



Assessment of *Aedes albopictus* mosquito behaviour in response to specific light sources.

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OBJECTIVE OF LIGHT AVOIDANCE TEST

These tests were performed by Biovecblok s.r.l., in the insectary of Camerino University.

MATERIALS

- Light source: mosquito repellent TRILED Baesten light and cold light (control)
 - Mosquitoes: *Ae. albopictus*: vector of dengue, yellow fever, chikungunya, zika and dirofilariasis.
- 8 cages (20x20x20cm) equipped with resting site (dark cardboard, 20x20x10cm).
- Large mosquito cage for hosting entire experimental set-up.
- Insectary room set up to maintain 28°C, 85% relative humidity and a 12 hour light/dark cycle mimicking day and night.
- Hemotek membrane feeding system to perform mosquito blood meal.

EXPERIMENTAL DESIGN

- The entire experiment (light source, cages, detection) was settled into an insectary room (see *Materials*) (Fig. 1).
- 4 small cages were placed with a 20cm stepwise increasing distance from the light source.
- A cardboard dividing a cage into a shady area for refuge and a bright zone structures each cage. The shade is generated by placing the cardboard in relation to light source.
- Each cage was equipped with sugar and blood feeding vessels.
- 25 female and 20 male adult *Ae. albopictus* mosquitoes (15 days after emergence) were added to each cage.
- After an adaptation time of 1h to light source, mosquitoes were fed using hemotek membrane feeding system for 4 hours.
- A control experiment was performed as described above, with cold light source.

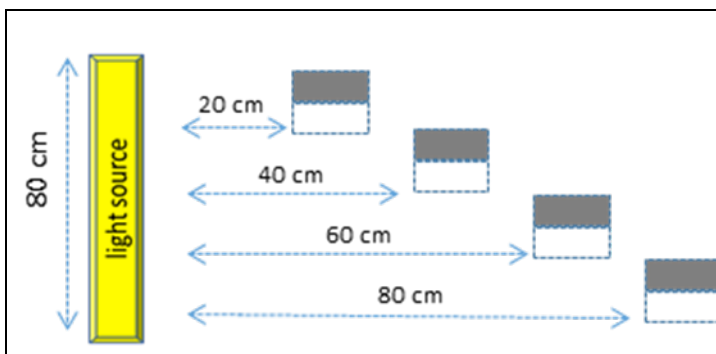


Fig. 1: Light avoidance experiment. Set-up of light source, cages and detection



ANALYSIS STRATEGY

Data collection: Feeding events were observed and recorded every 15minutes for each cage (Table 1).

Graphing: Feeding events for switched on periods were presented as plots. Feeding events in relation to distance from light source were plotted(Fig. 2).

Data collection (raw data).

	Cold light (control)				TRILED Baesten light (treatment)			
	20cm	40cm	60cm	80cm	20cm	40cm	60cm	80cm
30'	6	8	7	9	6	9	9	9
1h	2	2	1	1	1	2	3	2
1h30'	1	2	2	2	1	1	2	1
2h	1	3	3	1	1	2	1	2
2h30'	1	1	1	3	2	1	1	0
3h	3	1	1	1	0	0	0	0
3h30'	1	0	0	0	0	0	0	0
4h	0	0	0	0	0	0	0	0
Total biting	15/25	16/25	16/25	17/25	11/25	14/25	16/25	14/25
%biting	60%	64%	64%	68%	44%	56%	64%	56%

Table 1: the table shows the biting frequency (number of mosquitoes taking a blood meal) recorded for 4 hours into each cage located at different distances.

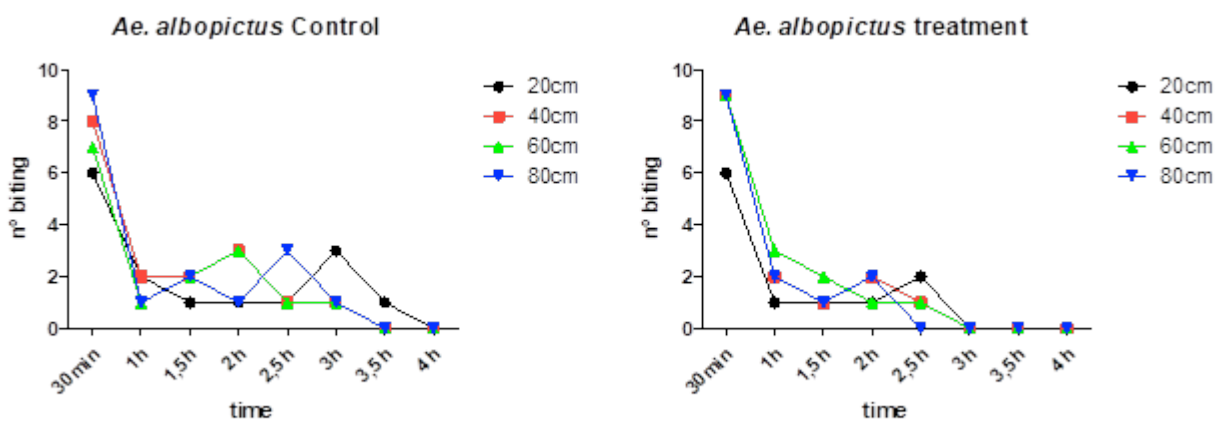


Fig. 2: Graph shows the biting decrease of insects expose to TRILED Baesten light compared to the control.

OBSERVATION

The experiment performed suggests that female mosquitoes of *Aedes albopictus* don't seem to be affected by the TRILED Baesten light compared to the control. We want to underline how, due to the extreme conditions we settle all the experimentation, this experiment does not mimic the field conditions, in which light will be placed between mosquitoes and the potential blood meal (Fig.3). The goal of this test, is to investigate the effect of TRILED Baesten light on mosquito biting behaviour, for this reason we have designed an experiment in which mosquitoes are placed in cage with a blood meal and thus almost forced to bite.

Mosquito biting behaviour is instinctive in all females mosquitoes that need the blood to develop their eggs and it's triggered and influenced by different factors. The results obtained in this experiment suggest that when placed at very close distance (lab conditions) mosquitoes and a potential blood meal, the effect of TRILED Baesten light on *Aedes albopictus* mosquitoes seems not to interfere with mosquito biting behaviour due to the severe conditions we settle the experiment. Eventually increasing the distance and the time required by mosquitoes to reach the blood may enhance TRILED Baesten light effect as it was documented in the field for *Culex quinquefasciatus* mosquitoes.

For this reason, further experiments in semi-field conditions, are required to observe and understand the effect of TRILED Baesten light when placed between mosquitoes and a potential blood meal

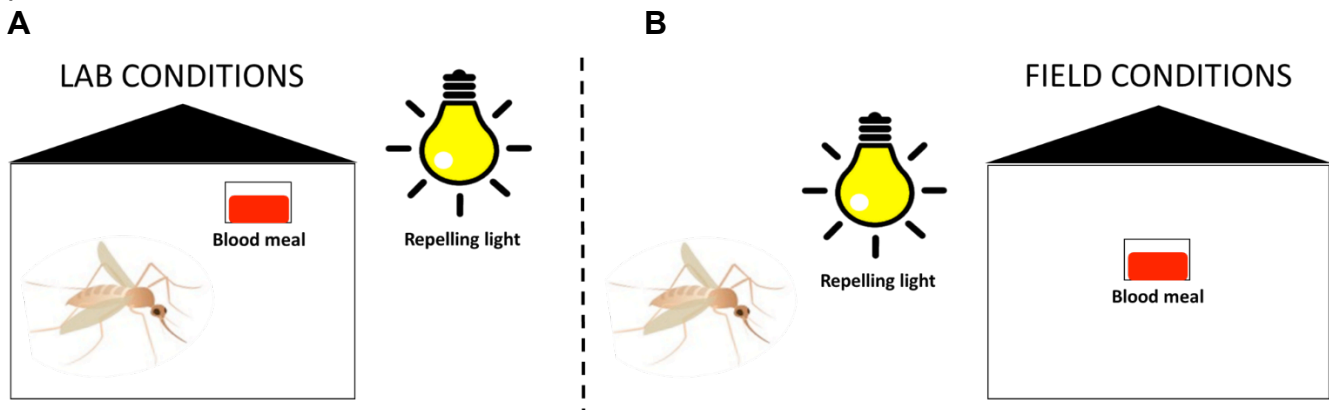


Fig. 3: A-Lab conditions, in which lights are placed outside the cage containing mosquitoes and the blood meal. B-Field conditions, in which lights will be placed between mosquitoes and the blood meal.

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