Mosquito Repellency of Essential Oils Derived from Lao Plants

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1. Abstract

Essential oils or extracts obtained from four plant species growing in Laos were tested for anti-mosquito activity in the field. Solutions of alcohol or acetone containing different concentrations of essential oil; 5, 10 or 19 v/v %, were prepared and tested. Mosquitoes were collected by human baits wearing oil impregnated mosquito nets around their shanks. The number of attracted mosquitoes was compared to the number attracted to positive and negative controls, i.e., human baits wearing nets impregnated with 19 % N,N-diethyl-meta-toluamide (DEET, NN-diethyl-methyl-benzamide) or untreated nets, respectively. A one way ANOVA analysis was conducted on the log-transformed total number of mosquitoes collected using each treatment. The statistical analysis showed that significantly fewer mosquitoes were attracted to the treatment harbouring 19 % oil of *Scutellaria angustifolia* than to the negative control**.** No significant decrease in attracted mosquitoes could be detected for the treatments containing oil from the plant *Litsea cubeba.* However, a tendency of repellency was indicated. Therefore, an investigation running over a longer period of time is desired for *Litsea cubeba.* No conclusions could be drawn for the remaining two species; *Tagetes patula* and *Citrus sinensis.*

2. Introduction

Mosquitoes are vectors of many infectious agents causing potentially severe diseases, including malaria, dengue fever, filariasis, yellow fever and different types of encephalitis. Out of these diseases, malaria is the one with the largest impact on global health, being endemic in 109 countries and leaving half of the world's population at risk of contracting the infection. Malaria is transmitted by *Anopheles* mosquitoes and is caused by protozoan parasites of the genus *Plasmodium*. The disease is annually causing around one million deaths globally, striking hardest against young children and pregnant women in tropical developing countries (Roll Back Malaria Partnership 2009). Laos is one of the countries in which malaria is endemic where nearly 100 % of the cases are due to *Plasmodium falciparum*. This is the most virulent species of the malaria parasites, being responsible for the majority of the fatal cases around the world. It was estimated that approximately 60 % of the Lao population was in high risk of getting malaria in 2008, even though the confirmed cases of the infection decreased by over 50 % between 2000 and 2008 (WHO 2009). Therefore, there is still an urgent need of good preventive measures in order to control or eradicate the disease from this area.

Since there is no effective vaccine available against malaria, and because of an increasing problem with resistance against conventional anti-malaria drugs, vector control plays a very important role in the fight against this, and other, mosquito-borne infections. Prevention of mosquito bites by natural insect repellents has been used for hundreds of years, and commercial repellents have been on the market since the early 20th century. The first product containing N,N-diethyl-meta-toluamide (DEET) was released in 1956 and still today, around 50 years later, DEET-containing products are the most commonly used commercial mosquito repellents globally (Peterson and Coats 2001). Though DEET is considered to be rather safe and has proved to function excellent as a mosquito repellent, there are still a few drawbacks with the substance. There have for example been reports of toxic effects, including impacts on the central nervous system, following usage (for example Katz et al. 2008, Imperial College 2002 and Briassoulis et al. 2001). Furthermore, it is not recommended to use DEET-containing products during longer periods of time, which makes DEET a repellent more suitable for usage by tourists than local inhabitants living in malaria endemic areas.

Many plants have evolved aromas that repel insects and other herbivores (Langerheim 1994 and Pålsson and Jaenson 1999); making essential oils extracted from plants potential sources of anti-mosquito material. Eucalyptus and citronella oils are two well documented natural substances which have proved to be efficient mosquito repellents (for example Batish et al. 2008, Trongtokit et al. 2005). These oils have thus become ingredients in a number of commercial mosquito repellents (Thavara et al. 2002). Other species found to have resident anti-mosquito activities are for example *Syzigum aromaticum*, *Cymbopogon nardus, Pogostemon cablin* and *Zanthoxylum limonella* (Trongtokit et al. 2005).

Since both the flora and the mosquito reservoir differ among countries, evaluations of the anti-mosquito activity of numerous plant species are needed in order to find efficient, useful repellents for the people living in areas with high prevalence of mosquito-borne diseases. In this study, the mosquito repellency of essential oils or extracts from four plant species commonly found in Laos is evaluated through collection of mosquitoes using human bait.

3. Material and Methods

3.1.1 Selection of plant species

Before the beginning of these experiments, people from 63 villages in Laos were interviewed about their traditional usage of botanicals products as mosquito repellents (C. Vongsombath and H. de Boer, unpublished data). Based on the results from these interviews, the following plants were selected for oil extraction: *Croton caudatus*, *Litsea cubeba*, *Scutellaria angustifolia*, *Citrus sinensis* and *Tagetes patula.*

3.1.2 Collection of plant material

L. cubeba, and a small fraction of the material of *C. caudatus,* was collected in the Xaysomboun district, Vientiane province, Laos, in June 2009. This material was kept in the freezer until steam distilled in December 2009. The remaining amount of *C. caudatus* was brought from outside Vang Vieng, Laos, on 11th of December 2009. *T. patula* was bought at a market in Vientiane Capital, Laos, on 22nd of December 2009 and *S. angustifolia* was picked outside Fuang, Laos, on 25th of December 2009. Also *C. sinensis* was brought from outside Vang Vieng, Laos, but in November 2009. The material was refrigerated until steam distilled in December.

3.2 Extraction and preparation of the essential oils

As demonstrated in Table 1, the essential oils were obtained from the bark of *C. caudatus*, the fruit of *L. cubeba*, all parts of *S. angustifolia* and the peel of *C. sinensis*. Attempts were also done to extract oil from the flowers of *T. patula*.

An amount of 0.3-1.1 kg at a time of the chosen plant parts was cut into small pieces and placed in a distillation flask. Water was added until approximately 80 % of the material was covered, whereupon the flask was placed in a distillation chamber and heated until boiling. Steam distillation was carried out for 2 hours, before the oily phase of the distillate was collected in a glass tube. The number of distillation sessions for the different species depended on the yield of essential oils. The extracted oils were stored refrigerated (in about +4 ^{o}C) until their mosquito repellent capacities were tested in the field.

Table 1: Yield of essential oils obtained from steam distillation of plant material from five different Lao plant species.

Species	Parts used	Fresh weight (kg)	Volume of oil (ml)	Yield (ml oil/kg material)
L. cubeba	Fruit	2.4	45	19
S. angustifolia	All parts	1.3	6	4.6
C. sinensis	Peel	2.0	4	2.0
C. caudatus	Bark	2.1	2	0.95
T. patula	Flowers	1.0	0	0.00

Since the yield of oil from *C. caudatus* was so low (Table 1), the species was removed from the experiment and its potential as a mosquito repellent therefore remains to be tested.

Steam distillation of *T. patula* did not generate any oil (Table 1); therefore extractions of the flowers were prepared in three solvents possessing different polarity characteristics.

A total of 100 g of the plant material was put into either 100 ml of water, hexane or 70 % ethanol. The mixtures were left standing at room temperature for one week. The plant material was removed from the solvents through two filtration sessions; one using a mosquito net and the other using filter paper.

The oils to be tested in the field were diluted in 99.5 % ethanol (*L. cubeba*) or in pure acetone (*S. angustifolia*, *C. sinensis*) until each one had a final volume of 12 ml and final oil concentrations of 5, 10 and 19 v/v %. To avoid rapid evaporation when applied to cloths, an additional volume of 20 µl coconut oil was added to each of the nine solutions.

3.3.1 Night-time experiment

The mosquito repellency of the essential oils extracted from *L. cubeba* and *S. angustifolia* was evaluated in the surroundings of the village Ban Nalang (Fuang district, Vientiane province, Laos) between 25th and 29th of December 2009. During three hours each night (6 p.m to 9 p.m.), two groups consisting of five volunteers each served as human bait and collected mosquitoes that landed below their knees. The other parts of the bodies, except hands and head, were covered by clothes. Test tubes and torches emitting red light were used during the collection. The mosquitoes caught were transferred to plastic boxes in which a wad of cotton soaked in ethyl acetate was put, resulting in death of the insects.

All members in both groups had before the beginning of the collection washed their legs with water and soap and tied a mosquito net (approximately 30 · 50 cm) to their shanks. Three persons in each group had both of their nets impregnated with 0.5 ml of the solutions containing 5, 10 or 19 v/v % of the essential oil in question. Besides, one person in every group served as a negative control and used untreated nets, while the remaining ones were positive controls and wore nets impregnated with Myggspray US 622 (Swedish commercial mosquito repellent) containing 19 % DEET. The volunteers switched treatment every night, so everyone in the groups tested all the concentrations of the oils and was both negative and positive controls before the experiment was completed.

Scattered trees and houses were present in the area in which the experiment took place. The volunteers were not sitting closer than 5 metres from each other. During the five days of the experiment, the temperature ranged from 24.0 to 27.3°C at the starting time and from 17.0 to 19.0°C at the time for finish. The humidity increased towards the ending time for the experiment every day and was never below 36 %, nor above 68 %. There was no wind or rain at the time for the experiment.

3.3.2 Day-time experiment

The same methods and procedures as for the night-time experiment were applied when testing the repellency of extracts of *T. patula* (diluted in hexane, ethanol or water) and the essential oil extracted through steam distillation of *C. sinensis*. However, these tests were performed during day-time in the Huay Yang reservation forest (15 km outside Vientiane Capital, Laos) between the 3rd and 5th of January 2010. During the initial two days, two collections of three hours each were carried out per day; one between 9 and 12 a.m. and one between 2 p.m. and 5 p.m. During the period before noon, the temperature ranged from 23.9 to 28.0°C and the humidity from 42 to 61 % whereas on the afternoons, it ranged from 27.1 to 31.4°C and from 32 to 53 %, respectively. There was no rain or wind at the time for the experiment.

3.4 Identification of mosquitoes

After the completion of the field testing, time was spent on identification of the collected mosquitoes. For this purpose, the computer program KeyMosq98 (Illustrated Key of Mosquitoes in Thailand, developed by the Department of Medical Science, Ministry of Public Health, Thailand, 1998) was used. Mosquitoes that proved difficult to identify were brought to the Malaria Control Centre, Vientiane, for comparison with specimens in their collection.

3.5 Calculations and statistical analyses

The mosquito repellency was calculated for all the different concentrations and solutions of the tested plant products through usage of the following formula:

% Repellency = [(No. control – No. test product) / No. control]* 100 (Pålsson and Jaenson 1999)

The efficacies of the oils as mosquito repellents were evaluated through a one-way ANOVA analysis of the log-transformed total number of mosquitoes caught for each treatment. If significant differences between the treatments were found, they were tested by Tukey's test. The same methods were used to assess if there were any significant differences between the days on which the experiments took place and in the number of mosquitoes attracted to the individuals functioning as human bait, respectively. Dissimilar data generated by one volunteer evaluating *S. angustifolia* was removed before performing the analyses, meaning that 56 collected mosquitoes were excluded.

On the data from the night-time experiment, separate ANOVA-analyses were also run on the log-transformed total number of mosquitoes belonging to the genera *Culex*, *Anopheles* and *Mansonia*.

3.6 Theoretical experiment

Based on the data collected in the field, a theoretical experiment was conducted in order to simulate what efficacy of the oils, obtained from *S. angustifolia* and *L. cubeba*, that would have been observed if the field work had proceeded for twice as many days. It was assumed that the same number of mosquitoes as recorded originally would have been attracted to the different treatments in a prolonged study; i.e. ANOVA analyses were performed in the same manner as before but on duplicates of the datasets.

3.7 Chemical analyses of the essential oils

Gas chromatography was performed to analyse the chemical compositions of the essential oils of *L. cubeba* and *S. angustifolia*. The analyses were run in a Varian 3400 gas chromatograph (Varian, Palo, Alto, CA) containing a DB-Wax capillary column of the length 30 metres, an inner diameter of 0.25 mm and a film thickness of 0.25 μm (produced by J&W Scientific, USA). The chromatograph was linked to a Finnigan SSQ 700, releasing electronic ionization. The analyses were conducted using the ion source at 150°C. The gas used was helium, having an inlet pressure of 67 kPa.

During the first minute of the analysis, the initial temperature of 40°C was unaltered. It was later increased by 5°C per minute until reaching 220°C, where status quo was allowed for 12 minutes. An injector temperature of 215°C was used. The retention times of the essential oils' volatiles were the basis for their separation. Identification was enabled using the reference databases Nist Library and Massfinder.

4. Results

4.1 Night-time experiment

4.1.1 Abundance and distribution of mosquito genera

A total number of 122 mosquitoes was collected (3 escaped) by the group evaluating the mosquito repellency of *S. angustifolia*. Out of the collected ones, 109 were identified to genus or species. As visualized in Table 2 the majority belonged to the genera *Mansonia*, *Anopheles* and *Culex*. The group testing *L. cubeba* collected 148 mosquitoes (24 escaped). Out of these, 132 were identified to genus or species. The majority belonged to the same genera as those collected by the other group (Table 2). A one-way ANOVA confirmed that there was no significant difference in catching efficiency among the collectors.

Evaluation of the distribution of mosquito genera through a one-way ANOVA demonstrated that there was no significant difference in the number of *Anopheles* and *Mansonia* attracted to any of the different *S. angustifolia* treatments. However, a significant difference could be detected for the number of *Culex* ($F=7.95$, $df=4, 19$, $P=0.001$). Tukey's test revealed that the negative control in this case differed significantly from all the other treatments, being greatest when compared to the solution with the highest concentration of oil. However, no significant difference in either the number of attracted *Anopheles*, *Mansonia* or *Culex* could be demonstrated for any of the different *L. cubeba* treatments.

Table 2: Proportions and numbers of mosquitoes in different genera, collected by all the human baits using cloths impregnated with essential oils of *S. angustifolia* and *L. cubeba*.

Genus	Fraction (%)		No. of mosquitoes	
	S. angustifolia	*L. cubeba*	*S. angustifolia*	*L. cubeba*
Armigeres	0.00	2.70	0	4
Aedes	0.00	0.00	0	0
Anopheles	15.6	25.7	19	38
Culex	30.3	24.3	37	36
Mansonia	43.4	36.5	53	54
Unidentified	10.7	10.8	13	16

4.1.2 Efficacy of L. cubeba *and* S. angustifolia *as mosquito repellents*

The total number of mosquitoes attracted to the differently treated mosquito nets, including both the collected and the missed ones, is along with the plants' calculated mosquito repellency shown in Table 3. Notable is that the negative control in the group testing *L. cubeba* on average attracted at least twice as many mosquitoes as the others. In the case of *S. angustifolia*, the difference between the negative control and the other treatments is even greater; in comparison to the 19 % oil solution, the negative control attracted nearly twelve times as many mosquitoes.

Table 3: Total number of mosquitoes attracted to human baits. The baits were using differently impregnated shank cloths, according to the demonstrated scheme. The calculated mosquito repellency is shown for each treatment.

Treatment	No. of mosquitoes		Repellency (%)	
	Total	Per occasion	Non-adjusted	Compared to 19% DEET
L. cubeba 5 %	24	4.8	64.7	91.6
L. cubeba 10%	26	5.2	61.8	87.5
L. cubeba 19 %	34	6.8	50.0	70.8
DEET, 19%	20	4.0	70.6	100
Negative control	68	14	---	---
S. angustifolia 5 %	25	6.3	64.8	73.0
S. angustifolia 10 %	15	3.8	78.9	88.9
S.angustifolia 19%	6	1.5	91.5	103
DEET, 19%	8	2.0	88.7	100
Negative control	71	18	---	---

The differences between the number of mosquitoes attracted to the treatments in the group testing *L. cubeba*, were through a one-way ANOVA shown to be non-significant (F=1.42, df=4, 24, P=0.265). In contrast, it could be confirmed that there indeed was a significant difference between the treatments in the group testing *S. angustifolia* (F=4.70, df= 4, 19, P = 0.012). Tukey's test further revealed that the positive control and the solution containing the highest concentration of oil were clearly separated from the negative control, as shown in Fig. 1.

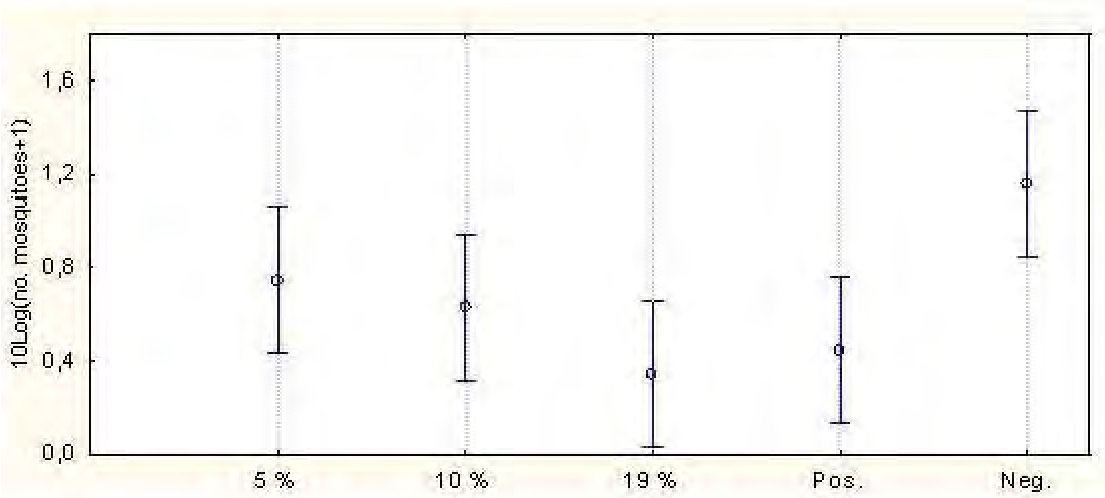

Fig. 1: The log-transformed total number of mosquitoes subjected to Tukey's statistical test. The vertical bars show the 95 % confidence interval. The mosquitoes were caught when trying to take a blood meal from the legs of human baits covering their shanks with mosquito nets either impregnated with different concentrations of essential oil of *S. angustifolia,* or with 19 % DEET (pos). The negative control group (neg), using untreated nets, is also shown.

4.1.3 Simulation of the anti-mosquito activity of S. angustifolia *and* L. cubeba *during a longer period of field work*

In the theoretical experiment aiming to investigate how it possibly would look like if more data had been available, new one-way ANOVAs and additional Tukey's analyses were performed on duplicates of the data sets. With this amount of data, the number of mosquitoes attracted to the treatments containing both 5 %, 10 % and 19 % of *S. angustifolia* was significantly less than the number attracted to the negative controls (F=10.96, df= 4, 39, P<0.0005). The same scenario could be demonstrated for the *Culex* mosquitoes alone (F=18.54 df=4, 39, P<0.0005). For *Mansonia*, a significant reduction of attraction was observed for the 19 % oil treatment (F=9.00, df=4, 39, P<0.0005). It could further be demonstrated that there were, in comparison to the negative control, significantly fewer *Anopheles* mosquitoes attracted to all of the different *S. angustifolia* treatments (F=6.81, df= 4, 39, P<0.0005). The results from all the Tukey's test here described is shown in Fig. 2.

For *L. cubeba*, analysis of the duplicated data set showed that there was a clear difference in mosquitoes number between the negative control and the treatment constituting of 5 % essential oil (F=3.19, df=4, 49, P=0.022).

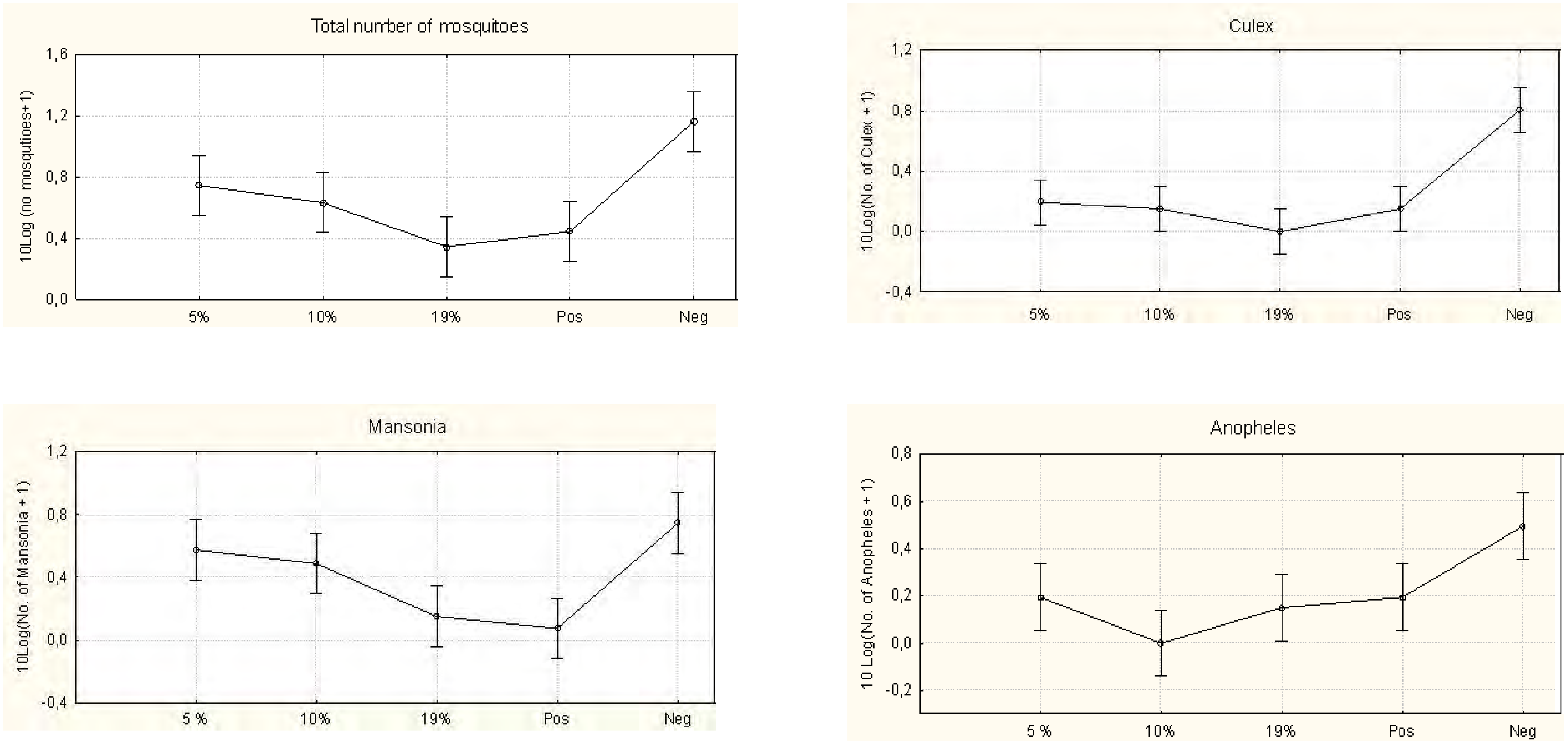

Fig. 2: Simulation of the differences in mosquito attraction to human baits covering their legs with mosquito nets impregnated with different concentrations of essential oil of *S. angustifolia*. The positive and negative control groups are shown. Data from five days of mosquito collection have here been duplicated, log-transformed and subjected to one way ANOVA analyses followed by Tukey's statistical test. As seen in the graphs, the tests show that solutions of *S. angustifolia* both repel mosquitoes in general and those belonging to the genera *Culex*, *Mansonia* and *Anopheles*. The horizontal bars show the 95 % confidence intervals.

4.2 Day-time experiment

4.2.1 Abundance and distribution of mosquito genera

The group evaluating *C. sinensis* collected 145 mosquitoes (and 10 escaped). A total number of 136 of the specimens were identified to genus or species. The vast majority belonged to the *Aedes* genus (Table 4). The group investigating the potential of *T. patula* as a mosquito repellent collected 104 mosquitoes. Out of these, 92 were identified to genus or species.

Table 4: Proportions and numbers of mosquitoes in different mosquito genera collected by human baits using cloths impregnated by substances from *T. patula* and *C. sinensis*.

Genus	Fraction (%)		No. of mosquitoes	
	T. patula	*C. sinensis*	*T. patula*	*C. sinensis*
Armigeres	18.3	10.3	19	15
Aedes	52.9	60.0	55	87
Anopheles	6.70	15.2	7	22
Culex	10.6	8.30	11	12
Mansonia	0.00	0.00	0	0
Unidentified	11.5	6.20	12	9

4.2.2 Efficacy of C. sinensis *and* T. patula *as mosquito repellents*

Neither essential oil extracted from *C. sinensis* nor extracts of *T. patula* could be shown to work as mosquito repellents, as demonstrated in Table 5. Notable is that, in the group testing *C. sinensis*, the calculated repellency of the positive control only reached 38.5 %. This is a result of the similar amount of mosquitoes collected by the positive and negative controls.

Table 5: The total number of mosquitoes attracted to human baits during five test sessions. The baits were using differently impregnated shank cloths, according to the demonstrated scheme. The calculated mosquito repellency is shown for each treatment.

Substance	No. of mosquitoes		Repellency (%)
	Total	Per occasion	
C. sinensis 5 %	43	8.6	0.00
C. sinensis 10%	18	3.6	30.8
C. sinensis 19%	53	10.6	0.00
DEET, 19%	16	3.2	38.5
Negative control	26	5.2	---
T. patula - ethanol	25	5.0	0.00
T. patula - hexane	32	6.4	0.00
T. patula - water	21	4.2	12.5
DEET, 19%	6	1.2	75.0
Negative control	24	4.8	---

4.3 Chemical composition of the essential oils

The results from the gas chromatography suggest that the essential oils extracted from both *L. cubeba* and *S. angustiolia* to a great extent is constituted of limonene. Other compounds found in both species are α-pinene, eucalyptol and camphene (Table 6). The analysis also showed that 26.7 % of the oil of *S. angustifolia* consists of fenchone, a compound which was not detected in *L. cubeba*.

Table 6: The chemical volatile constituents of the essential oils of *S. angustifolia* and *L. cubeba*.

S. angustifolia		***L. cubeba***	
Substance	Fraction (%)	Substance	Fraction (%)
Limonene	30.3	Limonene	48.1
Fenchone	26.7	4(10)-Thujene	9.50
α-Pinene	11.9	α-Pinene	8.62
Eucalyptol	9.20	6-Methyl-5-heptene-2-one	6.19
Camphene	6.92	β -Pinene	5.92
		Eucalyptol	4.16
		β -Myrcene	2.79
		Camphene	2.15
		β -Citral	2.02
		α-Citral	1.55

5. Discussion

In this experiment, it could be shown that *S. angustifolia* possesses potential mosquito repellent abilities, preventing bites from mosquitoes belonging to the genus *Culex* more than from those belonging to *Anopheles* or *Mansonia*. The difference in mosquito attraction, which was demonstrated to be significant, did only concern the treatment containing the highest concentration of essential oil; i.e. 19 v/v %. Interesting is that the calculated repellency of the 19 % *S. angustifolia* solution exceeds that of the positive control by giving as high protection as 91.5 %. The treatment containing 19 % DEET did in this case only provide 88.7 % protection.

No significant difference could be detected between the treatments within the group evaluating the mosquito repellency of *L. cubeba*. This means that not even the positive control was significantly separated from the negative control. Nevertheless, a clear tendency towards fewer attracted mosquitoes could be seen for the oil-containing treatments, as well as for the positive control, suggesting that there were too little data available to significantly detect eventual differences in mosquito attraction. It is worth to mention that this was the case also for *S. angustifolia* before the dissimilar data generated by one of the volunteers were removed.

A possible explanation to why the dataset of *S. angustifolia* was found to be much better when excluding one of the test persons is that the mosquito nets used might have been unevenly impregnated, leaving spots on the nets free from treatment. Mosquitoes are attracted to humans by a wide range of stimuli including body odours composed of carbon dioxide, lactic acid and moisture. Depending on the composition of these factors, individuals become more or less attractive to the insects (Lane and Crosskey 1996). In this case, the proximity to repellents might not have influenced the mosquitoes' biting behaviour to the extent that they always abstained from taking blood meals on repellent free spots on an otherwise attractive individual. The same scenario might also have taken place for the group testing *L. cubeba*. For the positive control in this group, the data generated resulted in a calculated repellency of only 70.6 %. As stated earlier, this was not enough for being significantly separated from the negative control. The efficacy of the solution containing 5 % oil correlated to 91.6 % of the protection provided by 19 % DEET, implying that *L. cubeba* should not be considered as non-functioning until more studies have been conducted. However, it has been reported elsewhere that neither solutions containing 10 % or 50 % of essential oil of *L. cubeba*, nor undiluted oil have the ability to completely repel the day-time biter *Aedes aegypti* (Trongtokit 2005).

To further investigate how *S. angustifolia* and *L. cubeba* may function as mosquito repellents, new analyses were run on duplicates of the datasets. It was assumed that the same number of mosquitoes would have been attracted to the different treatments if the experiment would have proceeded for twice as many days. The statistical analyses then showed that a significant decrease in mosquito attraction could be detected, also without removal of any data (data not shown), for all the tested treatments containing essential oil extracted from *S. angustifolia*. It could further be demonstrated that also *Anopheles* mosquitoes would be less keen to take blood meals if subjected to the treatments containing 10 and 19 % of oil. In the case of *Mansonia,* this was only found to be true for the highest concentration of oil. Also the 5 % treatment of *L. cubeba* attracted significantly fewer mosquitoes than the negative control. These implications are of course very promising and suggest that longer periods of field work are essential in order to generate reliable results during the dry season when relatively few mosquitoes are active. However, it is important to keep in mind that the assumptions done concerning the mosquitoes' biting behaviour are not necessarily reflecting the reality. Nevertheless, these findings unhesitatingly show that there are strong reasons to more thoroughly conduct research on the plant species mentioned.

When analysing the results obtained from this study, it is important to remember that the highest concentration of essential oil does not exceed 19 %. The reason for this is that the efficacy of the natural repellents, for practical reasons, should be directly comparable to the positive control, which in this case was constituted of 19 % of the active substance DEET. However, since the oils consist of a range of compounds, the active substances themselves do not constitute 19 % of the solutions. Therefore, this comparison does not have scientific relevance, but is solely restricted to comparisons of practical nature. For example, for the people living in Laos, it can be of high importance to know how effective a solution containing 19 % of essential oil is in comparison to a commercial, probably costlier, product. It is however probable that a higher concentration of the oils would generate an even greater effect, both for *S. angustifolia* and *L. cubeba*.

The chemical analyses of the volatiles from *S. angustifolia* and *L. cubeba* showed that a great portion of the oils is composed of limonene. Also α-pinene, eucalyptol and camphene could be recovered from both of the oils. All these compounds have previously been described as components of essential oils functioning as mosquito repellents (for example Jaenson et al. 2006 and Gillij et al. 2007); demonstrating that they do have important roles to play within this field. The relevance of conducting more studies on both *S. angustifolia* and *L. cubeba,* that both contain these compounds, is therefore indisputable. Interesting is also that 26.7 % of the essential oil of *S. angustifolia* are composed of fenchone, a compound that could not be recovered from *L. cubeba*. Also fenchone has been described as a compound found in essential oils repelling mosquitoes (for example Odalo et al. 2005). If a synergetic effect among the compounds found in *S. angustifolia* is the reason to why this species generated better results than *L. cubeba* remains to be tested.

No repellent effect could be demonstrated for *T. patula* or *C. sinensis*. However, noteworthy is that the positive control in the group evaluating *C. sinensis* only reached a calculated repellency of 38.5 %. This is partly a result of few collected mosquitoes by the negative control, against which the positive control was compared. During the day-time test sessions, it could also be noted that the mosquito activity differed remarkably within the forest in which the experiment took place. Therefore, the volunteers sitting in one area saw, heard and collected quite a lot of mosquitoes at the same time as others sitting elsewhere did not see a single one throughout the day. Therefore, it would have been desirable to have fixed positions that the volunteers rotated around. No conclusions can therefore be drawn from the data generated during day-time, since the lack of a stable study design can be considered to have influenced the results.

In conclusion, the results from the night-time experiment suggest that essential oil from *S. angustifolia* can be of great importance in prevention of mosquito-borne diseases such as Japanese encephalitis, bancroftian filariasis, malaria and brugian filariasis. The infectious agents causing these diseases are transmitted by mosquitoes belonging to the genera here investigated; the agents causing Japanese encephalitis and bancroftian filariasis are transmitted by *Culex*, the malaria-causing parasites by *Anopheles* and the pathogens causing brugian filariasis is vectored by night-biting mosquitoes such as *Anopheles* and *Mansonia* (Service 2008). However, all mosquito species belonging to the same genus does not necessarily behave in a comparable way (Service 2008); therefore *S. angustifolia* should be subjected to further, more thorough, studies in which also this fact is taken into consideration.

6. Acknowledgments

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