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EFFICACY OF MOSQUITO NETS TREATED WITH A PYRETHROID-ORGANOPHOSPHOROUS MIXTURE AGAINST *KDR*– AND *KDR*+ MALARIA VECTORS (*ANOPHELES GAMBIAE*)

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Summary:

In order to prevent the resistance of *Anopheles gambiae* s.l. to pyrethroids from spreading too quickly and to lengthen the effectiveness of insecticide impregnated mosquito nets, it has recently been suggested to use mixtures of insecticides that have different modes of action. This study presents the results obtained with tulle mosquito nets treated with bifenthrin (a pyrethroid) and chlorpyrifos-methyl (an organophosphorous) both separately and in mixture on two strains of *An. gambiae*, one sensitive to all insecticides, and the other resistant to pyrethroids. The values of KDt_{50} and KDt_{95} and the mortality induced with the mixture of bifenthrin (25 mg/m^2) and chlorpyrifos-methyl (4.5 mg/m^2) show a significant synergistic effect on the strain of *An. gambiae* susceptible to insecticides. However, the tested combination does not induce any synergistic effect on the VKPR strain selected with permethrin, but only enhances the effectiveness of the two insecticides taken separately.

KEY WORDS : bifenthrin, chlorpyrifos-methyl, synergy, mosquito net, *Anopheles gambiae*.

Résumé : EFFICACITÉ DE MOUSTIQUAIRES IMPRÉGNÉES AVEC UN MÉLANGE PYRÉTHRINOIDE-ORGANOPHOSPHORÉ CONTRE LES VECTEURS DU PALUDISME *KDR*– ET *KDR*+ (*ANOPHELES GAMBIAE*)

Afin d'éviter une extension trop rapide de la résistance d'Anopheles gambiae s.l. aux pyréthrinoïdes et de préserver durablement l'efficacité des moustiquaires imprégnées d'insecticide, il est depuis peu proposé d'associer des composés présentant des modes d'action différents. Cette étude présente les résultats obtenus avec des tulles moustiquaires traités à la bifenthrine (pyréthrinoïde) et au chlorpyriphos-méthyl (organophosphoré) séparément et en mélange sur deux souches d'An. gambiae, l'une sensible à tous les insecticides, et l'autre résistante aux pyréthrinoïdes. Les valeurs des KDt_{50} et KDt_{95} et de la mortalité induite avec le mélange de bifenthrine (25 mg/m^2) et de chlorpyriphos-méthyl (4,5 mg/m^2), montrent un effet de synergie significatif sur la souche d'An. gambiae sensible aux insecticides. En revanche, sur la souche VKPR sélectionnée à la perméthrine, l'association testée n'induit pas de synergie mais seulement un effet additif de l'efficacité des deux insecticides pris séparément.

MOTS CLÉS : *bifenthrine, chlorpyriphos-méthyl, synergie, moustiquaire,* Anopheles gambiae.

INTRODUCTION

Mosquito nets impregnated with insecticides are recommended for the control of the vectors of malaria, particularly in Sub-Saharan Africa where their effectiveness is no longer to be demonstrated (Lines, 1996). Nowadays, only the pyrethroid insecticides are used, because of their speed of action, their high repulsive and irritating capacity towards mosquitoes, and their low toxicity for humans. However, *Anopheles gambiae* s.l., the main vector of malaria in Africa, has become resistant to the majority of these insecticides (Chandre *et al.*, 1999; Koffi *et al.*, 1999). One strategy suggested for the management of resistance consists in combining a pyrethroid with an insecticide which has a different mode of action. This strategy proves worthy of being explored as the existence of positive interactions (synergistic effect) has been demonstrated in agriculture between pyrethroids and organophosphorous insecticides (Koziol & Witkowski, 1982; Asher *et al.*, 1986). In public health, a synergistic effect has recently been observed with mosquito nets impregnated with a pyrethroid (bifenthrin) and a carbamate (carbosulfan), on adults of *An. gambiae* susceptible to insecticides (Corbel *et al.*, 2002).

However, because carbosulfan turns into a more toxic compound, the toxicological risk of its use on mosquito nets has been questioned in relation to human safety (Anonymous WHO, 1986). For this reason the use of chlorpyrifos-methyl, an organophosphorous insecticide not so toxic for mammals (lethal dose 50 % for the rat from 1,630 to 2,140 mg/kg – Tomlin, 2000), has been considered a better candidate for use in combination with bifenthrin. This study was carried out in laboratory, on a population of *An. gambiae* not showing any mechanism of resistance, as well as on a population of the same species resistant to pyrethroids.

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MATERIAL AND METHODS

INSECTICIDES

Bifenthrin (2 methyl [1,1'-biphenyl]-3-yl)methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyclopropane-carboxylate) is a pyrethroid particularly effective against the vectors of malaria (Hougard *et al.,* 2002). Like all pyrethroids, this compound acts by modifying, along the axon, the kinetics of inactivation of the sodium channels which operate in the transmission of the nerve impulse in insects (Lund & Narahashi, 1983). The dosage recommended for the impregnations of mosquito nets is 50 mg of active ingredient (a.i.)/m^2 (Guillet *et al.,* 2001). The formulation used is a suspension concentrate (SC) at 80 g of a.i. per litre (Talstar®, FMC Corporation, P.O. Box 8, Princeton, New Jersey 08543, USA).

Chlorpyrifos-methyl (0,0-dimethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate) is an organophosphorous compound which operates on a different target than pyrethroids by inhibiting the acethylcholinesterase, an enzyme operating in the regulation of the nerve impulse in insects (Aldridge,1950). The experimental formulation used for our study was a suspension of microcapsules (CS) containing 460 g of a.i. per litre (GF298, Dow AgroScience, Norfolk PE30 2JD, UK).

BIOLOGICAL MATERIAL

The susceptible strain of *An. gambiae*, originating from Kisumu in Kenya (KIS), has been analyzed for many years and found to be free from any detectable insecticide resistance mechanism. The resistant strain of *An. gambiae* (VKPR) originating from Burkina Faso was already highly resistant to permethrin when collected in the field and has subsequently been maintained under constant permethrin selection at each generation (Darriet *et al.,* 1997). This strain was homozygous for the knockdown resistance (*kdr*) gene with a resistance factor (by topical application with permethrin) of about 40 fold compared to the KIS strain (Chandre *et al.,* 2000).

MOSQUITO NET IMPREGNATION

Pieces of netting (warp knitted multifilament polyester 100 denier, mesh 156, Vestergaard, Denmark), 25 × 25 cm, were treated by a volume of formulation suspension corresponding to the specific absorbency of the net (3.75 ml). For each insecticide tested separately, the bio-assays were carried out with concentrations not inducing more than 40 % mortality in the susceptible strain of *An. gambiae*. Beyond this value, it became difficult to demonstrate a synergistic effect because the results theoretically expected in the absence of any interaction [calculated by 1 – (% survival on bifenthrin × % survival chlorpyrifos-methyl)] would vary little from the results observed, except in the event of negative interactions where the percentage of mortality observed would be lower than the percentage of expected mortality (antagonism effect). Bifenthrin was thus tested at half the recommended dosage for operational use (25 mg a.i./m^2). Chlorpyrifos-methyl was tested at 4.5 mg a.i./ m^2. The bifenthrin/chlorpyrifos-methyl mixture totaled 25 mg/ m^2 of bifenthrin plus 4.5 mg/m^2 of chlorpyrifos-methyl.

TARSAL CONTACT

Knock-down and mortality were measured using WHO cone tests for adult mosquitoes (Anonymous WHO, 1998). The tests were conducted using the standard WHO plastic cones and a three minute exposure time. Five non blood-fed females two to five days old were introduced into the cones at a time. Four cones were applied at the same time onto the net sample and tests were carried out at 25° C ± 2 under subdued light. After exposure, the females were grouped into batches of 10 in 150 ml plastic cups and maintained at 27° C ± 2 and 80 % ± 10 RH with a honey solution provided. For each sample tested, 50 mosquitoes (ten cones) were used. Knock down rates were recorded after exposure at fixed intervals of time (every two to 10 min depending on *KD* rates) up to 60 min after exposure. KDT_{50} and KDT_{95} with 95 % confidence limits were calculated using log-probit analysis. Percentage mortalities were recorded after 24 h and compared by a Chi square test. All tests were replicated two to three times on different mosquito batches and conducted in parallel with a control where no insecticide was applied to the net. The results were corrected accordingly to Abbott (1925) when mortality in the control was above 5 %.

RESULTS

EFFICACY OF BIFENTHRIN

At 25 mg a.i./m ^{2}, bifenthrin induces 33.5 % mortality on the KIS strain and KDt_{50} and KDt_{95}, respectively, of 22 ± 1.5 and 82 ± 9.9 minutes. Mortality is only 4 % on the VKPR strain with higher KDt_{50} and KDt_{95} (> 120 min) (Table I).

EFFICACY OF CHLORPYRIFOS-METHYL

At 4.5 mg a.i./m^2, mortalities with chlorpyrifos-methyl were respectively of 22.4 % in KIS and 12.9 % in VKPR. As with other organophosphorous, chlorpyrifos-methyl did not induced *KD* effect (no fast acting insecticide), whatever the strain tested (Table I).

		Dose (mg/m²)	N	KDt_{50} (min) observed (expected)	KDt_{95} (min) observed (expected)	Mortality (%) observed (expected)	χ^2
Susceptible (KIS)	bifenthrin	25	155	22.2 ± 1.5 (-)	82.0 ± 9.9 (-)	33.5 (-)	–
	chlorp-met	4.5	147	ND	ND	22.4 (-)	–
	bifenthrin + chlorp-met	25 + 4.5	161	17.6 ± 1.1 (22.2)	57.4 ± 5.7 (82)	85.1 (48)	30.73 $P < 0,0001$
Resistant (VKPR)	bifenthrin	25	101	> 120 (–)	> 120 (–)	4.0 (–)	–
	chlorp-met	4.5	101	ND	ND	12.9 (–)	–
	bifenthrin + chlorp-met	25 + 4.5	103	> 120 (> 120)	> 120 (> 120)	10.7 (16.4)	1.07 $P = 0.3$

chlorp-met: chlorpyrifos-methyl; N: number mosquitoes tested; ND: not detectable.

Table I. – Activity of bifenthrin and chlorpyrifos-methyl formulations, alone and in combination, against susceptible and resistant strains of *Anopheles gambiae* (KDt_{50} and KDt_{95}, with their 95 % confidence intervals and mortality rates).

EFFICACY OF BIFENTHRIN/CHLORPYRIFOS-METHYL MIXTURE

The mixture made up of bifenthrin at 25 mg/m² and chlorpyrifos-methyl at 4.5 mg/m² produces a synergistic effect on the KIS strain which is perceptible both at the levels of *KD* effect and mortality. KDt_{50} and KDt_{95}, which were of 17.6 ± 1.1 and 57.4 ± 5.7 minutes respectively, decrease significantly ($P < 0.05$) compared to the *KDt* recorded with bifenthrin alone (KDt_{50} = 22.2 ± 1.5 min and KDt_{95} = 82 ± 9.9 min; no overlapping of the confidence intervals). In the same way, the mortality observed with the mixture (85.1 %) is close to twice higher than the mortality expected (48 %) ($P < 0.0001$). No similar phenomenon is observed on the VKPR strain at the level of the *KD* effect or mortality ($P = 0.3$) (Table I), because this mosquito strain is resistant to the depolarizing and repetitive firing action of pyrethroids.

DISCUSSION

The results recorded for the *KDt* and mortality with bifenthrin and chlorpyrifos-methyl mixture shows a synergistic effect on the strain of *An. gambiae* susceptible to insecticides. This confirms the data obtained with the combination of bifenthrin and carbosulfan on this strain of *An. gambiae* (Corbel *et al.*, 2002). On the VKPR strain however, the mixture tested does not induce any synergy but only enhances the effectiveness of the two insecticides taken separately. The existence of a synergy that is limited to the susceptible strain, nevertheless, offers prospects for the management of resistance as the probability of a simultaneous appearance of resistance to two insecticides with different modes of action would be less likely (Curtis, 1985). The *kdr* gene being semi-recessive, the heterozygous mosquitoes (RS) behave approximately in the same way as the susceptible homozygous mosquitoes (SS) with respect to mortality (Chandre *et al.*, 2000). The synergistic effect obtained from bifenthrin and chlorpyrifos-methyl may facilitate the elimination of the heterozygous individuals (RS), thus preventing the selection of the *kdr* mutant gene that confers tolerance to pyrethroids. On the other hand, since the combination tested on the strains of *An. gambiae* highly resistant to pyrethroids does not induce any synergistic effect, the concept of mixture could prove less advantageous in locations where resistant populations already exit. The pyrethroid insecticide having only little or no effectiveness, only the organophosphorous insecticide kills the resistant mosquitoes, which then simply results in an added effect to the mortality rate induced by the two insecticides.

The evaluation of mosquito nets impregnated with a mixture of a pyrethroid and chlorpyrifos-methyl must therefore now be conducted in experimental huts, not only in the areas where *An. gambiae* is susceptible to pyrethroids, but also in the areas where this mosquito is resistant (Darriet *et al.*, 2000).

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REFERENCES

ABBOTT W.S. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology,* 1925, *18,* 265-267.

ALDRIDGE W.N. Some properties of specific cholinesterase with particular reference to the mechanism of inhibition by diethyl *p*-nitrophenyl thiophosphate (E605) and analogues. *Biochemistry Journal,* 1950, *46,* 451-460.

ANONYMOUS. Carbamate insecticides: a general introduction. WHO mimeographed document Environmental Health Criteria 64, 1986, Geneva, WHO.

ANONYMOUS. Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticide on treated surfaces. WHO mimeographed document WHO/CDS/MAL/98.12, 1998, Geneva, WHO.

ASHER K.R.S., ELIYAHU M., ISHAAYA I., ZUR M. & BEN-MOSHE E. Synergism of pyrethroid-organophosphorous insecticides mixtures in insects and their toxicity against *Spodoptera littoralis* larvae. *Phytoparasitica,* 1986, *14,* 101-110.

CHANDRE F., DARRIET F., MANGUIN S., BRENGUES C., CARNEVALE P. & GUILLET P. Pyrethroid cross resistance spectrum among populations of *Anopheles gambiae* s.s. from Côte d'Ivoire. *Journal of American Mosquito Control Association,* 1999, *15,* 53-59.

CHANDRE F., DARRIET F., DUCHON S., FINOT L., MANGUIN S., CARNEVALE P. & GUILLET P. Modifications of pyrethroid effect associated with *kdr* mutation in *Anopheles gambiae. Medical and Veterinary Entomology,* 2000, *14,* 81-88.

CORBEL V., DARRIET F., CHANDRE F & HOUGARD J.M. Insecticides mixtures for mosquito net impregnation against malaria vector. *Parasite,* 2002, *9,* 255-259.

CURTIS C.F. Theoretical models of the use of insecticide mixtures for management of resistance. *Bulletin of Entomological Research,* 1985, *75,* 259-265.

DARRIET F., GUILLET P., CHANDRE F., N'GUESSAN R., DOANNIO J.M.C., RIVIÈRE F. & CARNEVALE P. Présence et évolution de la résistance aux pyréthrinoïdes et au DDT chez deux populations *d'Anopheles gambiae* s.s. d'Afrique de l'Ouest. WHO mimeographed document WHO/CTD/VBC/97.1001; WHO/MAL/97.1081, 1997, Geneva, WHO.

DARRIET F., N'GUESSAN R., KOFFI A.A., KONAN L.Y., DOANNIO J.M.C., CHANDRE F & CARNEVALE P. Impact de la résistance aux pyréthrinoïdes sur l'efficacité des moustiquaires imprégnées dans la prévention du paludisme : résultats des essais en cases expérimentales avec la deltaméthrine SC. *Bulletin de la Société de Pathologie Exotique,* 2000, *93,* 131-134.

GUILLET P., N'GUESSAN R., DARRIET F., TRAORÉ-LAMIZANA M., CHANDRE F. & CARNEVALE P. Combined pyrethroid and carbamate "two in one" treated mosquito nets: field efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus. Medical and Veterinary Entomology,* 2001, *15,* 105-112.

HOUGARD J.M., DUCHON S., ZAIM M. & GUILLET P. Bifenthrin: a useful pyrethroid insecticide for treatment of mosquito nets. *Journal of Medical Entomology,* 2002, *39,* 526-533.

KOFFI A.A., DARRIET F., N'GUESSAN R., DOANNIO J.M.C. & CARNEVALE P. Évaluation au laboratoire de l'efficacité insecticide de l'alpha-cyperméthrine sur les populations d'*Anopheles gambiae* de Côte d'Ivoire résistantes à la perméthrine et à la deltaméthrine. *Bulletin de la Société de Pathologie Exotique,* 1999, *92,* 62-66.

KOZIOL S.F. & WITOWSKI J.F. Synergism studies with binary mixtures of permethrin plus methyl parathion, chlorpyrifos and malathion on European corn borer larvae. *Journal of Economic Entomology,* 1982, *75,* 28-30.

LINES J. Mosquito nets and insecticides for net treatment: a discussion of existing and potential distribution systems in Africa. *Tropical Medicine and International Health,* 1996, *1,* 616-632.

LUND A.E. & NARAHASHI T. Kinetics of sodium channel modification as the basis for the variation in the nerve membrane effects of pyrethroid and DDT analogs. *Pesticide and Biochemical Physiology,* 1983, *20,* 203-216.

TOMLIN C.D.S. The pesticide Manuel. 12th ed. British Crop Protection Council, 2000, London, UK.

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