Laboratory and field evaluation of MAÏA®, an ointment containing N, N-Diethyl-3-methylbenzamide (DEET) against mosquitoes in Burkina Faso

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Research

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Abstract

Background

Malaria vector control relies upon the use of insecticide treated nets and the Indoor Residual Spraying. However, as the emergency of insecticide resistance in malaria vectors grows, the effectiveness of these measures could be limited. Thus, alternative tools are needed. In this context, repellents can play an important role against exophagic and exophilic mosquitoes. This study evaluated the efficacy of MAÏA® , a novel repellent ointment, in laboratory and field conditions in Burkina Faso.

For the Laboratory and field assessment, twenty volunteers were enrolled and trained for nocturnal collection of mosquitoes using Human Landing Catches (HLC).

Methods

In the laboratory tests, 2 mg / cm^2 of treatments (either the MAIA® or the 20% DEET) were used to assess median Complete Protection (CPT) against two species that includes *Anopheles gambiae* and *Aedes aegypti* following the WHO guidelines. For both species two strains consisting of susceptible and local strains were used. The susceptible strains were Kisumu and Bora Bora respectively for *Anopheles gambiae* and *Aedes aegypti*. For the field test, the median CPT of the MAIA was compared to that of a negative (70% Ethanol) and positive (20% DEET) after carrying out human landing catches in rural Burkina Faso in both indoor and outdoor settings.

Results

Laboratory tests showed median Kaplan–Meier Complete Protection Times (CPT) of 6 hours 30 minutes for *Anopheles gambiae* (Kisumu), of 5 hours 30 minutes for *Anopheles gambiae* (Goden, local strain) and of 4 hours for *Aedes aegypti* for both the local and sensitive strain. Thus, these laboratory results suggest MAÏA® is a good repellent against the three mosquito species.

Field tests showed that median CPT of 20% DEET and MAÏA® were similar (8 hours) and longer than that of the negative control (2 hours).

During these field test, in the field a total of 3,979 mosquitoes were caught. In this population, Anopheline represented 98.5% and the culicine (*Aedes*) making up the remaining 1.5%. Among anopheline mosquitoes, 95% belonged to *Anopheles gambiae* complex, followed by *Anopheles funestus*, and *Anopheles pharoensis*. The median CPT of 20% DEET and MAÏA® were similar (8 hours) and longer than that of the negative control (2 hours).

Conclusion

Results from the present studies showed that MAÏA® offers high protection against Anophelines biting indoor and outdoor and could play an important role in malaria control in Africa.

Background

Malaria is one of the deadliest diseases in many low- and middle-income countries, affecting mainly children and pregnant women in sub-Saharan Africa (1). Long-Lasting Insecticide-treated Nets (LLINs) have been regarded as the most effective method for controlling mosquitoes transmitting the malaria parasites. Since 2000, about one billion of nets have been distributed in Africa resulting in an incredible decline of malaria-related deaths on the continent between 2000 and 2015 (2–4).

However, this massive use of insecticide in public health in addition to that in agriculture caused a growing concern of insecticides resistance (5–7) and change their behaviors (8,9) of malaria vectors. For example a study conducted in Papua New Guinea showed a shift in mosquito biting from night to earlier hours in the evening after a nationwide distribution of LLINs (10). Similar changes in the behavior of *Anopheles funestus* have been observed in Benin and Senegal after insecticide treated nets achieved high level of coverage (9,10). Furthermore, studies have described that the scaling up in LLINs and IRS have also led to more outdoor biting in the *Anopheles gambiaes.l.* commonly considered as endophagic mosquitoes (11–13). A recent study in the Cascades region in Burkina Faso showed high level of insecticide resistance (14) and that more than 50% of the major vectors, *Anopheles gambiaes.l.,* were collected biting outdoor (15). These altered biting patterns - outdoor, early evening and morning biting habits- of Anopheles combined with resistance to insecticides showed that the mass distribution of insecticide treated nets alone eventually leads to a reduction in the efficacy of these interventions (16,17). A recent study highlighted that an increase in early evening biting could increase transmission not only because people are unprotected by nets, but also because there is a higher chance of malaria vectors becoming infectious (18). The development of new vector control tools in addition to LLINs is therefore necessary to protect people whenever they are not under a bed-net.

Topical repellents could play an important role in addressing this problem if they are effective and accepted by the population. A systematic review of repellent interventions and mathematical modeling has shown that “user compliance” is indeed one of the most decisive factors for the success of this intervention (19). In sub-Saharan Africa, ointments are used primarily by mothers and children every time after the shower to moisturize their skins. In Burkina Faso, ointments are applied on 80% of children every evening when mosquitoes start transmitting malaria (*Kadidia Ouedraogo et al.*, *in preparation*). From this insight, Maïa Africa, a company based in Burkina Faso, developed MAÏA®, a mosquito repellent ointment uniquely designed with local mothers, to be used every day within their families. The underlying idea is to leverage existing habits of mothers to protect their families from infectious bites whenever they are not under a net.

If the MAÏA® ointment is effective and accepted by the population, it could play a key role in reducing the probability of children experiencing infectious bites during the evening and be positioned as a complementary intervention to LLINs.

The aim of this study is, to evaluate the effectiveness of MAÏA® in both laboratory and field conditions, especially the median complete protection time offered by the product. Results from these evaluations are important for validating how effective is this new repellent product as behavioral responses to repellent differ between wild mosquitoes' populations and laboratory reared mosquitoes' populations (20).

Methods

Study area

Laboratory tests were conducted in May 2019 in the insectary of Centre National de Recherche et de Formation sur le Paludisme in Ouagadougou (CNRFP) in Ouagadougou, Burkina Faso. Field tests were carried out at Goden (12°25'N, 1°20'W) a site located at 15 km in the north-east of Ouagadougou, the capital city of Burkina Faso (**Figure 1**). Goden, is a rural village with Sudanian Savanna climate and rainfall under 900 mm annually. The ~800 inhabitants mainly belonging to the Mossi ethnic group, are mostly devoted to agriculture and growing few animals (e.g. pigs, dogs, goats and chickens) within their compounds. LLINs were distributed in 2016 to approximately 90% of the population (N'F S and MWG, Unpublished data). Goden is known for its high density of malaria vectors due to its proximity to the Massili river. The field study was carried out during the rainy season (August to November 2019) corresponding to the high vector density and high malaria transmission period. A preliminary assessment of the mosquito density on the collection site was carried out using human landing catches before the tests started.

Human volunteers' preparation

Healthy adult's male volunteers aged between 18 and 40 years, were enrolled in this study. The volunteers were instructed not to use fragranced soaps, perfume, tobacco, nor alcohol 12 hours before the start and throughout the experiments. To establish the amount of repellent required for application in the experiments, the surface area of the arm (for laboratory tests) or the leg (for field tests) of volunteers was determined using the following formula:

$$\text{Area} = \tfrac{1}{2} (C_w + C_e) D_{we}$$

Here C_w is the circumference of the wrist or ankle in cm, C_e is the elbow cubital fossa or the knee circumference in cm and D_{we} is the distance in cm between C_e and C_w (21). The amount needed for each volunteer was then determined depending on their forearm or leg length. In addition, the quantity of product left in bottles was weighed using a precision weighing balance (KERN & SOHN GmbH) to determine the amount applied by each volunteer.

Repellents

The MAÏA® a shea butter-based ointment containing 15% DEET (N, N-Diethyl-3-methylbenzamide) was received from Maïa Africa SAS. It was tested against an ethanolic solution of 20% DEET as positive control) and a negative control of 70% ethanol. The DEET is known as the standard repellent reference.

Laboratory evaluation

Strains of mosquitoes

Four strains of mosquitoes were used in the laboratory tests. This including Kisumu F57 and Bora bora F58 susceptible strains of respectively *Anopheles gambiae* and *Aedes aegypti*. In addition, local strains laboratory-colonized from rural areas of Goden, in Burkina Faso were also used hereafter named as "An-Goden" (*An. gambiae* local strain, F418) and "Loc-Aedes" (local *Aedes aegypti,* F318). These species are being maintained under a 12:12 (light: dark) photoperiod. During rearing, larvae were fed on fish food while glucose was used for adults. The temperature and relative humidity in the rearing room were 25 - 28 °C and 60 - 80% respectively. Moreover, the individual mosquitoes used in these experiments were five to ten-day-old nulliparous females starved from sugar solution for twelve hours before the experiment.

Evaluation in the laboratory

The laboratory experiments were conducted following the WHO guidelines for the arm-in-cage test (21). Cages were 45 x 45 x 45 cm screen enclosures. Two test cages were used, one for the repellent candidate and the other for the positive control. The test cages contained 200, 5 to 10-day-old females of one of the four mosquito strains: Kisumu, *An*-Goden, Bora bora and loc-*Aedes*. The experiment in the laboratory was carried out on at temperature ranging between 25 and 28 °C with the relative humidity being between 60 and 80%.

Overall, 2 mg of ointment were applied per square-centimetre (cm^2) on left forearm of each volunteer. A steel spatula was used to apply the ointment on the forearm of each volunteer prior to each experiment for avoiding absorption of part of the ointments through applier's skin during the application. Though, the positive control consisting of 1ml of the 20% DEET solution was applied to the right forearm of each volunteer.

Before exposure to the mosquito probing, the forearm was washed with odorless soap, dried, rinsed using 70% ethanol solution and then dried again. All volunteers wore latex gloves to protect their hands from mosquitoes biting. To assess the readiness of the mosquitoes to land, both left and right cleaned forearms of volunteers were exposed in the experimental cages for 30 seconds (or until 10 landings of mosquito were counted). Then, for each volunteer, the right forearm was treated from wrist to elbow using 1 ml of the 20% DEET solution whilst the left forearm was treated from the wrist to elbow too with the MAÏA® ointment. Thirty minutes after application of the repellent, the participant exposed the treated forearm in the test cage for 3 minutes. The procedure was then repeated every 30 minutes until the first bite occurred and the elapsed time to the first bite was recorded. The test was performed three times for each volunteer per mosquito species. Considering the difference in the relative periods of biting activity of

each mosquito species, the tests using *Aedes Aegypti* strains were carried out during the day between 09:00 am to 18:00 pm, whereas that of *Anopheles gambiae* were conducted between 5 pm and 5 am (21).

Field evaluation

Here, the lower legs of volunteers were washed with neutral soap, rinsed with 70% of ethanol solution and naturally dried. Once their legs treated volunteers were also asked to avoid rubbing, touching or wetting the repellent-treated area. Then, 2 mg of the MAÏA® per cm^2 (2.4 ± 0.2 g per 1189 ± 79.2 cm^2) and 2 ml per cm^2 of the 20% DEET (2ml ± 0.1 ml per 1189 ± 79.2 cm^2, as a positive control) were applied to volunteers lower legs, from the knee to the ankle. A total of twenty volunteers were recruited from the Goden village and trained for the nocturnal mosquito's collection using Human Landing Catches (HLC). Each volunteer was later randomly allocated to one of the five groups (2 for the MAIA®, 2 for positive control and 1 negative control) of four volunteers according to the treatment received. The experiments took place at five different households at each night of collection that were at least 20 meters apart as per WHO guidelines (21). This, in order to avoid biases due to competition in attractiveness to the mosquitoes

Mosquito collection started thirty minutes following treatments. Here, volunteers acting as bait, sat on a chair by pair and actively collected mosquito that landed on their treated lower leg using mouth aspirator and flash torch(22) for 45 minutes followed by a fifteen-minute break. During this collection, volunteers wore long-sleeved shirts, buttoned at the wrist, long trousers, closed shoes and latex gloves with a hat on their head. However, the treated lower leg was exposed to mosquito probing by rolling out the trousers up to the knee. During these experiments, mosquitoes were collected simultaneously at both indoor and outdoor environments between 7 pm to 6 am. Here to avoid biases introduced by individual attractiveness and skills(23,24) volunteers at the same household rotated between indoor and outdoor hourly. At the each of the household two groups of two people were constituted. A group collecting from 6 to 12 pm and the second from 12 to 6 am. The treatment rotated between households following a Williams balanced Latin Square design.

Collected mosquitoes were transferred into plastic caps covered, using a piece of untreated net, with a small hole at the bottom to allow mosquitoes to be easily aspirated into them. After collection mosquitoes were brought to the entomological laboratory of the CNRFP and morphologically identified using a stereo microscope and the identification keys (25).

Side effects

No side effects were observed or reported by any of the volunteers throughout the period of tests both in the laboratory and in the field.

Ethical clearance

Written informed consent was obtained from all volunteers recruited and household owners in this study. The study was approved by the institutional ethic committee of CNRFP under 2019/000008/MS/SG/CNRFP/CIB.

Data analysis

All data were collected on standard forms and were entered twice in a database by different people. Databases have been compared using Epi Info ™ 3.5.3, and inconsistencies were verified using the printed and corrected forms. The performance of the repellent was measured by calculating the repulsive efficiency and the median full protection time.

A Generalized Linear Mixed Model was used to further analyze the effect of the location (indoor versus outdoor) on the performance of the treatments. We also assessed whether there was any variation in the average number of bites received between treatments.

The median complete protection time (CPT) is defined as the interval of time between the beginning of the collection/test and the first mosquito landing. To estimate the median complete protection time of each treatment, a Kaplan-Meier survival analysis was performed for each vector species and strain used in the lab experiments and on field data through 'survival function' from R software - version 3.5.0 (2018-04-23). However, for the field test, the analysis was performed only on *Anophelesgambiae s.l.* as it was the most abundant species collected (~96% of the total collection). The analysis consisted of assessing the median CPT and the repulsive efficacy. The repulsive efficacy was calculated as a percentage of repulsion (% R) according to the formula % R = ((C-T) / C) * 100, where C is the number of mosquitoes collected on the treated legs of the two control treatments separately, and T is the total number of stinging mosquitoes on the volunteers legs treated with the test product (21).

Results

Laboratory tests

Overall, under laboratory conditions the relative repellency (median complete protection time) was higher for both MAÏA® and 20% DEET against *Anopheles gambiae* compare to *Aedes aegypti* (Table 1). In lab, MAÏA® performed well in repelling the four mosquito species used in this study. The median CPT were respectively 6.5 hours for *Anopheles gambiae* (Kisumu), 5.5 hours for *Anopheles gambiae* (Goden, local strain) and 4 hours for *Aedes aegypti* for both the local and sensitive strain. There was no significant difference between the two treatments for each of the experiment (Kisumu: χ^2 =2.1, p value = 0.14; Goden: χ^2 = 0.8, p value = 0.36; Bora bora: χ^2 =1.7, p value=0.19; and *Aedes aegypti* (local strain): χ^2 = 0.9, p value = 0. 35) indicating that both MAÏA® and 20% DEET have equally repellency for these strains. The Kaplan- Meier curves for MAÏA® and the 20% DEET respectively for Kisumu, *An*-Goden), Bora bora and Loc-Aedes are shown in **Figure 2**.

Field test

Mosquito species composition and biting behaviours

A total of 3,979 mosquitoes, stratified by treatment and species (Table 2), were caught using HLC. Anopheline represented 98.5% of the total catch, with mosquitoes of the culicine (Aedes) making up the remaining 1.5%. Among anopheline species, 99.6% belonged to *Anopheles gambiae* complex, followed by *Anopheles funestus* (0.1%), and *Anopheles pharoensis* (0.3%). The frequency of mosquitoes landing on the treated collectors, compared with the control subjects, varied according to the repellent used (Table 2). The hourly mosquitoes biting rate varied significantly between treatments ($df = 2$, $\chi2 = 426.22$, $p < 0.0001$). An average of 0.68 (95% CI: 0.51 – 0.91) bites of mosquito was received per person per hour for MAÏA® compare to 1.01 (95% CI: 0.76 – 1.33) for 20% DEET and 8.98 (95% CI: 6.56 – 12. 29) for the 70% ethanol. In addition, there was no variation between treatments according to the location (outdoor and indoor, $df = 2$, $\chi2 = 1.703$, $p = 0.42$). Overall, the ratio outdoor: indoor biting was 1.26 (95% CI: 1.25 – 1.27) showing that more bites were taking place outdoors compared to indoors ($df = 1$, $\chi2 = 5.79$, $p = 0.016$).

Repellency against mosquitoes

The repellency against *Anopheles gambiaes.l.* was stratified by time of collection. From 18 hours pm to midnight am (6 hours after the application) the percentage of repellency varied from 100% to 90% for MAÏA® and DEET. Between midnight am to 3 hours am (9 hours after application) the percentage was between 90% and 80% (**Figure 3**). After 3 h am (10 hours after application) this percentage was under 80% for the 20% DEET but the MAÏA® was still over 80%. The MAÏA® gave a high percentage of repellency however, no difference in the repellency was observed between both treatments during the first 9 hours after their applications.

When data were stratified by location of the mosquito's biting, the trend was the same for indoor and outdoor. No difference was observed during the first 9 hours between the MAÏA® and the 20% DEET. Therefore, these results show that the MAÏA® can protect both indoor and outdoor.

Complete protection time

The median CPT of 20% DEET and MAÏA® were estimated at 480 minutes (8 hours) against 120 minutes (2 hours) for the negative control (Table 3).

When the median CPT is considered for outdoor collections it was respectively 480 minutes for 20% DEET and MAÏA®, and 120 minutes for Control (Table 4). For indoor collections, these estimates were 480, 450 and 60 minutes respectively for 20% DEET, MAÏA® and Ethanol (Table 4). Statistical analyses showed that there was no difference in the median CPT between 20% DEET and MAÏA® ($df = 1$, $\chi2 = 0.2$, $p = 0.7$). However, there was a significant difference between median CPT as estimated for MAÏA® and the control ($df = 2$, $\chi2 = 106$, $p < 0.0001$, **Figure 4**). Even when the collection was stratified by location this difference still occurred in both indoor (**Figure 5 (A)**; $df = 2$, $\chi2 = 41.6$, $p < 0.0001$) and outdoor collections; $df = 2$, $\chi2 = 66.7$, $p < 0.0001$) **Figure 5 (B)**.

Discussion

The results of this study demonstrated that MAÏA®, a shea butter-based ointment with 15% DEET provides high protection against mosquitoes in Goden, a rural area of Burkina Faso.

Both tests under the field and laboratory conditions suggested that MAÏA® has equal repellency effect as the 20% DEET ethanolic solution during the period of collection. Similar results were also found in both indoor and outdoor environments. The percentage of repellency when using the MAÏA® varied between 100% and 80% over the major malaria vector biting activities period which occurs between 6 pm to 6 am. The median CPT were also similar and estimated around 480 min. The MAÏA® and 20% DEET ethanolic solution were found to provide up to 90% of repellency during the first-six-hours after their applications. Furthermore, results suggested that using MAÏA®, the average hourly bites received is significantly lower (less than 1 bite per hour) compare to that of both 20% DEET and negative control. Overall, it can be argued that MAÏA® could provide protection to people before they go to bed.

Previous studies in the same locality comparing the repellency between three different repellents found that DEET, IR3535, and KBR 3023 were effective against *Anopheles gambiae* s.l. and other Afrotropical vector mosquitoes (26). In this study authors showed that protection from KBR 3023, DEET and IR3535 were still high against Anophelines for up to 10 hours' post-exposure. In contrast, results from the current study indicated that the relative repellency was 100% for ~ 8 h. However, our result was similar to that from recently study in Ethiopia comparing DEET (N, N-diethyl-1,3-methylbenzamide) and MyggA (p-methane diol) and other laboratory- products (20% neem oil and 20% chinaberry oil), the mean CPT was 8 hours for DEET whist estimated at 6 hours for MyggA (27). It can be argued that these 8 hours of repellency may be enough to protect against earlier vector biting at both indoor and outdoor before residents get protection from the insecticide-treated nets deployed indoors.

Today 11 countries are classified as having high burden of malaria (2) in the world. In these countries malaria vector control activities are still based on the use of insecticides either in the form of indoor spraying or by promoting the large-scale use of insecticide-treated mosquito nets. These strategies can effectively reduce the number of malaria cases (28), however the major challenge is still the resistance of malaria vectors to different classes of insecticides and the shifts in the feeding and resting behaviors with the tendency of biting and rest outdoors. For example, a study in the cascades region in Burkina Faso indicate that in addition to insecticide resistance, more than 50% of the malaria vector biting were taking place outdoor. Therefore, new and supplementary methods are urgently needed to complement these tools in the perspective of malaria elimination (29). In accordance with the spirit of locally adapt-integrated ways of vector and disease control (30), repellents can usefully complement existing control strategies and provide an additional tool in the management of insecticide resistance. In the context of the widespread resistant vector to insecticide and the tendency of mosquitoes to bite outside houses, there is need to add this MAÏA® ointment in the basket of vector control tools in Sub-Saharan malaria high burden countries. In addition, although contained only 15% of DEET, MAÏA® showed promising in protection against vectors biting.

The originality of MAÏA® comes from the formulation based on the local butter massively used in the rural area of West African and contributing to women economic income. This means that the promotion of use of local endogen strategies can also sustain the malaria control in these burden countries, but also improve the economic situation in African women with limited resource. Additionally, it has been shown that shea butter is a source of anti-inflammatory and anti-tumor promoting compounds (31). Another interesting compound in shea butter is cinnamic acid which is known for its antibacterial, antifungal, and antiviral properties (32). Shea butter is a wonderful product which both moisturizes and heals the skin. Clinical studies have shown it to be safe for skin (33). These indicate that MAÏA® ointment will not only protect from mosquito-borne diseases but also against other microorganisms.

Conclusion

MAÏA®, a novel ointment formulated with shea butter massively used in West Africa to moisturize the skin of children, showed high repellency against laboratory reared and wild malaria vectors. Therefore, in the context of the widespread vector resistance to insecticide and the growing tendency of mosquitoes to bite outside houses, there is need to add this MAÏA® ointment into the basket of vector control tools in in Sub-Saharan countries with high malaria burden.

Declarations

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Not applicable.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was approved by the institutional ethic committee of CNRFP under 2019/000008/MS/SG/CNRFP/CIB. All volunteers involved in this study were recruited upon a written

informed consent form and they were informed that they are free to leave the study at any time without any repercussion.

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Authors' contributions

GWM, NFS and AS designed the study, AT and GN conducted the laboratory and field data collection and drafted the manuscript, AS and GWM performed the data analysis, interpreted the results and participated in manuscript writing. NFS and AG contributed to the design of the study and provided comments upon the manuscript. FL provided comments upon the manuscript. FL and GN sought the funding to support all laboratory and field experiments. All authors read and approved the final manuscript.

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Tables

Table 1. Median Complete Protection Times (CPT) in minutes and their 95% Confidence Intervals (CI) against mosquitoes' strains according the tow treatments (20% DEET and MAÏA®) under laboratory conditions.

	An. gambiae Kisumu		*An. gambiae* Goden		*Ae. aegypti* Bora bora		*Ae. Aegypti* local	
	DEET	MAÏA®	DEET	MAÏA®	DEET	MAÏA®	DEET	MAÏA®
Median. CPT	390	390	300	330	270	240	240	240
Lower.CI	368	334	272	216	252	239	212	225
Upper.CI	412	446	328	444	288	241	268	255

Table 2: Total number of common mosquitoes collected after treatment of 20% DEET, MAÏA® and Ethanol 70%.

Mosquito's species	Treatment		
	DEET	Ethanol	MAÏA®
Anopheles gambiae sensu lato (s.l.)	686	2660	480
Anopheles funestus	1	2	1
Other anopheles	1	9	3
Culicine	7	106	23

Table 3: Estimated complete protection time (mn) with 95% CI, against *Anopheles gambiae s.l.* for 20% DEET, MAÏA® and Ethanol 70%.

	***Anopheles gambiae* s.l.**		
	DEET	Ethanol	MAÏA®
median CPT	480	120	480
Lower.CI	454	91	448
Upper.CI	506	149	512

Table 4: The estimated complete protection times (mn) with 95% CI, against *Anopheles gambiae s.l.* for 20% DEET, MAÏA® and Ethanol 70%, in indoor and outdoor.

	Anopheles gambiae s.l					
	Indoor			**Outdoor**		
	20%DEET	Ethanol	MAÏA®	20%DEET	Ethanol	MAÏA®
Median CPT	480	60	480	480	120	450
Lower CI	440	<60	440	447	95	428
Upper CI	521	NA	521	513	145	472

Figures

Figure 1

Study area

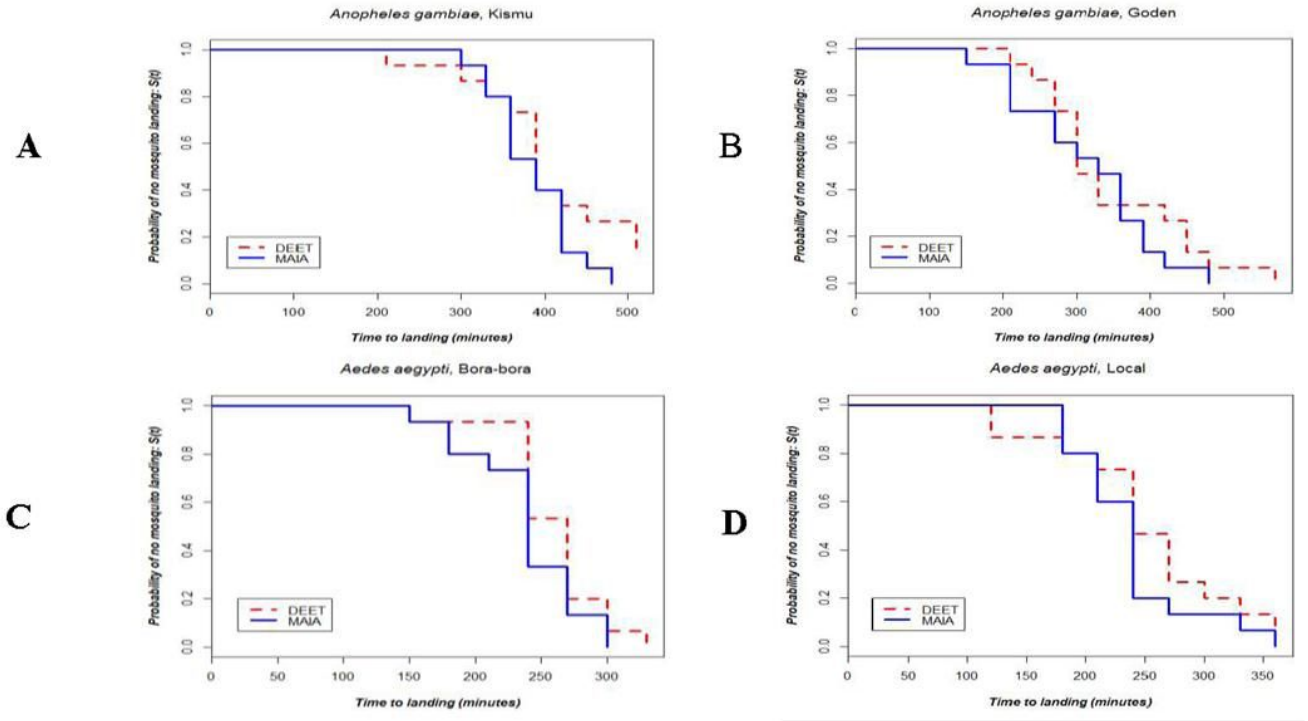

Figure 2

Kaplan–Meier plots for 20% DEET and MAÏA® tested against the four species on five volunteers.

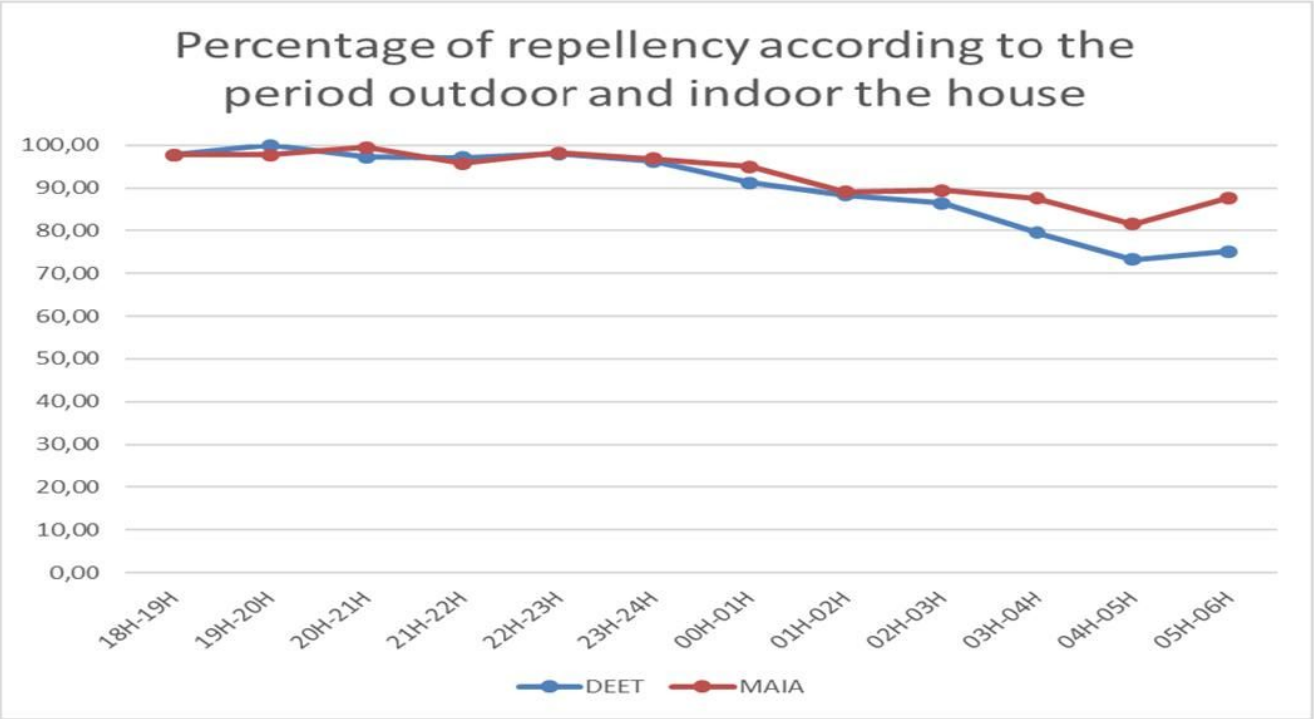

Figure 3

Repellency of 20% DEET and MAÏA® both indoor and outdoor collection.

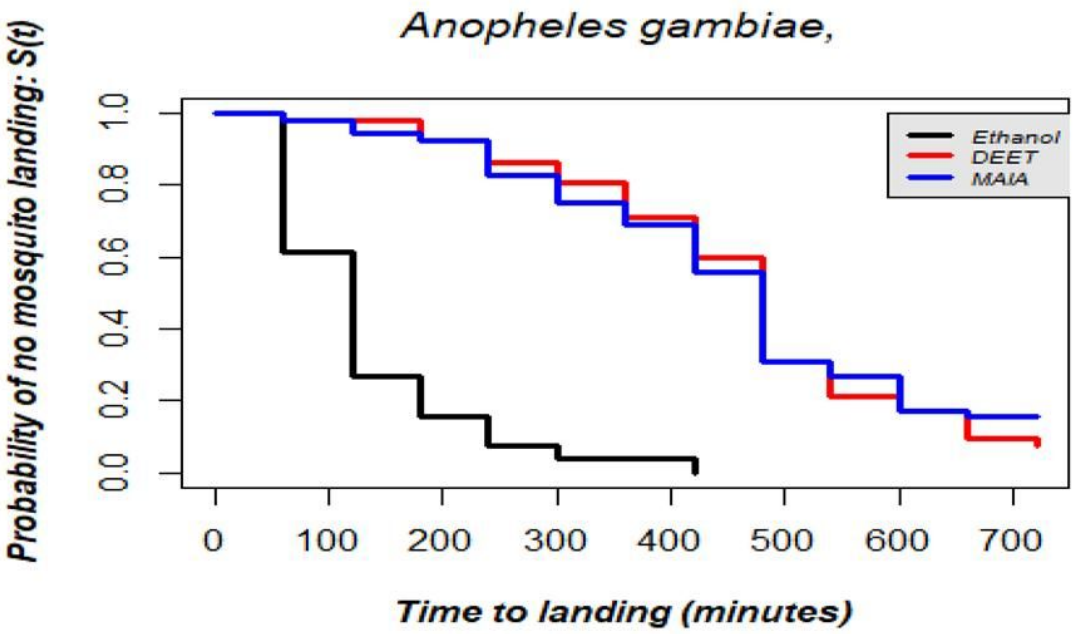

Figure 4

Estimate probability of no mosquito landing for each treatment according to the time when accounting for the effect of location (outdoor and indoor) of collections in the analysis.

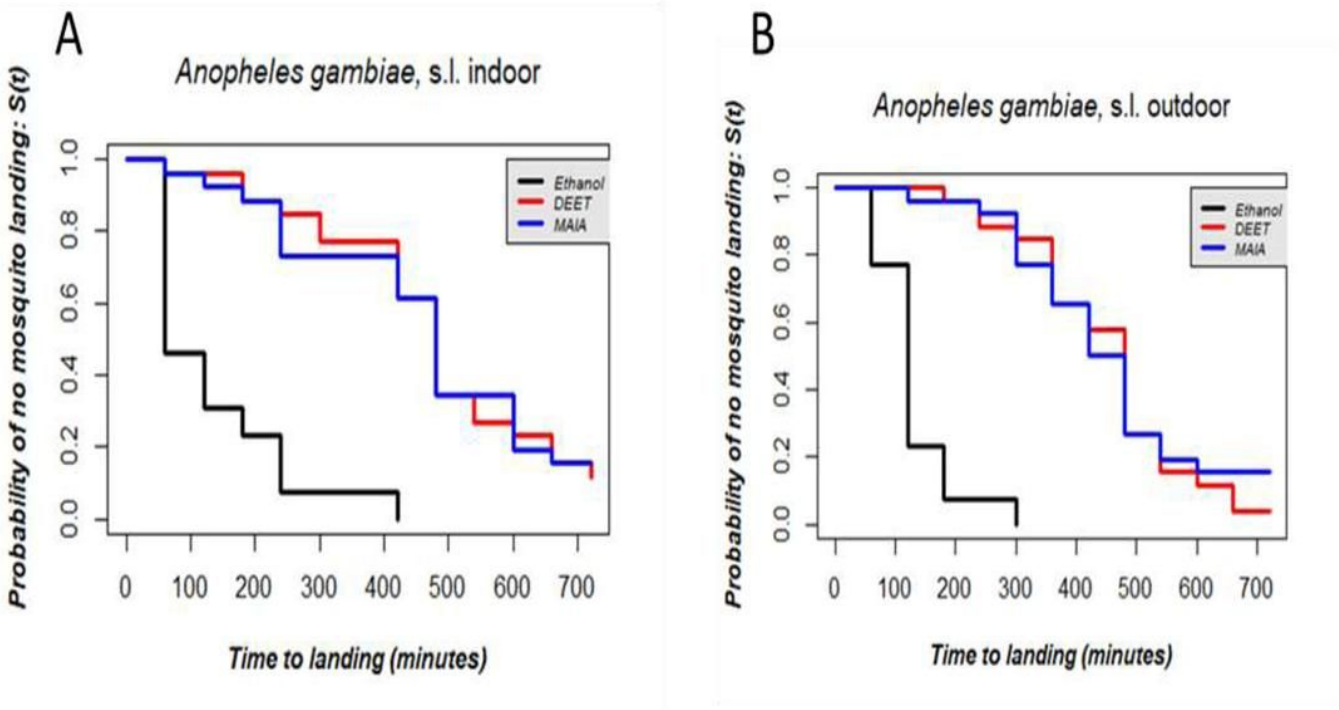

Figure 5

Estimated probabilities of no mosquito landing for each treatment according to the time at indoor (A) and outdoor locations (B).