

COMPARISON OF THE EFFECT OF TWO EXCIPIENTS (KARITE NUT BUTTER AND VASELINE) ON THE EFFICACY OF *COCOS NUCIFERA*, *ELAEIS GUINEENSIS* AND *CARAPA PROCERA* OIL-BASED REPELLENTS FORMULATIONS AGAINST MOSQUITOES BITING IN IVORY COAST

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Summary :

Repellents in the form of dermal pomades are recommended as a protection against awakening and bedtime mosquito bites. If synthesis repellents are available, they are nevertheless not common and the prices remain out of reach for the communities concerned. The people therefore have to resort more and more to traditional concoctions, some of which have been shown to be effective. After demonstrating that oil-based formulations (lotions, creams, pomades) of *Cocos nucifera* (coconut), *Elaeis guineensis* (oil palm) and *Carapa procera* (gobi) were effective against mosquitoes, it became necessary to study the impact of the two excipients used in their manufacture, on the effectiveness of the repellents. Experiments were carried with *Anopheles gambiae* and *Aedes aegypti* under laboratory conditions and any other mosquitoes collected under field conditions in Ivory Coast. The laboratory results indicate that the average protection times obtained with formulations with karite nut butter as excipient (54.8 ± 37.0 mn and 74.6 ± 26.4 mn respectively on *An. gambiae* and *Ae. aegypti*) are higher than those recorded with vaseline as excipient (respectively 42.7 ± 30.0 mn and 60.8 ± 33.9 mn). On the other hand, under field conditions, the biting rate percentage reduction obtained with the products with karite nut butter and vaseline excipient were similar (respectively 29.8 % and 35.9 % for all mosquitoes collected and 45.7 % and 47.4 % against *An. gambiae*). Nevertheless, the use of karite nut butter on repellent products should be encouraged because its sale price is very lower (10 time less) than the vaseline's.

KEY WORDS : mosquito, individual protection, natural repellent, excipient, karite nut butter, vaseline, Ivory Coast.

Résumé : COMPARAISON DE L'EFFET DE DEUX EXCIPIENTS (BEURRE DE KARITÉ ET VASELINE) SUR L'EFFICACITÉ DE FORMULATIONS À BASE D'HUILE DE *COCOS NUCIFERA*, D'*ELAEIS GUINEENSIS* ET DE *CARAPA PROCERA* CONTRE LES PIQÛRES DE MOUSTIQUES EN CÔTE D'IVOIRE

Les répulsifs en application dermique sont recommandés contre les piqûres de moustiques aux heures d'éveil et avant d'aller au lit. Si des répulsifs de synthèse sont disponibles, leur utilisation n'est cependant pas courante car leur prix de vente reste dissuasif pour la plupart des communautés concernées et ils ne sont pas tous exempt de toxicité. Les populations ont donc de plus en plus recours à des recettes traditionnelles dont l'efficacité de certaines d'entre elles a été démontrée par ailleurs. Après avoir montré que des formulations (lotions, crèmes, pommades) à base d'huiles de *Cocos nucifera* (coco), d'*Elaeis guineensis* (palmiste) et de *Carapa procera* ("gobi") étaient efficaces vis-à-vis des moustiques, il importait de voir l'impact des deux excipients utilisés dans leur fabrication sur l'efficacité de ces produits répulsifs. Les travaux se sont déroulés au laboratoire sur deux espèces culicidiennes – *Anopheles gambiae* et *Aedes aegypti* – et sur le terrain en zone de savane de Côte d'Ivoire. Les résultats de laboratoire indiquent que les temps moyens de protection obtenus avec les formulations à excipient beurre de karité ($54,8 \pm 37,0$ mn et $74,6 \pm 26,4$ mn respectivement sur *An. gambiae* et *Ae. aegypti*) sont supérieurs à ceux enregistrés avec les répulsifs à excipient vaseline (respectivement $42,7 \pm 30,0$ et $60,8 \pm 33,9$ mn). En revanche, dans les conditions de terrain, les pourcentages de réduction des piqûres de moustiques obtenus avec les produits à excipient beurre de karité et vaseline ne présentent pas de différence significative (respectivement 29,8 % et 35,9 % pour toutes les espèces confondues et 45,7 % et 47,4 % en ce qui concerne *An. gambiae*). Toutefois, l'utilisation du beurre de karité dans la production des formulations de répulsifs est souhaitable dans la mesure où son prix de vente est très bas (10 fois moins) par rapport à celui de la vaseline.

MOTS CLÉS : moustique, protection individuelle, répulsif naturel, excipient, beurre de karité, vaseline, Côte d'Ivoire.

Among the preventive measures recommended by WHO in 1989 against malaria, special importance is given to individual and collective protection methods against mosquito bites. Pyrethroids impregnated bed nets bring excellent protection to users at least during their sleep. Sunset to the

one of the persons, there is a risk of malaria transmission, even if the majority of *Anopheles* species infestations is met between 23 hours and 04 hours in the morning (Dossou-yovo *et al.*, 1999). Besides, nuisance caused by *Aedes aegypti* and *Culex quinquefasciatus* necessitates also an individual protection before hours of using bed nets. Several natural or synthetic repellents are used against biting insects pests (Curtis *et al.*, 1989). Synthetic products are generally sold in order to protect against mosquito bites. Nevertheless, they are prohibitively expensive for the communities concerned (Hougard *et al.*, 1998) and have

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to be used carefully (Combemale *et al.*, 1992). Some studies show that traditional natural repellents were effective against mosquito bites (Sharma *et al.*, 1993; Yao, 2000; Konan *et al.*, 2002) and against black-flies (Pitroipa, 2000; Sylla, 2002). In the face of the growing interest in traditional preparations, has become necessary to test several formulations, to select and to propose the most efficient. Since these formulations being made with excipients, it imported to study their impact on the effectiveness of these repellents. This study give the effect of two excipients (karite nut butter and vaseline) on the effectiveness of *Cocos nucifera* (coconut), *Elaeis guineensis* (oil palm) and *Carapa procera* (gobi) oil-based formulations against mosquitoes.

MATERIALS AND METHODS

The different formulations of oil palm, coconut and gobi oil-based were locally produced with karite nut butter or pure vaseline as excipient. Six formulations were obtained with 75 % of mixed oil to 25 % of excipient, six other were realized with 50 % of mixed oil to 50 % of excipient and the six final formulations with 25 % mixed oil to 75 % of excipient. Their repellent capacity was tested against mosquitoes under laboratory and field conditions. In the laboratory, the tests consisted to determine protection time which is calculated to first bite, confirmed by the second and third bites within 15 min (Schrek, 1977; Curtis *et al.*, 1994; Frances *et al.*, 1993; Frances *et al.*, 1998). For each test, 60 female mosquitoes, aged three-five days were placed in cages (30 cm × 30 cm × 30 cm). The mosquitoes were deprived of any juices for six hours before the beginning of the experiment to increase their aggressiveness.

Three adult volunteers served as experimental host: each volunteer used one forearm for treated and the other one for untreated control. The forearm (wrist to elbow) were treated with one gram of repellent product, the hand protected by a glove. The untreated forearm was introduced first into the mosquito cage to test the anthropophily of the mosquitoes. After the first bite, time is noted and the test continues. The treated forearm was then introduced into the same mosquito cage. The time of the confirmed bite was noted. Each test was realized with three different persons in order to offset any personal differences in attractiveness to mosquitoes and with *An. gambiae* and *Ae. aegypti* while respecting their natural biting period. The tests were done with excipients before the products. The average protection time recorded comparison was realized by an analysis of variance.

In field conditions, evaluation was conducted according to the classical method of night biting catches on human subjects (Baudon *et al.*, 1991). The volunteers were kept on antimalaria prophylaxis. Six catching-points were chosen outdoors. For each catching session, one untreated volunteer was used as control. The five others applied or one of the excipients or the repellent (1 gram measured with a small spoon), knee to ankle. The catching session last from 22 hours to five hours. The mosquitoes collected during each hour were counted and identified. For each formulation, eight consecutive night catches were carried out. The collectors were alternately used as treated and untreated. A rotation of repellents was done for each collector to compensate for personnel differences in catching ability and attractiveness to mosquitoes. The repellent products effectiveness was estimated by biting rate calculated for treated and untreated collectors. Percentage protection was estimated as $100 \times (\text{control-treatment})/\text{control}$. The protection factors thus obtained were compared by χ^2 tests.

RESULTS

EFFECTIVENESS IN THE LABORATORY CONDITIONS

The protection of treated forearms was four and 15 seconds respectively with the vaseline and karite nut butter, while it was of five seconds on untreated forearm. The two used excipients were not repelled to mosquitoes.

The average protection times varied according to mosquitoes and excipient (Table I). Against *An. gambiae*, it varied between 121 ± 9 minutes (maximum) and 12 ± 2 minutes (minimum) when the formulations with karite nut butter as excipient, and 103 ± 18 minutes and 11 ± 3 minutes for vaseline. With *Aedes aegypti*, the protection was between 177 ± 10 minutes and 34 ± 4 minutes for karite nut butter, and between 101 ± 9 minutes and 40 ± 8 minutes for vaseline.

EFFECTIVENESS UNDER FIELD CONDITIONS

The 7,525 mosquitoes collected were composed of *Anopheles* (30.44 %), *Aedes* (0.66 %), *Culex* (0.66 %) and *Mansonia* (68.22 %). The mosquitoes biting rate was $73 \pm 16,7$ on the control while it was $71 \pm 15,8$ p/h/n and 69 ± 20 p/h/n respectively with the vaseline and karite nut butter. The percentage reduction in the biting rate of all mosquitoes collected, varied of 20 % to 37 % with the formulations with karite nut butter excipient and of 21 % to 41 % with those with vaseline. Against *An. gambiae*, they were in the order of 58 % to 47 % and of 51 % to 61 % for the same excipients (Table II).

Mosquitoes	Products	Karite nut butter	Vaseline	Statistical tests
<i>Anopheles gambiae</i>	G1	121.3 ± 9.0	103.0 ± 18.9	p = 0.023 S
	G2	96.8 ± 13.5	66.0 ± 6.0	p = 10 ⁻⁵ S
	G3	40.3 ± 8.1	28.0 ± 6.1	p = 0.003 S
	P1	83.3 ± 8.5	65.4 ± 18.2	p = 0.017 S
	P2	40.2 ± 6.3	42.5 ± 13.5	p = 0.64 NS
	P3	32.2 ± 4.9	20.5 ± 4.7	p = 9.10 ⁻⁹ S
	C1	46.2 ± 7.8	33.1 ± 2.8	p = 0.0002 S
	C2	20.4 ± 3.9	15.1 ± 1.8	p = 0.0019 S
	C3	12.5 ± 2.5	11.5 ± 3.2	p = 0.474 NS
<i>Aedes aegypti</i>	G1	177.1 ± 10.1	101.0 ± 9.5	p < 10 ⁻⁶ S
	G2	92.6 ± 14.1	66.1 ± 6.7	p = 10 ⁻⁴ S
	G3	83.7 ± 14.1	23.0 ± 3.2	p < 10 ⁻⁶ S
	P1	91.5 ± 10.6	82.7 ± 8.6	p = 0.072 NS
	P2	82.2 ± 6.2	49.2 ± 6.3	p < 10 ⁻⁶ S
	P3	44.7 ± 5.5	28.6 ± 9.4	p = 0.0004 S
	C1	111.0 ± 18.9	127.6 ± 9.2	p = 0.030 S
	C2	56.5 ± 6.1	69.3 ± 15.4	p = 0.034 S
	C3	34.8 ± 4.9	40.3 ± 8.7	p = 0.123 NS

P < 0.05, S; P > 0.05, NS.

G: "gobi" oil 1: 75 % of mixed oil to 25 % of excipient.

P: oil palm oil 2: 50 % of mixed oil to 50 % of excipient.

C: coconut oil 3: 25 % of mixed oil to 75 % of excipient.

Table I. – Impact of excipients on the average protection times obtained with vegetable oil-based repellent products in the laboratory.

	Products	Karite nut butter	Vaseline	Statistical tests
All mosquitoes collected	G1	37.33 % (366)	41.26 % (343)	p = 0.279 NS
	G2	29.45 % (412)	37.67 % (364)	p = 0.0012 S
	P1	41.71 % (334)	31.90 % (390)	p = 0.0076 S
	P2	26.35 % (422)	25.83 % (425)	p = 0.858 NS
	C1	23.97 % (406)	27.34 % (388)	p = 0.268 NS
	C2	20.03 % (427)	21.34 % (420)	p = 0.644 NS
<i>Anopheles gambiae</i>	G1	58.23 % (71)	61.17 % (66)	p = 0.733 NS
	G2	44.12 % (95)	45.29 % (93)	p = 0.865 NS
	P1	46.29 % (58)	40.74 % (64)	p = 0.509 NS
	P2	35.19 % (70)	31.48 % (74)	p = 0.614 NS
	C1	55.71 % (31)	68.57 % (22)	p = 0.327 NS
	C2	47.17 % (37)	51.42 % (34)	p = 0.732 NS

P < 0.05, S; P > 0.05, NS; () : number of mosquitoes.

G: "gobi" oil; C: coconut oil; P: palm oil.

1: 75 % of mixed oil to 25 % of excipient; 2: 50 % of mixed oil to 50 % of excipient.

Table II. – Impact of excipients on mosquitoes protection rates on field conditions with vegetable oil-based repellent products.

DISCUSSION AND CONCLUSION

The average protection times and the percentage protection obtained with the different formulations demonstrated their repellency. With citronella (*Cymbopogon* sp.) essential oil, Mc Alister (1949), Osmani *et al.* (1972) and Abu Shady *et al.* (1983) successively obtained, in the laboratory, protection times of 79, 240 and 150 minutes respectively against *Aedes aegypti*, *Culex fatigans* and *Culex pipiens*. Yao (2000) obtained, in the laboratory, protection times of 33, 58 and 186 minutes against respectively *An. gambiae*, *Ae. aegypti* and *Culex quinquefasciatus* with *Lippia multiflora* essential oil at a dilution of 10 %. The repel-

lency of our products is very short duration than those observed by Schreck (1977) with the DEET and the DMP respectively against *Ae. aegypti* (426 minutes) and *An. quadrimaculatus* (415 minutes).

In our case, the products were made with karite nut butter and used by populations in villages and that seem to give them satisfaction. For our part, we wanted to verify this effectiveness to scientific level. Besides, the vaseline being a neuter composite, we associated it in order to determine the role of the karite nut butter in the effectiveness of these traditional products. The following investigation is the research of actif ingredient and the formulation (cream, pommade or lotion) the better one for tropical environment.

The laboratory tests results indicate that formulations with karite nut butter excipient were more effective than the vaseline one. In fact, except formulations of palm oil-based to 50 % (F = 0.219; p = 0.645) and of coconut oil-based to 25 % (F = 0.535; p = 0.474), protection times of products with karite nut butter excipient against *An. gambiae* were higher than those obtained with vaseline excipient. With *Ae. aegypti*, except the palm oil lotion (P1) and the coco nut oil pommade (C3), palm and gobi oil-based formulations with karite nut butter excipient are more repellency than the one with vaseline. On the other hand, coconut oil lotion (C1) and cream (C2) with vaseline are more effective than the one with karite nut butter.

In some villages, populations used that products to protect themselves to the night during some ceremonies. Under the circumstances, we are decided to test our products by all night human landing catches in order to quantify their efficacy in that situation. Under field conditions, the excipient doesn't seem to have an influence on the effectiveness of formulations. In fact, except the gobi cream (G2; p = 0.0012) and the palm lotion (P1; p = 0.0076), protection factors obtained with the different products is comparable with the two excipients. The difference noted between laboratory and field results could be hand to tests conditions. The laboratory tests were carried in a room and began at latest at 19 o'clock. While the field tests was conducted out'doors and began at 22 o'clock in order to respect *An. gambiae* natural biting period. Nevertheless, according to Buescher *et al.*, (1983), the use of excipient in formulations preparation doesn't have habitually any impact on their effectiveness; only their concentration counts. In the laboratory conditions, the karite nut butter seems better than vaseline. Besides, looking at the field results, excipient have just had to do not influence to natural repellent products effectiveness in their simple formulation. Also, the sale price of a kilogram of vaseline being about 10 superior time to one of karite nut butter, the use of this last excipient, available locally, would allow for a reduce more the price to repellents product formulations.

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