



EFFICACY EVALUATION OF "STEAM-X" AGAINST *Cimex lectularius*

Test completed on
March 10, 2024



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1. TEST INDEX-CARD

Study conducted for:	TECNOVAP srl Via dei Sassi, 1/a – 37026 Pescantina (VR) - Italy
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Supervisor of the study:	Dr. Andrea Drago
Responsible of the study:	Matteo Cecconello
Tested product:	STEAM-X
Species used for the test:	<i>Cimex lectularius</i>
Test start date:	February 26, 2024
Test completion date:	March 10, 2024



2. TEST OBJECTIVE

To evaluate the efficacy of the "STEAM-X" Steam System against the target species through a simulated use test.

3. SUMMARY

Target insect: *Cimex lectularius*

Formulation of the tested machinery: The machinery used for the test is a steam generator, "STEAM-X," equipped with a superheater gun. Distilled water was used for the treatment.

Method of testing: A shelter is created by placing two surfaces on top of each other to create a small gap. The shelter is then placed in a plastic arena where 5 insects (of mixed stage and sex) are placed inside the slit. After a period of acclimatization, the treatment was performed by applying steam around the shelter. The knockdown time and mortality at 24 h is then evaluated.

The same procedure was performed by placing 10 eggs in the shelter that are observed for 1 week after treatment.

4. MATERIALS AND METHODS

4.1 SPECIES OF INSECTS USED FOR THE TEST AND BREEDING CONDITIONS

The bed bugs used for the test came from the laboratory of Entostudio.

The bedbugs are kept in the laboratory at 27°C and 75% R.H.. The photoperiod lasts 12 hours. The bedbugs are confined into a falcon containing a piece of black cardboard and closed with a tulle netting. Once a week the container is placed tulle side down on a Parafilm sack filled with defibrinated rabbit blood in order to let the bed bug's feed. The females lay the eggs on the cardboard.

The colony originated in 2022 from bedbugs provided by Czech University of Life Sciences Prague (CZU).

4.2 DETAILS OF THE TESTED MACHINERY

Name: EVO SANITIZER

Description of machinery tested: The test was carried out with steam generator mod. Evo sanitizer (boiler power 2 kW/h, steam production 3.8 Kg/h at 165°C - 6 bar), and steam gun with superheater. The compatible models for the test performed are the following: Evo Sanitizer, Evo Blu, Evo 304, Steam Box Mini, Junior Star, Carmen Plus, Carmen Mini, Gal e Tosca.



Fig. 1 Machinery used for the test

4.3 DOSAGE APPLIED

Each shelter was treated for 4 seconds, applying steam perimeterwise at a distance of 20 cm.

4.4 TEST-ROOM SPECIFICATIONS

The test was performed in a room measuring 3.15 x 2.90 x 3.03(h) m corresponding to a surface of 9.13 m² and a volume of 27.7 m³. The room is washable thanks to a resin floor and varnished walls. The room is completely white. The climatic parameters (temperature and humidity) and the illumination are automatically controlled and regulated: the illumination is provided by led lights 4000°K solar-spectrum. The test was performed at 300 Lux intensity, 25±1°C and 60±5% Relative Humidity.

The room has an adjustable air extraction system that is capable to change the air up to 2800 m³/h.



4.5 EQUIPMENT

For the test procedure, the following devices were used as specified:

- CompactSteam humidifier (CAREL S.p.A.);
- TFA, digital thermo-hygrometer to measure environmental parameters;
- Nimex Ni 2600, digital lux meter to measure light intensity;
- Daikin FDXM25F2V1B (indoor unit) and Daikin RXM25M (outdoor unit), for the air conditioning temperature;
- BITICINO H4684-AM5864, for photoperiod management;
- Arena SAMLA 78 x 56 x 18 cm (Volume 56 L)
- Stereo-microscope ZEISS Stemi 508;
- Steam generator, mod. Evo sanitizer.

4.6 DEFINITION OF TEST AND REPLICATE

Test: is the complete evaluation procedure of a product. It is given by the set of replicates performed.

Replicate: corresponds to a treated arena and a control arena. The procedure begins with the introduction of the insects into the container.

4.7 EVALUATION PARAMETERS

Dead: Individuals which do not show any movement and which do not react to external stimuli (i.e. when touched with a pair of tweezers) are considered as dead.

Moribund: after the test starting the individuals that react to stimuli but are unable to move in a coordinated manner, e.g. to upright itself or walk properly, are considered as "moribund". These insects left into the arena. If moribund insects turn dead, the data is reported to the moment when they were observed as moribund.

Alive: Individuals which do not show any behavioural alterations.



4.8 TEST DESIGN

The test was performed in a plastic arena measuring 78 x 56 x 18 (h) cm, having an area of 0.43 cm² and a volume of 56 L.

Each arena corresponds to a replica.

A shelter was placed in the center of the arena (Figure 2), the shelter was made by placing two surfaces, non-porous (ceramic tile, vitrified side) and porous surfaces (wood tile), one on top of the other so as to create a gap of about 4 mm.

For each type of surface, 3 treated replicates and 3 control replicates were performed.

For each replicate, 5 insects (of mixed sex and stage) were used.

The insects are introduced 15 minutes before treatment, during this acclimatization period the light is left on so as to encourage the insects to stay inside the slit.

Before applying steam, the machinery used is prepared as per the directions below:

- Distilled water is introduced into the reservoir
- The gun with superheater is connected to the steam outlet of the machine
- The boiler is then turned on for about 5 minutes, the time to reach the required working operating pressure (165°C - 6 bar)
- Steam condensate is discharged into a container for about 1 minute in order to standardize the pressure of the machinery before application.

Once the minimum power of the machinery was set, steam was applied around the shelter for 4 seconds while maintaining a distance of 20 cm.

At the end of the application, the shelter was opened and the culling time was evaluated (Figure 3). The culled insects were then moved to a plastic container where 24-hour mortality was recorded.

The same procedure was performed, on shelters created with nonporous surfaces, using 10 eggs for each replicate. After application of the product, the eggs were moved to a plastic container and observed for one week to check for hatching or not.

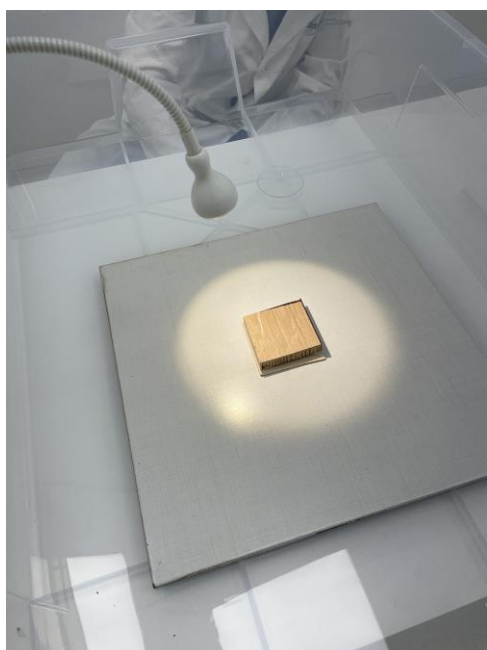


Fig 2. Shelter used for the test



Fig 3. Steam-treated bugs.



5. RESULTS

5.1 ADULTS AND NYMPHS

The following are the results obtained.

Tab. 1: Results for Wood surface

February 26, 2024	Treated (N. knocked down insects)			Mean (%) ± SEM	Control (N. knocked down insects)			Mean (%) ± SEM
Time (min)	Treat. 1	Treat. 2	Treat. 3		Cont. 1	Cont. 2	Cont. 3	
2	5	5	5	100.00±0.00	0	0	0	0.00±0.00
5	5	5	5	100.00±0.00	0	0	0	0.00±0.00
Alive 24 h	0	0	0	0.00±0.00	5	5	5	100.00±0.00
Moribund 24 h	0	0	0	0.00±0.00	0	0	0	0.00±0.00
Dead 24 h	5	5	5	100.00±0.00	0	0	0	0.00±0.00

Tab. 2: Results for ceramic tile surface

February 26, 2024	Treated (N. knocked down insects)			Mean (%) ± SEM	Control (N. knocked down insects)			Mean (%) ± SEM
Time (min)	Treat. 1	Treat. 2	Treat. 3		Cont. 1	Cont. 2	Cont. 3	
2	5	5	5	100.00±0.00	0	0	0	0.00±0.00
5	5	5	5	100.00±0.00	0	0	0	0.00±0.00
Alive 24 h	0	0	0	0.00±0.00	5	5	5	100.00±0.00
Moribund 24 h	0	0	0	0.00±0.00	0	0	0	0.00±0.00
Dead 24 h	5	5	5	100.00±0.00	0	0	0	0.00±0.00



5.2 EGGS

The following are the results obtained.

Tab. 3: Results for *Cimex lectularius*

March 03, 2024	Treated Tiles (N. of eggs hatched)			Mean (%) ± SEM	Control Tiles (N. knocked down insects)			Mean (%) ± SEM
	Treat. 1	Treat. 2	Treat. 3		Cont. 1	Cont. 2	Cont. 3	
N. of egg per replicate	10	10	10	100.00±0.00	10	10	10	100.00±0.00
Hatched 1 day	0	0	0	0.00±0.00	5	4	3	40.00±5.77
Not Hatched 1 day	10	10	10	100.00±0.00	0	0	0	0.00±0.00
Hatched 2 days	0	0	0	0.00±0.00	6	8	6	66.67±6.67
Not Hatched 2 days	10	10	10	100.00±0.00	0	0	0	0.00±0.00
Hatched 3 days	0	0	0	0.00±0.00	9	8	9	86.67±3.33
Not Hatched 3 days	10	10	10	100.00±0.00	0	0	0	0.00±0.00
Hatched 4 days	0	0	0	0.00±0.00	10	10	10	100.00±0.00
Not Hatched 4 days	10	10	10	100.00±0.00	0	0	0	0.00±0.00
Hatched 5 days	0	0	0	0.00±0.00	10	10	10	100.00±0.00
Not Hatched 5 days	10	10	10	100.00±0.00	0	0	0	0.00±0.00
Hatched 6 days	0	0	0	0.00±0.00	10	10	10	100.00±0.00
Not Hatched 6 days	10	10	10	100.00±0.00	0	0	0	0.00±0.00
Hatched 7 days	0	0	0	0.00±0.00	10	10	10	100.00±0.00
Not Hatched 7 days	10	10	10	100.00±0.00	0	0	0	0.00±0.00



6. CONCLUSIONS

Based on the tests conducted, the following was found:

Nymphs and adults:

In the treated replicates, for both surfaces tested, culling was 100% after 2 minutes and mortality 100% after 24 hours. No culling and mortality values were recorded in the control replicates.

Eggs:

In the treated replicates, no eggs completed hatching during the 7-day evaluation period.

In control replications (those not treated with steam), however, all eggs hatched after 4 days of observation.

Ponte San Nicolò,

Reported on **May 06, 2024**

Signed

Andrea Drago PhD