

Centro Colaborador de la ORGANIZACION MUNDIAL DE LA SALUD/ ORGANIZACIÓN PANAMERICANA DE LA SALUD para Investigaciones sobre Susceptibilidad y Resistencia a Insecticidas en insectos vectores de la enfermedad de Chagas y Dengue

Evaluation of the pediculicidal effect of Polyphenol-HT1® on *Pediculus humanus capitis*

**CENTRO DE INVESTIGACIONES DE PLAGAS E INSECTICIDAS
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Requested by

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Buenos Aires July 27, 2023

Applicant company *NOVA MENTIS LTD.*

Study: Evaluation of the pediculicidal effect of Polyphenol-HT1 on *Pediculus humanus capitis*

Laboratory: Centro de Investigaciones de Plagas e Insecticidas (CIPEIN-CONICET-UNIDEF)

Study Managers:

<i>Dr. Ariel Toloza</i> <i>Study Director</i>	<i>Fecha: 28/July/2023</i>
<i>Dra. Claudia Vassena</i> <i>Director of CIPEIN</i>	<i>Fecha: 28/July/2023</i>
<i>Dra. Laura Mugas</i> <i>Associate researcher</i>	<i>Fecha: 28/July/2023</i>

REPORT SUMMARY

Applicant company: NOVA MENTIS.

Laboratory: Pest and Insecticide Research Center (CONICET- UNIDEF-CITEDEF)
Buenos Aires. Argentina.

Date: 27 July, 2023

Formulations evaluated: Polyphenol-HT1® (65%).

Title of the study: Evaluation of the pediculicidal effect of Polyphenol-HT1 on *Pediculus humanus capitis*)

Insects: *Pediculus humanus capitis*, adults & nymphs III.

Methodology: The immersion method consists of applying the product for 10 minutes. Knockdown symptoms are recorded after 10 minutes post treatment, and mortality of head lice is measured after 8 hours.

Results:

Product	knockdown effect (%) adults & nymphs III ($\times \pm \text{ES}$)	Mortality (%) adults & nymphs III ($\times \pm \text{ES}$)
Polyphenol-HT1 (3%)	0 ± 0	$14,7 \pm 4$
Polyphenol-HT1 (20%)	0 ± 0	$16,1 \pm 2$
Pure water	0 ± 0	0 ± 0

1. AIM

At the request of Enrico Altieri, Development Director of the company NOVA MENTIS LTD., the evaluation of the pediculicidal effectiveness of the Polyphenol-HT1 lotion was carried out on adults and nymphs III of *Pediculus humanus capitis* (head louse).

2. FORMULATED

The product was delivered by the company to be evaluated and its composition was not determined by CIPEIN. The label contained the following information:

Hydroxytyrosol. Purity >99%. CAS #: 10597-60-1. Lot: 220215.

3. MATERIALS AND METHODS

Insects: Lice (*Pediculus humanus capitis*) were collected by using a fine metal comb from educational establishments that were previously studied by the CIPEIN (Tolosa et al. 2008).

All lice collected in the same school were considered to belong to the same population.

The insects were collected from the heads of children 6-12 years old using a fine comb and sent to the CIPEIN where they were conditioned at 18° C and 75% RH (to reduce the dehydration suffered by the insect when removing it from their micro-habitat).

Adult lice and third instar nymphs were selected for use in the pediculicidal trial (Figure 1).

Figure 1. Collected insects that were used in the bioassays.



4. PREPARATION OF THE SAMPLES

- Preparation of a 3% solution

Sample delivered by customer (W/V): 65%; 65g/100ml

VF 10 ml (W/V): 3% (WITH DISTILLED WATER)

$V = 3\% \times 10\text{ml} / 65\% = 0.462 \text{ ml}$ equivalent to 462 μl

462 μl of HT was pipetted with a validated automatic pipette (BPL) and made up to volume with distilled water in a 10ml flask.

OBSERVATIONS: Due to the presence of white particles in suspension when the dilution was performed, the diluted formulation was protected from light during handling.

- Preparation of a 20% solution

$V = 20\% \times 10\text{ml} / 65\% = 3,077 \text{ ml}$ equivalent to 3077 μl is made up to 10 ml with distilled water.

5. BIOASSAY

Adulticide bioassay consisted of at the exposure of at least 15 selected head lice that were immersed for 10 minutes in 1 ml of the formulation to be evaluated (3 and 20% dilutions). Then, they were transferred to a metal mesh and placed on Whatman No. 1 filter paper.

They were left in the recovery period for 10 minutes, during that period, insects were inactivated by immersion but not intoxicated, and recovered their normal activity (Gallardo et al. 2012). The number of affected lice after 10 minutes of exposure were registered (Figure 2).

Figure 2. Immersion bioassay of adults



Considering that the product to be evaluated may not possess knockdown effect, treated insects were kept at 18 °C and 75% RH until the endpoint of 8 hours to record mortality.

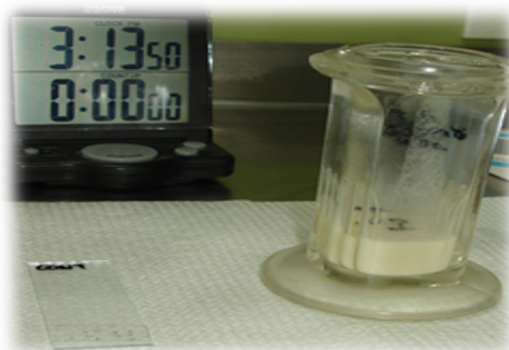
The evaluation of the samples was carried out in a minimum of three independent tests. Groups of insects immersed in water were used as a control.

Ovicidal bioassay :

Groups of 10-20 late development viable eggs were selected as previously by Toloza et al. (2008). Late eggs showed black eye spots and clearly visible appendages of the embryo. Tested eggs were fixed to a microscope slide by a double-face adhesive tape.

Slides containing eggs were immersed in the tested pediculicide for 10 min (Fig. 3). Then, head louse eggs were incubated at 28 ± 1 °C and $75 \pm 5\%$ RH (closed containers with saturated salt solutions). Mortality data of treated eggs were recorded 5 days after the eclosion of controls. Treatments and controls were replicated three times. The criterion for mortality was eggs with closed operculum or eggs with opened operculum and the nymph inside.

Figure 3. Immersion bioassay of viable eggs.



6. EFFECTIVENESS RESULTS IN ADULTS AND NYMPHS III

The average percentage of the three independent trials using the different batches of turned (10 minutes) and dead (8 hours) lice are summarized in Table 1.

Table 1. Percentage of knockdown head lice (10 minutes) and dead (18 hours)

Product	knockdown effect (%) ($\times \pm ES$) n: 30	Mortality (%) ($\times \pm ES$)
Polyphenol-HT1 (3%)	0 ± 0	$14,7 \pm 4$
Polyphenol-HT1 (20%)	0 ± 0	$16,1 \pm 2$
Water	0 ± 0	0 ± 0

7. EFFECTIVENESS RESULTS IN VIABLE EGGS WERE SELECTED AS PREVIOUSLY (NITS)

Mortality data of treated eggs were recorded 5 days after the eclosion of controls. Treatments and controls were replicated three times. Three independent trials using the different batches lice are summarized in Table 2.

Table 2. Percentage of mortality in viable eggs (NITS)

Product	Mortality (%) ($\times \pm ES$) n:30
Pure sample delivered by customer (W/V): 65%; 65g/100ml	10 $\pm$ 0
Water	0 $\pm$ 0

7. CONCLUSIONS

The Polyphenol-HT1 pediculicidal lotion delivered by Enrico Altieri was evaluated in either adulticide or ovicide bioassay test on adults, nymphs III and eggs of *P. humanus capitis*.

Immersion of lice in Polyphenol-HT1 lotion in three independent trials did not produce knockdown effect of the exposed insects.

The percentage of lice affected at 8 hours for the 3 and 20% dilutions was of 14 and 16%; respectively.

The negative control (water) had neither knockdown nor mortality of the exposed lice.

Lice eggs (nits) were evaluated with the sample in its pure state (without dilutions) and they died 100%.

8. REFERENCES

- A. Gallardo, Gastón Mougabure-Cueto, Claudia Vassena, María Inés Picollo, Ariel Ceferino Toloza. 2012. Comparative efficacy of new commercial pediculicides against adults and eggs of *Pediculus humanus capitis* (head lice). *Parasitology Research* 110: 1601- 1606.
- A.C. Toloza, C. Vassena and MI Picollo. 2008. Ovicidal and adulticidal effect of monoterpenoids against permethrin-resistant human head lice *Pediculus humanus capitis*. *Medical and Veterinary Entomology* 22: 335-339.