1. Executive Summary

The bite-preventing efficacy of one repellent treated fabric sample (steeped in a solution of IR3535 prior to testing) was evaluated in a cage test study with yellow-fever mosquitoes (*Aedes aegypti*) and one volunteer.

After drying, the IR3535 textile sample was attached to the forearm of one volunteer (male, 30 years). Consecutively, the sample was exposed to a host-seeking cage population of yellow-fever mosquitoes. During a maximum test period of 2 minute, the number of landing and biting mosquitoes was documented. An untreated sample was used to determine the biting permeability of the fabric.

The untreated sample could easily be pierced by the test mosquitoes. During control tests 15 landings were counted and 10 bites occurred within 60 seconds.
Compared to the control tests, the treated sample showed a repelling and bite reducing effect. When the treated sample was tested, only 3 landings were counted and 1 bite occurred during 120 seconds.

NOTE: the use of one mosquito species and one volunteer only provides an indication on the potential of a treated fabric.

2. General Information

Sponsor	Kim & Friends Inc. 119, SK Twintech Tower B-304 Gasan digital 1-ro Geumcheon-gu Seoul, Korea
Testing Facility	Biogents AG Weissenburgstr. 22 D-93055 Regensburg
Principal Investigator	 signed Sergej Sperling, M. Sc. Biology Date: 27.01.2017
Time Frame	Receipt of the test samples: 25.01.2017 Experimental start date: 25.01.2017 Experimental termination date: 26.01.2017 Data analysis and report: 26.01.2017

3. Material and Methods

3.1. Test Samples

The test fabric and the solution to treat the fabric prior to testing was provided by Kim & Friends Inc., Korea. The Solution was labelled IR3535

The fabric sample was steeped in the provided solution for 1 min, dried for 1 h and then used.

Test samples were kept at 20 – 22°C until the start of the test.

3.2. Test Insects *Aedes aegypti*

Female mosquitoes of the genera *Aedes* were reared according to the standard protocol at a temperature of 27° C, a relative humidity of 60 – 80 % and a 12:12 hour photo period. The light period (150 Lux) was set from 8:00 to 20:00. After hatching of the eggs, larvae were kept in a water basin (30 x 30 x 10 cm) filled with a 1:1 mixture of tap- and deionised water and fed with fish food flakes (Tetra Min®). Pupae were transferred to a cage (40 x 30 x 20 cm) provided with sugar solution (10% dextrose). Mosquitoes at an age of 9-13 days after emergence were used for the repellent tests.

3.3. Test Room

Cage tests were performed in an air-conditioned room (4,6 x 3,4 x 2,6 m) without windows. The temperature and relative humidity of the room air were 26 ±1°C and 65 ± 5% r.H. The light intensity was 350 Lux.

3.4 BG Test Cages

BG test cages are an in-house improvement of conventional test cages for the evaluation of mosquito repellents (Obermayr et al., 2010). The cages have a volume of 27.000 cm³ (41 x 41 x 16 cm). Four sides of a cage are made of acrylic glass, the floor is made of metal sheet and the rear side is covered by a gauze sleeve. The floor sheet is equipped with a test window (size: 56 cm²; 14,8 x 3,8 cm). In between tests, cages are connected to a ventilation system that provides clean, warm and humid air (26 ± 1°C, 70 ± 10% r.H.) to remove remaining host odours and repellent volatiles from the air inside the cage
Each cages is filled with populations of 30 mosquitoes, those are lured out of their rearing cages by a natural stimulus (human hand) to ensure that only host-seeking females are used for the test.

Fig. 1: BG Test Cage (front view). The air ventilation system is connected to the cage (black arrow). The arm is exposed at the test window in the metal floor sheet (black rectangle). The rear side is covered by gauze.

3.5. Test Procedure

3.5.1. Zero Control

Prior to an efficacy test, the biting activity of the test mosquitoes is verified with the other, untreated forearm of the test person. A minimum of 10 landings have to be observed in 30 seconds, otherwise mosquitoes are added to the cage or 30 new mosquitoes are used.

3.5.2. Test Proper

The test sample is attached to the forearm of one test person (male, 30 years) by metal clips in a way that it is not stretched tight to the skin. Short after attaching the treated surface is exposed to the test mosquitoes at the test window in the floor of the test cage. During a maximum testing time of 2 minutes, the number of mosquitoes landing and biting is documented. In addition, the test situation is digitally recorded.

4. Results

4.1. Efficacy of the treated fabrics

Table 1 shows the results from tests of treated fabric samples.

Tab. 1: BG screening cage tests of the IR3535 treated sample including control. The number of landings and bites is given for each tested fabric. The tests were performed with *Ae. aegypti* and one volunteer (n=1).

Sample N°	Number of landings	Number of Bites / Time	Control of Biting-Activity (see 3.5.1)	Bite reduction
Untreated fabric	15	13 / 60 sec	10 / 15 sec	-
Fabric steeped in provided solution of IR3535	3	1 / 120 sec	10 / 13 sec	96%

The untreated sample could easily be pierced by the test mosquitoes. During control tests 15 landings were counted and 13 bites occurred within 60 seconds.
When the treated sample was exposed to the test mosquitoes, only one bite was counted in 2 minutes, representing a 96% bite reduction compared to the untreated sample.

Conclusion: The fabric used was very thin and easily accessible for the mosquitoes, i.e. they had no difficulties to pierce it as shown by tests of the untreated samples.
However, the same fabric steeped in a solution of IR3535 had a bite reducing and repelling effect. It would be interesting to evaluate, how long the repelling effect lasts after the treatment.

NOTE: the use of one mosquito species and two volunteers only provides an indication on the potential of a treated fabric. For safe results, at least 5 volunteers should be involved.

The following figures show the test samples during cage tests. Pictures were taken after approximately 30 seconds for the untreated sample and 60 seconds for the treated one.

Fig. 2: Untreated sample; 10 mosquitoes sit on the surface.

Fig. 3: Treated sample (steeped in IR3535); 1 mosquito sits on the test surface.

References

Obermayr, U., Rose, A. and Geier, M. (2010). A Novel Test Cage with an Air Ventilation System as an Alternative to Conventional Cages for the Efficacy Testing of Mosquito Repellents. Journal of Medical Entomology 47 (6): 1116-1122 / DOI: 10.1603/ME10093