the reduction in blood feeding in the treatment house compared to the control huts.

$$BFI (\%) = (BF_c \times BF_t)/BF_c \times 100$$
(BF_c and BF_t are the number of blood-fed mosquitoes collected

respectively control and treatment huts).

- Overall mortality calculated for each treatment, corresponds to the proportion of mosquitoes dead (included immediate dead and delayed dead).

$M (\%) = Dt/Nt \times 100$ (Dt and Nt correspond to the total number of dead mosquitoes found and the total number of mosquitoes).

- The immediate mortality (IM) is the proportion of mosquitoes found dead in the morning hour during the collection (hut, under bednet and *verandah* included).

$IM (\%) = (ID/Nt) \times 100$ (ID and Nt are respectively the total number of dead mosquitoes and Nt the total number of mosquitoes collected in the hut (under bednet and *verandah* included).

- Delayed mortality corresponds to the proportion of mosquitoes dead 24 h later, from alive collected mosquitoes.

$DM (\%) = ND_{24}/NT_{24} \times 100$ (ND_{24} is the number of dead mosquitoes after 24 h, and NT_{24} is the number of alive mosquitoes collected).

- Non-blood-fed mortality (NBFM) corresponds to the proportion of unfed mosquitoes found dead in the hut (under bednet and *verandah* included).

$NBFM (\%) = (Nbfd/Nt) \times 100$ (Nbfd is the number of non-blood-fed mosquitoes and Nt is the total number of collected mosquitoes).

The confidence limits of these parameters were calculated and the proportions were compared using a chi-square test with a significance limit of 0.05.

RESULTS

Mosquito abundance in experimental huts

A total of 2265 mosquitoes, belonging to different

genera and species, were collected during 120 nights of the study period in experimental huts (Table 1). Anophelines formed 68.9% of the total catch, and mosquitoes of the genera *Aedes*, *Culex* and *Mansonia* made up the remaining 31.1%. Among the anophelines, 1392 (61.5% of the total) belonged to the *An. gambiae* complex. *An. rufipes* was the second most abundant species with 107 (4.7%) specimens collected, followed by *An. funestus*, with 60 (2.6%) specimens. The remaining collected mosquitoes were culicines (639 mosquitoes, 28.1%), aedines (59 mosquitoes, 2.6%) and *Ma. uniformis* (8 mosquitoes, 0.4%). The distribution of the mosquitoes according to the treatment was not statistically significant ($p > 0.05$).

Dynamics of mosquito population

The density of the mosquitoes collected during the study per week collection according to the mosquito species is summarized in Fig. 1. Density of all the mosqui-

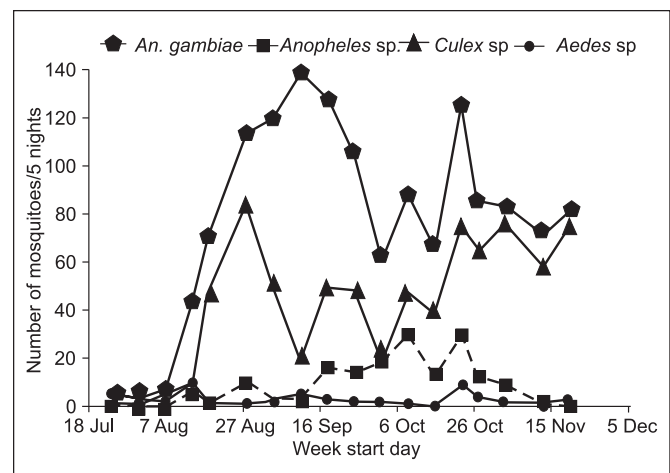


Fig. 1: Dynamics of mosquitoes collected per week in the huts. The mosquitoes collected during five day age pooled according to their species or genes.

Table 1. Number and total proportion of mosquitoes collected in experimental huts according to their species and bednet type

Mosquito species	Treatments						Proportion (%)
	Control	Fendona 6SC®	K-O TAB®	Interceptor®	PermaNet®	Interceptor® 20 ⁺	
<i>Anopheles gambiae</i>	210	270	242	241	232	197	58.12
<i>Anopheles funestus</i>	9	6	9	15	11	10	2.51
<i>Anopheles rufipes</i>	10	15	19	19	30	14	4.47
<i>Culex decens</i>	75	167	128	102	84	80	26.56
<i>Culex quinquefasciatus</i>	0	1	1	1	0	0	0.13
<i>Culex nebulosus</i>	11	13	9	12	14	10	2.88
Other <i>Culex</i> species	1	13	25	8	11	5	2.63
<i>Aedes aegypti</i>	1	1	2	1	3	3	0.46
<i>Aedes</i> sp	8	5	9	1	9	14	1.92
<i>Mansonia uniformis</i>	0	0	1	2	0	5	0.33

⁺Interceptor net after 20 washes.

toes increased from July with the beginning of the rainy season to a peak in August for *An. gambiae s.l.* and other anophelines (138 and 16 mosquitoes, respectively), which corresponds to the peak of rainfall in the locality. For *Culex* sp., a density peak of 84 was in August. The densities decreased from the middle of September and there was another peak at the end of October, which corresponds to the end of the rainy season and then density decreased until the end of the study.

Mortality rate

To comply with the study objectives, only the *An. gambiae* complex mosquito data were analyzed. The overall mortality rates of the hut-collected mosquitoes (Table 2) were 85.4, 77.5 and 77.7% for the PermaNet[®], unwashed and 20 times washed Interceptor[®] nets respectively. The differences between these three treated nets were not statistically significant ($\chi^2=2.6$, $p > 0.1$). The overall mortality in the control hut was 13.3%, significantly lower than that for the treated bednets. The mortality of the non-blood-fed mosquitoes was higher in the Interceptor[®] net than in the PermaNet[®]. The immediate mortality followed the same trend as the overall mortality and the difference was not statistically significant.

The overall mortality was 78.2% with the Fendona 6SC[®]-treated nets and 75.5% with the K-O TAB[®]-treated nets (Table 2). There was no significant difference between the two treated nets, but the treated nets killed significantly more mosquitoes than the untreated nets.

Blood feeding

The blood-fed mosquito proportions were significantly higher in the untreated net arm than in the treated

net arms (Table 2): 74.29%, in untreated net vs PermaNet[®], 49.4%; untreated vs Interceptor[®], 54.7%; untreated vs Interceptor[®] after 20 washes, 56.8%. The differences between the three types of treated bednets were not statistically significant. The reductions in blood-feeding seen in the treated nets compared with that in the untreated nets were 33.5, 26.4 and 23.5% for PermaNet[®], Interceptor[®] and Interceptor[®] after 20 washes, respectively. The long-lasting nets, including the 20 x-washed Interceptor[®], significantly reduced mosquito blood-feeding relative to the untreated bednets.

The blood-feeding rates for the Fendona 6SC[®]-treated nets and the K-O TAB[®]-treated nets (Table 2) were 51.8 and 54.3%, respectively. The reductions in terms of blood-feeding compared with the control were 30.3 and 26.9% for the Fendona 6SC[®]-treated nets and the K-O TAB[®]-treated nets, respectively. The treated bednets significantly reduced mosquito blood-feeding compared with the untreated nets.

Entry rate

The total number of *An. gambiae* mosquitoes entering the hut (collected in the hut, on the floor and under the bednets) (Table 2) did not vary significantly according to the type of treatment of the nets. The Fendona 6SC[®]-treated nets and K-O TAB[®]-treated nets did not reduce the mosquito entry rate compared with the untreated nets.

Induced exophily

The exophily rates were 43.3, 60.8, 63.8 and 54.8% for the untreated bednets, PermaNet[®], Interceptor[®] and 20 x-washed Interceptor[®], respectively (Table 2). The long-lasting nets of PermaNet[®], Interceptor[®] and 20x-

Table 2. Number and percentage of *Anopheles gambiae s.l.* collected in experimental huts

	Treatments					
	Control	Fendona 6SC [®]	K-O TAB [®]	PermaNet [®]	Interceptor [®]	Interceptor [®] 20 ⁺
Total	210	270	242	241	232	197
Exophily (%)	43.3 ^a	59.5 ^b	64.7 ^b	60.8 ^a	63.8 ^a	54.8 ^a
95% confidence limits	(36.5–49.5)	(51.7–68.4)	(48.1–86.9)	(59.3–63.9)	(55.4–78.7)	(45.3–71.4)
Induced exophily (%)	–	37.4	25.6	40.4	47.3	26.6
Blood-fed (%)	74.29 ^a	51.8 ^b	54.3 ^b	49.4 ^b	54.7 ^b	56.8 ^b
95% confidence limits	(67.7–80.3)	(24.6–76.2)	(39.9–73.8)	(38.8–57.8)	(38.4–57.8)	(40.7–67.4)
Blood-feeding inhibition (%)	–	30.3	26.9	33.5	26.4	23.5
Overall mortality (%)	13.3 ^a	78.2 ^b	75.5 ^b	85.4 ^b	77.5 ^b	77.7 ^b
95% confidence limits	(4.1–35.4)	(63.13–96.7)	(61.2–93)	(79.7–91.4)	(56.9–97.3)	(61.41–93.5)
Immediate mortality (%)	4.3	75.9	73.7	78	81.9	71.6
Delayed mortality (%)	9	73.1	5.3	6.9	4.3	14.2
Non-blood-fed mortality	12.8	90	80.3	85.1	88.2	86.6

Numbers on the same line sharing the same superscript letter are not statistically different; ⁺Interceptor net after 20 washes.

washed Interceptor[®] increased exophily by approximately 40, 47 and 27%, respectively, compared with the control untreated net, but the difference was not statistically significant.

The exophily rates were 59.5 and 64.7% for Fendona 6SC[®]-treated nets and K-O TAB[®]-treated nets, respectively. The Fendona 6SC[®]-treated nets and the K-O TAB[®]-treated nets significantly increased exophily by about 37.4 and 25.6%, respectively, compared with the untreated bednets.

DISCUSSION

In the present study, the Fendona 6SC[®] kit for bednet dipping, containing alpha-cypermethrin, and the Interceptor[®] bednets were evaluated in experimental huts in the field for their efficacy against *An. gambiae s.l.* malaria vectors, and compared with the efficacy of the well-known K-O TAB[®] and PermaNet[®].

Most of the studies carried out on PermaNet[®] bednets in the laboratory as well as in the field have demonstrated their efficacy against *An. gambiae s.l.* and other malaria vectors, even after as many as 20 washes^{28–36}; in contrast, the effects of Interceptor[®] bednets have yet to be well-documented^{37–39}. In our study, the long-lasting nets, Interceptor[®] and PermaNet[®], performed equally well against *An. gambiae s.l.* and both performed significantly better than the untreated bednets in terms of induced exophily, mortality after 24 h and blood-feeding inhibition. The 20×-washed Interceptor[®] performed effectively as well as the unwashed Interceptor[®] and PermaNet[®]. The blood-feeding rate and blood-feeding inhibition values obtained in the present study are higher than those of Graham *et al*²⁸ and Asidi *et al*³⁵, but are consistent with the findings of Dabire *et al*³³. Our exophily rate values are higher than those of Asidi *et al*³⁵. The overall mortality was higher than those of Dabire *et al*³³ and Asidi *et al*³⁵, which was likely due to resistance to pyrethroid insecticide in the areas where these researchers worked, however, our data were consistent with the findings of Graham *et al*²⁸.

Regarding the conventionally treated nets, the K-O TAB[®] treatment kit has demonstrated high efficacy against anopheline mosquitoes when used for bednet treatment^{22, 28, 29, 40}. The Fendona 6SC[®] has not been evaluated in terms of the kit formula, but the alpha-cypermethrin-treated bednets have been found to be efficacious^{35, 41}. In our results, there was no difference between Fendona 6SC[®]-treated bednets and K-O TAB[®]-treated bednets in terms of induced exophily, indicating that the repellent effects of these two treatments are

equivalent, but significantly higher than those of the untreated bednets. The induced exophily values for the K-O TAB[®] insecticide are lower than those obtained by Malima *et al*⁴¹ and higher than the results obtained by Darriet *et al*⁴² with deltamethrin-treated bednets. The blood-feeding inhibition was also equivalent for these two treatments, but was significantly higher than that induced by the untreated bednets. Our blood-feeding proportions are higher than those of reported by Darriet *et al*⁴², Malima *et al*⁴¹ and Graham *et al*²⁸. The overall mortality was equivalent for the Fendona 6SC[®]- and the K-O TAB[®]-treated bednets, but these were significantly higher for the untreated bednets. The mortality rates are consistent with the findings of Malima *et al*⁴¹ and Graham *et al*²⁸, but lower than those obtained by Darriet *et al*⁴² and Asidi *et al*³⁵.

This study has shown that the Interceptor[®] and PermaNet[®] bednets had comparable efficacy against *An. gambiae s.l.* malaria vectors in experimental hut evaluation, as did as the two insecticides kits K-O TAB[®] and Fendona 6SC[®] used to treat bednets. A recent international study⁴³ has highlighted the overall progress of bednet coverage. From 2000 to 2007, the mean bednet coverage progressed from 1.3 to 18.5%, but 89.6 million children are still unprotected. Lines and Addington⁴⁴ attributed this progress largely to the competition in terms of bednet production and distribution, which has reduced the retail price in rural markets.

CONCLUSION

In Burkina Faso, impregnation of mosquito nets has been restricted to sites located close to health centers; this situation has made impregnation relatively inaccessible to rural populations¹⁸. The availability of impregnation kits like K-O TAB[®] and Fendona 6SC[®] in the market could be an opportunity for many users to impregnate their nets by themselves. Most of the bednets recommended by the WHO are in the market in Burkina Faso, so the availability of such impregnation kits could increase the availability of bednets on the market and subsequently increase the bednet coverage rate.

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