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IN SITU TEST: COTTON SHEETS AGAINST MOSQUITO BITES IN INDIA.

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ABSTRACT

Historically, fabrics were considered as a source of warmth and protection against weather conditions. Nowadays, fabrics can be converted into smart textiles and through this process new properties are conferred to them. For that purpose, microcapsules can play an important role in that they can be used within many application areas including medicine or pharmaceuticals. Malaria, dengue fever and other diseases are typically spread through mosquito bites. This is a concern of many authorities in affected countries and significant research is being conducted today in order to reduce incidence. The aim of the study reported here is not only to demonstrate the effectiveness of microcapsules on cotton fabrics as a prevention to mosquito bites but also to test this in situ. Different fabrics were prepared and tested in two Indian regions. Laboratory tests were performed according to a standard designed by the Swiss Tropical laboratory. Results demonstrated that the fabrics´ repellence to mosquitos could be considered as very good and that the repellent effect of the microcapsules was maintained for more than 10 laundry cycles. Furthermore, our experiments conducted in situ confirmed the effectiveness of the technology.

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KEY WORDS

Fabric, mosquito, repellent, microcapsules, in situ.

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INTRODUCTION

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Humans interact with insects. They are a part of the natural world and there are thought to be between 5 to 10 million specices, of whom only 1 million have been identified. Microcapsule technology can confer a number of different properties into faric, one of which is mosquito repellence. For the purpose of mosquito repellence, microencapsulation has been demonstrated as one of the most effectives methods (Moreti et al. 2004, Maji et al 2007). Microcapsules have been used for many different applications including in medicine (Moreti et al. 2004, Maji et al 2007), cosmetics ((Moreti et al. 2004), pharmaceuticals (Berger et al 2004, Muzzarelli et al 2004, Majeti, Ravi Kumar 2000, Senjković, Jalŝenjak, 1981, Magnin et al 2004, Hatefi, Amsden 2002, Berger et al 2004) food (Heinzelmann et al 1999, Wibowo et al 2005, Downham 2000, Dewettinck, Huyghebaert 1999, Wen-Tao et al 2005, gouin 2004) etc. However, since Nelson Gordon (Nelson 19991) published his paper regarding the possibility to incorporate microcapsules into fabrics, there have been many articles focused on different issues such as the fabric application procedure (Monllor et al 2007, Bonet-Aracil et al 2015, Bonet-Aracil 2012) and the microencapsulation procedure (Carvalho et al 2016, Hirech et al 2003).

Nowadays it is well known that there has been an increase in insect resistance to insecticides. Mosquito bites can be mild, producing some itching, but such bites can lead to the transmission of illnesses such as dengue fever or malaria (Lee et al 2016, Carter, Mendis 2002). According to the World Health Organisation nearly half of the world's population is at risk of malaria. In 2015, there were roughly 212 million malaria cases and an estimated 429 000 malaria deaths (WHO 2015).

Different products are used as active core for mosquito repellence. Some of them came from natural oils such as citronella oil (Solomon et al 2012), geraniol and lemon eucalyptus (Liu et al 2014), other products are focused on synthetic chemicals such as the N,N-diethyl-3-methylbenzamide, which has been demonstrated as the most effective product for mosquito repellence (Liu et al 2015, Kamsuk et al 2007), picaridine (Thavara et al 2002) , N,N-diethyl-m-touluamide (DEET) or permetrin.

One suitable support for repellence products could be textile fabrics which include the active product for repellence. Permetrin and DEET are the most common repellents used with textiles (Anuar, Yusof 2016). Some studies have considered not only the level of repellence but the weathering of the fabric. In these studies, we have seen how the effect of teh repellent diminishes as time goes by, particularly where tropical weather has been simulated (Gupta et al 1989). There are different tests to determine repellency efficiency (Anuar, Yusof 2016). Some of them are based on female mosquitos kept without any food for a period of time (minimum 4 hours), then later placed in a box by a gate on one side and on the opposite side there is another gate (exit) where they can find some food. The fabric is placed on the top of the box so that mosquitos must fly over the fabric to reach the exit gate. From this basis, the number of mosquitos that pass through the fabric in order to find some food are evaluated. Another method described is called the cone test. Mosquitos are allowed to come in through the narrow part of a cone, with the fabric placed on the opposite side. The number of mosquitos placed on the fabric in a period of time is evaluated. Finally, Anuar et al. describe a test where the arm covered with a fabric is placed inside a box and the number of mosquitos bites placed on the arm are registered (Anuar, Yusof 2016).

Some tests are based on the effect the particle size can have on the release (Liu et al 2015) or the time necessary to release a quantity of active ingredient (Solomon et al 2012). The aim of this paper is to compare the effectiveness of cotton fabrics for mosquito repellence when treated with microcapsules. The main features of this paper would be a comparison between laboratory tests and in situ tests. Some cotton fabrics were prepared. Part of these fabrics were sent to a laboratory and some sheets were sewed and sent to India. In situ tests were performed in two areas.

Results showed both tests demonstrated high repellence levels for the treated fabrics, and the effect lasting for at least for 12 weeks when the fabric was washed once a week. Thus, the originality of this paper is based on the coparison between laboratory results and real use of the fabrics.

EXPERIMENTAL

Materials

Cellulosic fibres are considered to confer good comfort to people and are widely used for clothes or home textiles. The fabric used in our tests was a 100% cotton twill fabric with 120 g/m^2, which had been chemically and optically bleached in an industrial process.

The microcapsules contained an active ingredient based mainly on citrodiol and an aminoplaste shell, which were developed by interfacial polymerization by INNOVATEC. An acrylic binder, Colorcenter STK-100 (Color-center S,L.) was used to bind the microcapsules onto the cellulosic fibres.

Methods

Padding microcapsules

Two dispersion units carrying 30 g/L and 60 g/L of microcapsules containing 5 and 10 g/L of binder respectively were prepared. Furthermore, in order to determine the behaviour of the binder two fabrics were prepared with 5 and 10 g/L of binder without any microcapsules. For padding, a horizontal foulard, was used. Foulard work was performed in order to obtain a pickup (bath absorption) around 89–90%. Thermal treatment in the form of hot air was applied to cure the binder and to induce microcapsule adhesion onto the fiber surface.

SEM microscopy

For surface observation, a Scanning Electron Microscope (SEM) Scanning Microscope JEOL JSM-6300 was used. Each sample was fixed on a standard sample holder and sputter coated with a gold–platinum mixture. Samples were then examined with suitable accelerating voltage and magnification.

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Particle Analysis

A piece of fabric was washed according to ISO 105. C10: 2010. Once the fabric was washed wastewater was collected and analysed in order to determine the number of microcapsules being washed out of the fabric. The particle size distribution of the microcapsules was measured by a Coulter® Counter apparatus (Multisizer Z2, Coulter Electronics, Northwell, UK). The particle size was expressed as the equivalent volume diameter and two replicates were performed for each batch of microcapsules. To reduce error an average curve was calculated and analyzed. This test was used to determine the laundry effect on fabrics containing microcapsules.

Laboratory mosquito repellence

The laboratory test was conducted according to the Swiss Tropical Institute standard STI-Norm MV-02. According to this test a piece of the tissue A, the repelling efficacy against mosquitoes of which was to be compared with tissue B, is placed on top of a mosquito cage of 30 x 30 x 30 cm covered with gauze. A bowl filled with water of 38 ± 1°C is put on the piece of tissue. The warmed surface area measured 65 cm2 and has the role of an enticement. The mosquito cage contains 100 female mosquitoes of the species Aedes aegypti. The number of mosquitoes sitting on the warmed tissue is counted after 1 and 5 minutes. Four independent counts are done. The same procedure is performed using a piece of tissue B. The reduction of landings after 5 minutes is given as the final result. A separate population of mosquitoes is used for each test-sample.

In situ procedure

The number of bites every volunteer showed, was controlled every day for a period of 12 weeks. Two different areas in two Indian Non Governmental Organizations (NGO) (Anantapur and Bangalore) were involved in the project. This test was carried out in two NGO facilities and people were given instructions about how to place the sheet prior to going to bed every night as they were not used to doing so. In order to establish a control test, the placebo effect was controlled by sharing sheets without microcapsules. Every sheet was referenced and only the researchers knew which sheets had been sprayed with microcapsules and which had no microcapsules. The study was completed with a sample population of 151 subjects,

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75 of which belong to the treatment group and 76 to the control group. One of the main constraints in this study was the limitation of the sample size in relation to the number of people living in the NGOs participating in this study.

IN SITU TEST	PEOPLE	Weeks	Laundries
Treated sheets	75	12	1 per week
Placebo sheets	76	12	1 per week
TOTAL	151	12	12

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Every morning the number of new bites on the participants' skin were registered. Mosquito repellence efficiency was calculated as the reduction in % in the number of bites according to the equation 1:

$$\%\ Reduction = \frac{NB\_W - NB\_MICS}{NTB}\ x\ 100 \qquad (1)$$

Where:

NB_W = Number of bites from individuals on the white sheet

NB_MICS = Number of bites from individuals on the sheet treated with microcapsules

NTB = Total number of bites (NB_W + NB_MICS)

According to this equation 100% of repellence would only be obtained when no bites occur on the treated sheets and there were some bits on the white sheets. This is just to avoid to consider 100% of repellence if there are not mosquitoes.

RESULTS AND DISCUSSION

Fabric characterization

Cotton sheets were analysed in order to determine whether the application procedure had been carried out properly. Figure 1 shows the fabric surface of cotton fibres once the treatment had been applied. The microcapsules presence is clearly observed on the fibres surface.

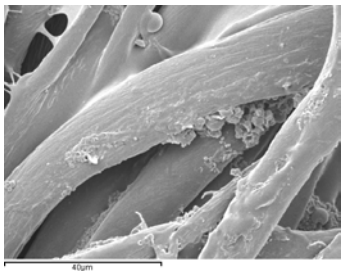

Figure 1. Cotton sheets treated with microcapsules.

In order to determine how long microcapsules could remain on the fibres' surface, and the optimal microcapsules concentration, the treated fabrics were washed for different cycles and wastewater was analysed according to the number of particles. Figure 2 shows there are microcapsules in the wastewater.

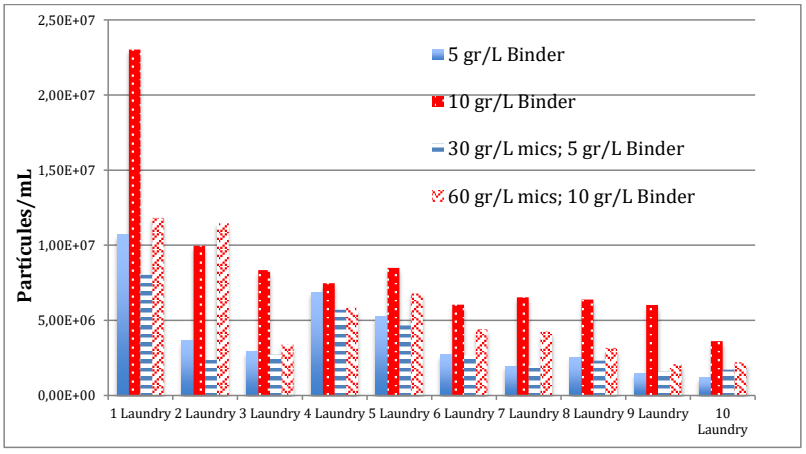

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Figure 2. Number of particles in the wastewater after every laundry cycle.

Apparently, according to the data presented in figure 2, the wide majority of microcapsules are removed from the fabric on the first 5 laundry cycles and for the 6th or subsequent washing cycles the loss of microcapsules seems to be practically constant. This data also shows that there is still some remaining microcapsules after the 10th cycle, something that can be verified by the observation of fibres on SEM as shown in figure 3 below. As expected, an increase in the binder concentration is reflected in the higher number of particles in the wastewater. Something similar is observed when comparing samples with 30 g/L and 60 g/L of microcapsules – we see more particles in the wastewater. Surprisingly, the number of particles decreases when microcapsules are included in the recipe when compared with the same concentration of binder without microcapsules. This fact could be explained as being a result of the fact that part of the binder is not lost into the wastewater as it is keeping the microcapsules adhered to the fibre surface, and the mechanical action of the washing is not enough to break the crosslinking between the cellulosic fibre and the microcapsule.

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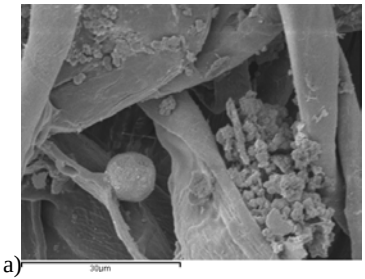
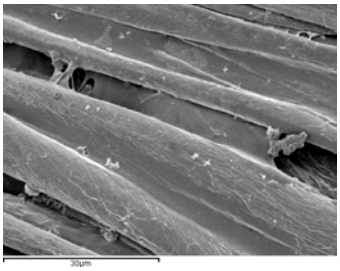
a)  b) 

Figure 3. Cotton sheets after laundry. a) 5 laundry cycles; b; 10 laundry cycles;

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Considering the desired effect is to maintain the adherence of the microcapsules as long as possible on the fabric to prevent mosquito bites, we decided to develop sheets with the higher concentration of microcapsules, and the selected recipe was 60 g/L of microcapsules and 10 g/L of binder.

Mosquito repellence

According to a standard

As stated previously, the mosquito repellence test was performed according to the standard STI-Norm MV-02. Table 1 presents the results of repellence from the laboratory test and for the fabric treated with 60 g/L of microcapsules and 10 g/L of binder.

Table 1.- Repellence results from cotton fabrics.

SAMPLE	REPELLENCE (%)
Cotton fabric	90
Cotton fabric with 10 laundry cycles	60

According to the Swiss Tropical Institute, repellence results between 40% and 49% are acceptable, between 50% and 69% are classified as good and higher than 69% as very good. When results from table 1 are analysed, we can conclude that microcapsules treatment can functionalise the fabric up to a very good level of repellence, and when fabrics have been treated for 10 laundry cycles the results still remain at the level of 'good' repellence. The laboratory scale analysis reflects the effectiveness of the treatment: however, this should be tested in situ.

In situ test

As it has been previously described, the test was conducted in two areas (SGIS, TEL-NEK). Results are expressed as the number of bites participants showed in the morning following a night with either treated or untreated fabric. Table 2 shows the difference between the number of bites from persons with a treated sheet with microcapsules (treated) or the number of bites from persons with a placebo sheet (white).

Table 2. Number of bites in every area for every week.

	SGIS		TEL-NEK	
WEEK	TREATED	WHITE	TREATED	WHITE
1	10	14	64	89
2	5	82	26	82
3	164	161	4	43

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4	0	43	42	72
5	470	490	2	44
6	3	9	3	112
7	137	195	20	82
8	56	53	0	52
9	3	12	0	66
10	37	65	2	73
11	28	85	6	30
12	1	5	6	13

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We can clearly observe an irregular number of bites when results are compared both between weeks and comparing the two regions where the tests were conducted. Considering it is an in-situ test, parameters such as the number of mosquitos, the weather conditions (wind, rain, etc) could not be controlled. However, it is evident that the people sleeping on the sheets treated with mosquito repellent microcapsules (treated column) show fewer bites than the people sleeping on the sheets without any treatment (white column).

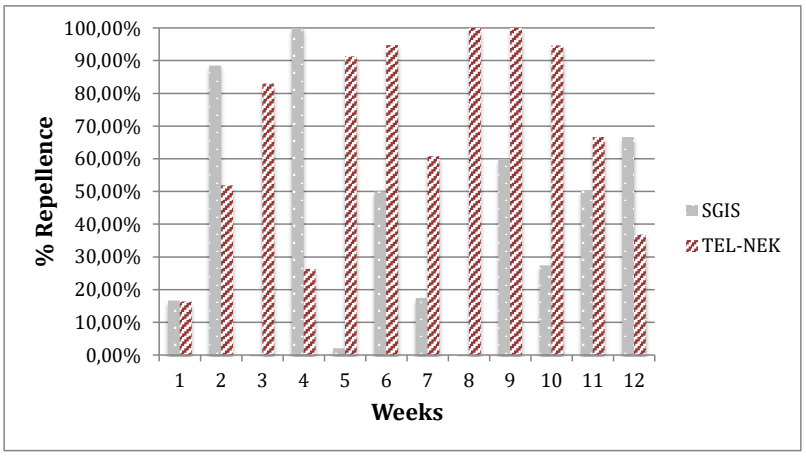
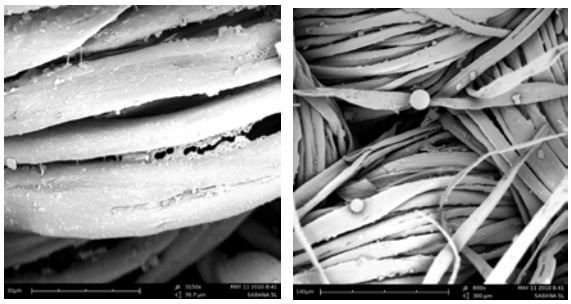


Figure 4. % Repellence from the sheets.

When considering the reduction in the number of bites, figure 4 shows the percentage of repellence. Despite the graphic not showing any tendency, it is

evident that the reduction in the number of bites is higher than the 60 % in the majority of weeks and across both areas.

Considering that sheets were washed in the river water nearby the NGOs facilities, SEM analysis demonstrates that once the sheets had been washed, some microcapsules still remain in the fibres as can be seen in figure 5.



a) b)

Figure 5. Cotton sheet after 5 laundries in the river water. a) broken micorcapsules; b) still active microcapsules

Figure 5 shows the fabric surface after having washed the sheets for 5 cycles in the river. Figure 5a shows evidence that some microcapsules have been broken however, figure 5b (from the same piece of fabric) shows some spherical microcapsules still remain on the fabric. Particle analysis from river washed samples have not been included, as it was not possible to collect these samples. Figure 6 shows not only broken or spherical microcapsules but some dust on the fibres due to the washing procedure of laundering in the river.

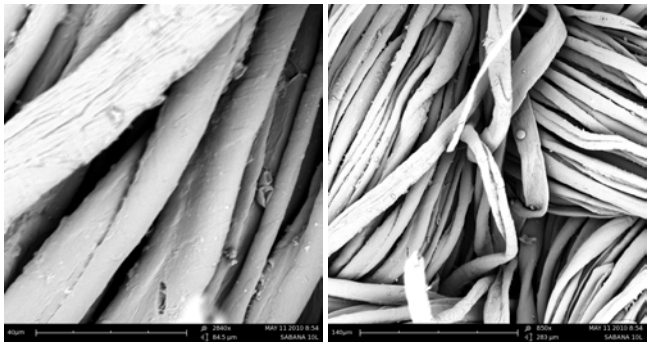


Figure 6. Cotton sheet after 10 laundries in India a) depleated micorcapsules; b) still remaining active microcapsules

It is evident that the laundry processes applied to the fabric do not match those from a developed country as the fabric was rinsed in a river without any soap and the mechanical action was not similar to that which a washing machine generates. This has allowed microcapsules to remain in the fabric for more than twelve weeks.

The hypothesis contrasts have determined that the use of the bed-sheets treated with insect repellent reduced in a global way the number of bites received by the subjects in comparison with the subjects who stayed overnight with non-treated sheets.

CONCLUSIONS

The tests conducted reflect the functionalisation of cotton fabrics by conferring mosquito repellence to the sheets. In situ tests show a wide variety of results and do not show a clear tendency mainly due to the fact that there are external conditions such as the weather, frequency and hunger of mosquitos, etc., which can be controlled in a laboratory setting. Laboratory tests showed the high effect of mosquito repellence on the treated cloths with citrodiol microcapsules. Moreover, in situ tests demonstrated the efficiency of the mosquito repellence. Furthermore, results provide evidence that there is a strong relationship between the laboratory tests and the in situ results. Fabrics classified as having 'good' repellence from the laboratory tests can reduce considerably the number of bites on people. Thus - and considering mosquito bites can transmit serious diseases - fabrics with the anti-

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mosquito microcapsule treatment would reduce the risk of disease contraction in some regions.

To sum up, we can conclude that results from this paper reveal that the addition of microcapsules on cotton fabrics show an improvement in the level of mosquito repellence of treated cellulosic sheets and that this reduces the number of bites on people sleeping with treated bed clothes. As a general evaluation we point out that the repellence obtained was considered reasonably good and on future studies we will test some other essential oils.

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