Pyrethrum: A Mixture of Natural Pyrethrins Has Potential for Malaria Vector Control

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ABSTRACT Pyrethrum is a natural mixture of six insecticidal esters, recognized for low mammalian toxicity and limited persistence in the environment. In this study, World Health Organization standard bioassays were used to evaluate the performance of pyrethrum against both susceptible and pyrethroid-resistant *Anopheles gambiae* s.s. The results showed that the intrinsic activity of pyrethrum was similar to that of permethrin but lower than that of deltamethrin against susceptible mosquitoes. However, pyrethrum was less affected by the presence of the *kdr* mutation than synthetic pyrethroids (with lower resistance ratios) and showed good knock-down effect, repellency, and blood-feeding inhibition against the pyrethroid-resistant strain. In laboratory condition, mosquito nets treated with 500–1,000 mg/m^2 (pyrethrum) remained effective, i.e., >80% mortality and/or >95% K_D effect, for 9 mo. Conversely, the efficacy and residual activity of pyrethrum (Pynet 5% EC) on substrates was not conclusive, especially concerning mud, which is a porous substrate (mortality <80% after 3 mo at 2 g/m^2). These findings suggested that pyrethrum may be a potential alternative candidate for the impregnation of mosquito nets and textiles in areas where resistance to pyrethroids has become problematic.

KEY WORDS *Anopheles gambiae*, pyrethrum, inseticide-treated nets, indoor residual spraying, pyrethroid resistance

Currently, long-lasting insecticidal nets (LLINs) are one of the two main interventions (other equally promoted is indoor residual spraying [IRS]) of the World Health Organization (WHO) global strategy for malaria control (WHO 2005). Unfortunately, pyrethroid resistance caused by *kdr* mutations (Chandre et al. 1999, Ranson et al. 2000) and/or increased detoxification through higher metabolic activity (Etang et al. 2006, Corbel et al. 2007) has become widespread for *An. gambiae* in Africa and may impede the efficacy of vector control operations (N′Guessan et al. 2007). Few effective and safe insecticides are proposed as alternatives to pyrethroids for use in mosquito control, especially for the impregnation of mosquito nets. Pyrethrum commercialized by the Pyrethrum Board of Kenya is a natural mixture of six organic esters derived from the flowers of a plant in the genus *Chrysanthemum*, which belongs to the family Compositae. The combination of these components accounts for the kill and knockdown properties of pyrethrum extract. Pyrethrum is recognized for low mammalian toxicity (Sattelle and Yamamoto 1988) and its nonpersistence in the environment (Katsuda 1999). It rapidly knocks down and kills a wide variety of insect pests, such as cockroaches, mosquitoes, fleas, and houseflies, for example (Silcox and Roth 1994). As is true for synthetic pyrethroids, pyrethrum targets the voltage-gated sodium channels of neuronal membranes, inducing paralysis and death of the insect (Salgado et al. 1983, Sattelle and Yamamoto 1988). Despite its long history of use, very few cases of specific resistance to pyrethrum have been described, and these have mainly arisen as a result of cross-tolerance conferred by the insects developing resistance to other insecticides (Cochran 1995). The fact that pyrethrum may have a different profile from synthetic products in terms of patterns of cross-resistance may be of great interest for controlling pyrethroid-resistant *An. gambiae* in field situations.

In this study, we characterized, under standardized laboratory conditions, the performance of pyrethrum-based products (technical grade and an EC formulation) against susceptible and *kdr*-resistant strains of *An. gambiae* in comparison with standard pyrethroids (i.e., deltamethrin and permethrin). We first established the dose/response relationships of pyrethrum

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Table 1. Efficacy of pyrethrum against susceptible (SS) and pyrethroid-resistant (*kdr*) mosquitoes of *An. gambiae* by topical application

Strain	Slope (±SD)	LD_{50} ng/mg female (95% CI)	LD_{95} ng/mg female (95% CI)	RR_{50} (95% CI)	RR_{95} (95% CI)
SS	2.1 (±0.1)	1.9 (1.6–2.1)	11.5 (9.2–15.2)	—	—
Kdr	3.1 (±0.2)	13.0 (11.8–14.3)	44.1 (37.1–55.2)	5.4[a] (4.7–6.2)	3.0[a] (2.3–3.9)

[a] Significantly different from 1.0 ($P < 0.05$).

by topical applications and tarsal contact to deposits on filter papers and estimated the discriminating dosage (DD) of the compound. The efficacy, repellency, and residual activity of a pyrethrum-based formulation (Pynet 5% EC) were also studied on different substrates (mud, wood, cement) and netting materials.

Materials and Methods

Mosquito Strains. Two laboratory strains of *An. gambiae* were used. The reference susceptible strains of *An. gambiae* (Kisumu), originating from Kenya, are free of any detectable insecticide resistance mechanism. The pyrethroid-resistant strain (VKPR), originating from the Kou Valley in Burkina Faso, was already resistant to pyrethroids when it was collected in the field and has been exposed to constant pyrethroid selection in the laboratory until it became homozygous for the *knock down* mutation (Darriet et al. 1997). Resistant and susceptible strains were checked every 3 mo for resistance status and S and R genotype.

Insecticides and Substrates. Bioassays were made with technical grade and formulated product of pyrethrum. The active ingredient (purity 25%) and formulation (Pynet 5% EC) were provided by the Pyrethrum Board of Kenya (Nakuru, Kenya). Deltamethrin (100%) and Permethrin *cis:trans* 25:75 (94.6%) were provided by Agrevo (Berkhamsted, UK). The nets used for impregnation were white polyester multifilament, 100 denier, mesh 156, from Siam-Dutch Mosquito Netting Co (Bangkok, Thailand).

Bioassay Procedures. All bioassay procedures used in this study are fully described in the *WHO guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets* (WHO 2006). The laboratory conditions used were the same for all tests (temperature, 27°C; 80% RH).

Topical Application. Topical application allows an estimation of the intrinsic activity of a pesticide on a given pest species (WHO 2006). Technical grade pyrethrum was tested against both susceptible and *kdr*-resistant strains of *An. gambiae.* A volume of 0.1 μl of insecticide solution at the required concentration was applied on the pronotum of each female using a microcapillary. Females treated with pure acetone served as controls. Mortality rates were recorded 24 h after exposure. Data were analyzed by the log-probit method of Finney (1971) using Probit software (Raymond et al. 1997). They were expressed in nanograms of insecticide per milligram of body weight. Results were corrected by the formula of Abbott (1925) in the case of control mortality >5%.

Tarsal Contact with Treated Filter Papers. Tarsal contact tests were run using filter papers treated with technical grade pyrethrum. Filter papers were treated using acetone solutions of insecticide and silicone oil as the carrier (WHO 2006). Mortality was recorded 24 h after exposure, corrected by the formula of Abbott if necessary (Abbott 1925), and data were analyzed by Probit software (Raymond et al. 1997). Filter papers impregnated with pyrethrum, permethrin, and deltamethrin, at the WHO discriminating dosage, were tested to assess the mortality rates after 24 h, the KDT_{50} and KDT_{95} (time after which 50 and 95% of mosquitoes were knocked-down, respectively), and their 95% CL.

Net and Substrate Impregnations. Netting material (25 by 25 cm) was impregnated with the Pynet EC 5% formulation, which was diluted in deionized water to the required concentration. The size required for hard substrate samples was 12 by 12 cm to fit with the larger diameter of the standard WHO cone. Cement, mud, and wood were added to petri dishes. Once the substrates dried, they were treated by spraying a precise homogeneous residual film of the EC formulation on the substrate using a pressurized handled-sprayer (model Berthout F2; Berthout, France). All samples (nettings and substrates) were stored in climatic chamber at 27 ± 2°C, 80 ± 10% RH, and 12/12 light/dark until use.

Table 2. Comparative insecticidal activity of pyrethrum, permethrin, and deltamethrin against susceptible (SS) and pyrethroid-resistant (*kdr*) mosquitoes of *An. gambiae* using treated filter paper (expressed in wt:wt percentage of active ingredient in silicone oil)

Insecticide	Strain	Slope (±SD)	LD_{50} (%) (95% CI)	LD_{95} (%) (95% CI)	RR_{50} (95% CI)	RR_{95} (95% CI)
Pyrethrum	SS	3.6 (±0.15)	0.10 (0.09–0.11)	0.29 (0.27–0.32)	—	—
	Kdr	3.3 (±0.14)	0.48 (0.46–0.51)	1.54 (1.41–1.7)	4.8 (4.3–5.3)	[a]
Permethrin	SS	3.7 (±0.17)	0.078 (0.075–0.083)	0.22 (0.2–0.24)	—	—
	Kdr	4.7 (±0.29)	0.74 (0.7–0.77)	1.66 (1.52–1.85)	9.4 (8.3–10.6)	[a]
Deltamethrin	SS	4.0 (±0.21)	0.0008 (0.00075–0.00083)	0.002 (0.0018–0.0022)	—	—
	Kdr	2.0 (±0.16)	0.043 (0.038–0.049)	0.27 (0.20–0.42)	55.1 (49.6–61.1)	138 (106–180)

[a] RR_{95} not statistically different from RR_{50} because regression lines for susceptible and resistant strains are parallel ($P > 0.05$).

Table 3. Mortality and knock down effect of pyrethrum, permethrin, and deltamethrin, at the WHO DDs, against susceptible (*SS*) and resistant (*kdr*) *An. gambiae*

Insecticide	Strain	*N*	Mortality	KDT_{50} (min) (95% CI)	KDT_{95} (min) (95% CI)
Pyrethrum. (1%)	SS	300	100%	3.6 (2.9–4.3)	7.0 (5.4–9.1)
	Kdr	307	90%	11.7 (11.3–11.9)	19.9 (19.0–21.0)
Permethrin (0.75%)	SS	300	100%	15.8 (15.3–16.2)	26.1 (25.0–27.3)
	Kdr	300	72%	57.9 (56.0–60.3)	99.3 (90.8–111.6)
Deltamethrin (0.05%)	SS	202	100%	14.2 (13.4–15.5)	21.4 (17.9–25.6)
	Kdr	303	80%	29.0 (27.3–30.8)	45.7 (41.6–50.2)

WHO Cone Test. To evaluate the efficacy of the EC formulation of pyrethrum, 50 non–blood-fed females 2–5 d of age were exposed for 3 min to nets (25 by 25 cm) impregnated with increasing concentrations of the insecticide (WHO 2006). Females exposed to untreated nets and deltamethrin-treated nets (25 mg/m^2) were used as negative and positive controls, respectively. Two net samples were used for each test, and results were pooled for analysis (50 by 2 = 100 mosquitoes). Knock down was measured 60 min after exposure and mortality after 24 h. To evaluate the EC formulation of pyrethrum on cement, wood, and mud, 45 susceptible non–blood-fed females 2–5 d of age were exposed to each substrate for 30 min. Two replicates were used for each test, and results were pooled for analysis (45 by 2 = 90 mosquitoes). Knock down was measured 60 min after exposure and mortality after 24 h.

Tunnel Tests. Mortality and blood feeding inhibition induced by pyrethrum treated nets were evaluated using a tunnel apparatus (WHO 2006). The system is composed of a square glass cylinder, 25 cm high, 25 cm wide, and 60 cm long, with a square of netting with nine 1-cm-diameter holes fixed into a frame that slots across the tunnel, dividing it into two chambers. A guinea pig is housed in the bait chamber unconstrained in a cage, and in the other chamber, 100 unfed female mosquitoes 5–8 d of age were released at dusk and left overnight in the dark. The following morning, the numbers of mosquitoes found alive or dead and fed or unfed in each compartment were scored.

Results

Intrinsic Activity of Pyrethrum Against SS and kdr *An. gambiae*. The LD_{50} and LD_{95} of pyrethrum determined by topical applications were 1.9 and 11.5 ng/mg of female body weight on SS mosquitoes and 13.0 and 44.1 ng/mg on pyrethroid-resistant mosquitoes, respectively (Table 1). The slopes of the regression lines were low and significantly different between SS and *kdr* mosquitoes (95% confidence intervals do not overlap, $P < 0.05$). Mortality was significantly lower for resistant individuals than susceptible ones (RR_{50} and RR_{95} were 5.4 and 3.0, respectively).

With tarsal contact, pyrethrum and permethrin acted in a similar range of concentrations against SS mosquitoes (LD_{50} were 0.10 and 0.078%, respectively), whereas deltamethrin acted at far lower concentrations (LD_{50} was 0.0008%; Table 2). Considering the observed LD_{100} of pyrethrum on susceptible mosquitoes (0.5%), the diagnostic dosage (DD) for *An. gambiae* should be 1%. Interestingly, the level of cross-resistance was lower for pyrethrum (RR_{50} = 4.8) and, to a lesser extent, for permethrin (RR_{50} = 9.4) than it was for deltamethrin (RR_{50} = 55).

Mortality and the K_D effect of pyrethrum, permethrin, and deltamethrin, at the WHO DD, against SS and RR mosquitoes are shown in Table 3. All pyrethroids induced 100% mortality for SS individuals, whereas mortality decreased to 90, 80, and 72% for *kdr* mosquitoes with pyrethrum, deltamethrin, and permethrin, respectively. Results showed that the KDT_{50}

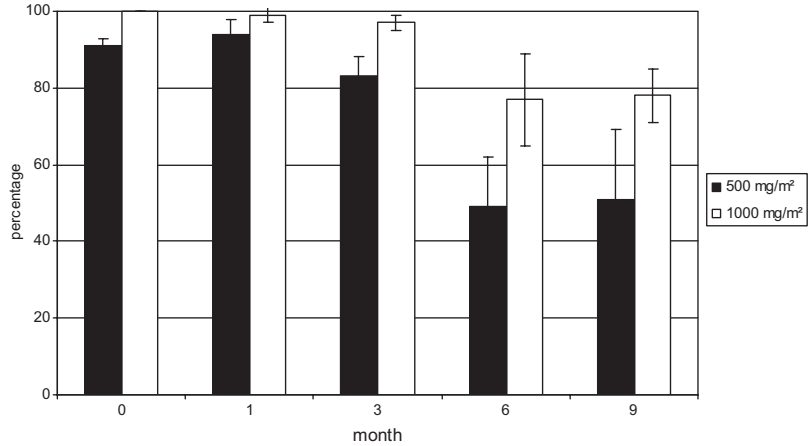

Fig. 1. Residual efficacy of pyrethrum-treated nets (Pynet 5%EC) at 500 and 1,000 mg/m^2 against susceptible *An. gambiae*.

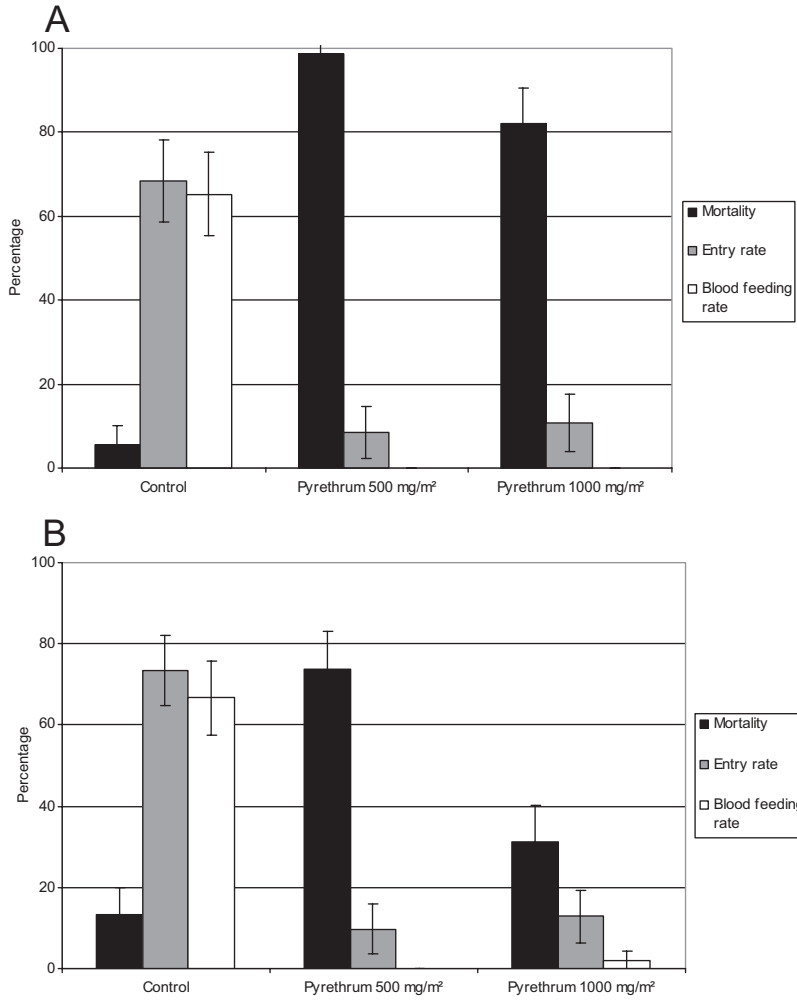

Fig. 2. Efficacy, in tunnel apparatus, of netting treated with pyrethrum at 500 and 1,000 mg/m^2 against susceptible (A) and pyrethroid-resistant (B) *An. gambiae*.

and KDT_{90} of pyrethrum were two to four times lower than that of permethrin and deltamethrin for SS mosquitoes (Table 3). Interestingly, the presence of the *kdr* mutation did not greatly affect the KD effect of pyrethrum (KDT_{95} of 19.9 min) compared with deltamethrin (KDT_{95} of 45.7 min) or permethrin (KDT_{95} of 99.3 min).

Efficacy and Residual Activity of Pyrethrum on Nets and Substrates. Preliminary studies carried out on SS mosquitoes showed that the first concentrations of pyrethrum causing >80% mortality and/or >95% KD effect after 60 min (WHO requirements) on nets were 500 and 1,000 mg/m^2 (data not shown). At these two concentrations, the KD effect of pyrethrum-treated samples was 100% and lasted 9 mo in laboratory conditions (data not shown). Mortality caused by pyrethrum remained within WHO requirements for 9 mo at the highest dosage (1,000 mg/m^2), whereas it significantly dropped below 80% after 6 mo at 500 mg/m^2 (Fig. 1).

With the tunnel apparatus, both 500 and 1,000 mg/m^2 pyrethrum showed good performance against SS mosquitoes in terms of mortality (>80%), entry rates (<15% entry rates), and blood-feeding inhibition (100% BFI; Fig. 2A). One should note that the highest dose of pyrethrum killed significantly less mosquitoes than the lower one (82 and 99%, respectively, $P < 0.05$). With the pyrethroid-resistant strain, mortality induced by pyrethrum at 500 mg/m^2 was once again significantly higher than that recorded at 1,000 mg/m^2 (74 and 31%, respectively; $P < 0.05$). Despite the presence of the *kdr* mutation, low entry and blood-feeding rates (<20%) were recorded with pyrethrum at 500 and 1,000 mg/m^2 (Fig. 2B).

As for the netting materials, a preliminary study on pyrethrum efficacy was performed using susceptible *An. gambiae* with different dosages and substrates (wood, cement, and mud). The first concentrations causing >80% mortality and >95% KD effect were 1 and 2 g/m^2 for all substrates (data not shown). At 1

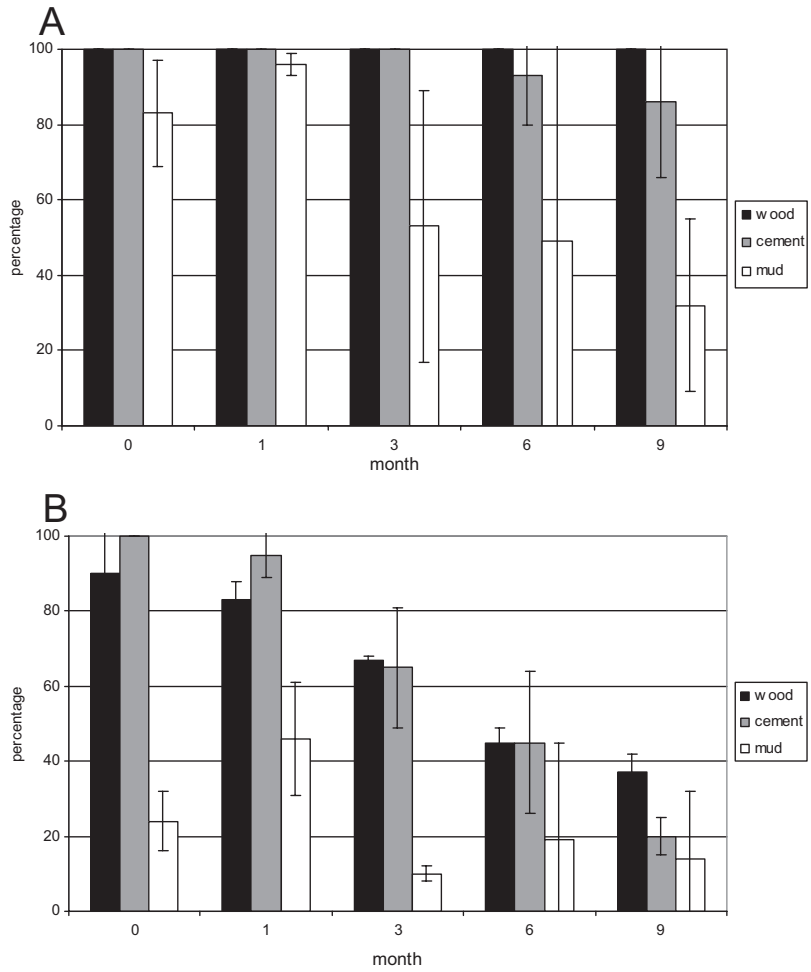

Fig. 3. KD effect (A) and mortality (B) of pyrethrum (2 g/m^2) on different substrates against susceptible *An. gambiae*.

g/m^2 pyrethrum, both mortality and KD effect were low (<20%) after 3 mo in laboratory conditions of storage (except on wood, which is a nonporous substrate). At 2 g/m^2 pyrethrum (Fig. 3), mortality significantly dropped below 80% after 3 mo on cement and wood, whereas KD effect remained >95% for 9 mo. Mud was the substrate on which pyrethrum showed the lowest efficacy and persistence. In addition, high variability was observed between muds replicates, explaining the large confidence intervals observed.

Discussion

The intrinsic activity of pyrethrum against susceptible mosquitoes was found to be similar to that of permethrin but was much lower than other pesticides, such as pyrethroids, fiprols, and neonicotinoids (Table 4 taken from Corbel et al. 2004). However, the toxicity of pyrethrum was not strongly affected by the presence of the *kdr* mutation in *An. gambiae*, as indicated by the relatively low resistance ratios observed with topical application and filter paper bioassays. In contrast, bifenthrin and permethrin showed higher resistance ratios (RR_{50} of 12 and 30, respectively) than pyrethrum with the same *An. gambiae* strain (Chandre

Table 4. Comparative adult toxicity of several insecticides against susceptible reference strains of *An. gambiae and Ae. aegypti* (taken from Corbel et al. 2004)

		Topical applications (LD_{50} in ng/mg female)	
		An. gambiae	*Ae. aegypti*
Pyrethroids	Deltamethrin	0.018	—
	Bifenthrin	0.14	0.077
	Permethrin	1.02	0.24
	Pyrethrum	1.90	0.28[a]
Fiprols	Fipronil	0.040	0.020
Neonicotinoids	Dinotefuran	0.18	7.1

Data not available with these chemicals.
[a] Unpublished data (LIN-IRD).

et al. 2000, Bonnet et al. 2004). The WHO treated filter papers also showed that the DD of pyrethrum was 1% for susceptible *Anopheles* mosquitoes. At this concentration, a strong KD effect was noted for both susceptible and pyrethroid-resistant strains compared with permethrin and deltamethrin.

WHO cone tests carried out on pyrethrum-treated netting (Pynet 5% EC) showed that unwashed nets treated at 500 and 1,000 g/m^2 fulfilled WHO requirements (i.e., >80% mortality and/or KD > 95%) for a period of 9 mo in laboratory conditions of storage (100% KD for both concentrations). The evaluation carried out in tunnel apparatus confirmed the good performance of pyrethrum-treated nets in terms of mortality (>80%), entry rate (<15%), and blood-feeding inhibition (>95%). This suggests that pyrethrum may be applied to nets at dosages similar to those recommended for permethrin, i.e., between 500 and 1,000 mg/m^2 providing that this product presents no risk for human health. As the efficacy of pyrethrum-treated nets was found to rapidly decrease after a few washing cycles (data not shown), the search for long-lasting technologies for mosquito net treatment should be encouraged.

Conversely, the efficacy and residual activity of pyrethrum Pynet 5% EC on substrates was not conclusive, especially concerning mud, which is a porous substrate. Mortality fell below 80% after 3 mo on all substrates (cement, mud, and wood). It has been previously observed that porous surfaces are heterogeneous for the absorption of insecticides, which may lead to variable results (Darriet et al. 1998, Rojas de Arias et al. 2003, Romi et al. 2005). This study suggests that dosages <2 g/m^2 would be not appropriate for indoor residual spraying.

In conclusion, this study showed that pyrethrum may be promising for malaria vector control, especially for the impregnation of bednets and textiles, as it showed strong KD effect and high excito-repellency against resistant mosquitoes that are particularly important for achieving good personal protection. Further field studies (e.g., experimental huts) are now needed before efficacy and cost-effectiveness of the product can be totally assessed.

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References Cited

Abbott, W. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Bonnet, J., V. Corbel, F. Darriet, F. Chandre, and J. M. Hougard. 2004. Topical applications of pyrethroid and organophosphate mixtures revealed positive interactions against pyrethroid resistant *Anopheles gambiae.* J. Am. Mosq. Control Assoc. 20:438–443.

Chandre, F., F. Darriet, L. Manga, M. Akogbéto, O. Faye, J. Mouchet, and P. Guillet. 1999. Status of pyrethroid resistance in *Anopheles gambiae* s.l. Bull. WHO 77: 230–234.

Chandre, F., F. Darriet, S. Duchon, L. Finot, S. Manguin, P. Carnevale, and P. Guillet. 2000. Modifications of pyrethroid effect associated with *Kdr* mutation in *Anopheles gambiae.* Med. Vet. Entomol. 14: 81–88.

Cochran, D. G. 1995. Insect resistance to pyrethrins and pyrethroids, pp. 234–238. *In* J. E. Casida and G. B. Quistad (eds.), Pyrethrum flowers: production, chemistry, toxicology and uses. Oxford University Press, Oxford, United Kingdom.

Corbel, V., F. Chandre, C. Brengues, M. Akogbéto, F. Lardeux, J. M. Hougard, and P. Guillet. 2004. Dosage-dependent effects of permethrin-treated nets on the behaviour of *Anopheles gambiae* and the selection of pyrethroid resistance. Malar. J. 3: 22.

Corbel, V., R. N'Guessan, C. Brengues, F. Chandre, L. Djogbenou, T. Martin, M. Akogbéto, J. M. Hougard, and M. Rowland. 2007. Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. Acta Trop. 101: 207–216.

Darriet, F., P. Guillet, F. Chandre, R. N'Guessan, J.M.C. Doannio, F. Rivière, and P. Carnevale. 1997. Présence et évolution de la résistance aux pyréthrinoïdes et au DDT chez deux populations d'*Anopheles gambiae* s.s. d'Afrique de l'Ouest. Document WHO/CTD/VBC/97.1001, WHO/MAL/97.1081. World Health Organization, Geneva, Switzerland.

Darriet, F., P. Guillet, R. N'Guessan, J.M.C. Doannio, A. Koffi, L. Y. Konan, and P. Carnevale. 1998. Impact of resistance of *Anopheles gambiae* s.s. to permethrin and deltamethrin on the efficacy of impregnated mosquito nets. Med. Trop. 58: 349–354.

Etang, J., E. Fondjo, F. Chandre, I. Morlais, C. Brengues, P. Nwane, M. Chouaibou, H. Ndjemai, and F. Simard. 2006. First report of knockdown mutations in the malaria vector *Anopheles gambiae* from Cameroon. Am. J. Trop. Med. Hyg. 74: 795–797.

Finney, D. J. 1971. Probit analysis. Cambridge University Press, Cambridge, United Kingdom.

Katsuda, Y. 1999. Development and future prospects for pyrethroid chemistry. Pest. Sci. 55: 775–782.

N'Guessan, R., V. Corbel, M. Akogbéto, and M. Rowland. 2007. Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. Emerg. Infect. Dis. 13: 199–206.

Ranson, H., B. Jensen, J. M. Vulule, X. Wang, J. Hemingway, and F. H. Collins. 2000. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. Insect Mol. Biol. 9: 491–497.

Raymond, M., G. Prato, and D. Ratsira. 1997. Probit and logit analysis program, version 2.0. Praxème R&D, Montpellier, France.

Rojas de Arias, A., M. J. Lehane, C. J. Schofield, and A. Fournet. 2003. Comparative evaluation of pyrethroid insecticide formulations against *Triatoma infestans* (Klug): residual efficacy on four substrates. Mem. Inst. Oswaldo Cruz 98: 975–980.

Romi, R., R. Raharimanga, E. M. Rakotondraibe, R. Dommarco, A. Santillo, A. Sorbo, and G. Majori. 2005. Assessment of the residual efficacy of three pyrethroids for the intradomiciliary control of malaria vectors in the Highlands of Madagascar. Ital. Med. Trop. 10: 39–45.

Salgado, V. L., S. N. Irving, and T. A. Miller. 1983. The importance of nerve terminals depolarization in pyrethroid poisoning insects. Pestic. Biochem. Physiol. 20: 169–182.

Sattelle, D. B., and D. Yamamoto. 1988. Molecular targets of pyrethroid insecticides. Adv. Insect Physiol. 20: 147–213.

Silcox, C. A., and E. S. Roth. 1994. Pyrethrum for pest control. *In* J. E. Casida and G. B. Quistad (eds.), Pyrethrum flowers: production, chemistry, toxicology and uses. Oxford University Press, Oxford, United Kingdom.

World Health Organization. 2005. World malaria report 2005. Document WHO/HTM/MAL/2005.1102. World Health Organization, Geneva, Switzerland.

World Health Organization. 2006. Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets. Document WHO/CDS/WHOPES/GCDPP/2005.11. World Health Organization, Geneva, Switzerland.

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