



Efficacy and selectivity of *Sextonia rubra* wood extracts and formulation in the control of *Aedes aegypti* strains

Emmanuelle Clervil, Jean-Bernard Duchemin, Nadine Amusant, Edmond Wozniak, Didier Azam, Maïra Coke, Dominique Huteau, Rémy Le Guével, Hélène Solhi, Isabelle Dusfour, et al.

► To cite this version:

Emmanuelle Clervil, Jean-Bernard Duchemin, Nadine Amusant, Edmond Wozniak, Didier Azam, et al.. Efficacy and selectivity of *Sextonia rubra* wood extracts and formulation in the control of *Aedes aegypti* strains. *Journal of Pest Science*, inPress, 10.1007/s10340-024-01747-4 . hal-04454494v1

HAL Id: hal-04454494

<https://hal.science/hal-04454494v1>

Submitted on 13 Feb 2024 (v1), last revised 13 Feb 2024 (v2)

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Efficacy and selectivity of *Sextonia rubra* wood extracts and bio-based formulation for the management of insecticide resistant *Aedes aegypti* in French Guiana

Emmanuelle Clervil^{a,b}, Jean-Bernard Duchemin^b, Nadine Amusant^c, Edmond Wozniak^d,
Didier Azam^e, Maïra Coke^e, Dominique Huteau^e, Rémy Le Guével^f, Hélène Solhi^f,
Isabelle Dusfour^g, Emeline Houël^{a,h*}

^a CNRS, UMR EcoFoG, AgroParisTech, Cirad, INRAE, Université des Antilles, Université de Guyane, 97300 Cayenne, France

^b Institut Pasteur de la Guyane, Vectopôle Amazonien Emile Abonnenc, Unité Entomologie Médicale, 23 avenue Pasteur, BP6010, 97306 Cayenne cedex, France

^c CIRAD, UMR EcoFoG, AgroParisTech, CNRS, INRAE, Université des Antilles, Université de Guyane, 97310 Kourou, France

^d W.Ed. Consulting, 81290 Viviers-les-Montagnes, France

^e Unité Expérimentale d'Ecologie et d'Ecotoxicologie Aquatique, INRA-U3E, 35042 Rennes, France

^f Plate-Forme ImpACcell, SF BIOSIT, Université de Rennes 1, Rennes, France

^g Département de santé globale, Institut Pasteur, Paris ; MIVEGEC, UMR IRD 224-CNRS 5290, Université de Montpellier, Montpellier, France

^h Sorbonne Université, CNRS, Laboratoire de Biodiversité et Biotechnologies Microbiennes, LBBM, Observatoire Océanologique, 66650 Banyuls-sur-Mer, France; emeline.houel@cnrs.fr; ORCID 0000-0003-4888-2332

Abstract:

Sextonia rubra is an Amazonian tree known for the larvicide activity of its wood extracts against *Aedes aegypti*. However, the lipophilic character of this extract represents an

obstacle to the development of vector management products. The purpose of this study was to assess the feasibility, efficacy and selectivity of a water-soluble formulation based on a *S. rubra* wood extract characterized by its content in the larvicidal compounds rubrenolide and rubrynlolide. Using reference and wild strains, the larval mortality, oviposition behavior, hatching response, and fitness of the mosquitoes exposed to the extract were evaluated. Laboratory eco- and cytotoxicity bioassays were concurrently performed. The extract showed larvicide efficacy alongside with no repulsive effect on oviposition, thus supporting its interest for the treatment of breeding sites. The formulation exhibited strong acute toxicity, leading to 100% larval mortality in 2 to 4 days either in dry or rainy season at the test dose of 102 µg/ml under semi-operational conditions, with a residual activity maintained during the 5 weeks of test. The formulation allowed for a noticeable gain in selectivity compared with the extract, with lower immobilization rates for *D. magna* and *C. riparius*, and no significant toxicity towards human cells lines (IC₅₀ > 100 µg/ml). These results highlight the potential of a *S. rubra*-based formulation for the management of *Ae. aegypti* larvae, including strains showing resistance to conventional insecticides.

Keywords: vector control, botanical insecticide, semi-field trials, non-target effects, oviposition

Key Message

- *S. rubra* wood extract formulation is easily usable in aqueous media, hence to treat breeding sites
- Semi-field assays highlighted acute toxicity and residual activity of the formulation over 5 weeks
- The formulation is more selective than the extract in eco- and cytotoxicity assays
- *S. rubra* crude extract has no effect on *Ae. aegypti* oviposition behaviour

- *S. rubra* sawmill waste can be valorized as an insecticide formulation

Introduction

Globalization and extensive urbanization have promoted a worldwide expansion of the anthropophilic invasive mosquito *Aedes aegypti* L. (Diptera, Culicidae), notably due to the proliferation of breeding sites that can retain stagnant water (Carvalho and Moreira 2017). This species is causing numerous human casualties as the main vector of several arboviruses (Gloria-Soria et al. 2016; Jones et al. 2021). In the absence of clinically approved vaccine or antiviral therapy, except in the case of yellow fever virus, the vector control strategies based on the use of insecticides are of crucial importance. Dengue fever, and Zika or chikungunya viruses are in particular strongly circulating in French Guiana, where vector control strategies have followed one another (Epelboin et al. 2018; Bailly et al. 2021; de Thoisy et al. 2021). Nowadays, this French overseas territory faces a strong resistance of *Ae. aegypti* populations towards pyrethroids as adult vector-control products (Dusfour et al. 2015; Faucon et al. 2017; Guidez et al. 2020; Senthil-Nathan 2020; Epelboin et al. 2021). Bti (*Bacillus thuringiensis* var. *israelensis*) is currently used to reduce the density of *Ae. aegypti* larvae, without the apparition of resistance phenomena (Darriet et al. 2010; Kumar et al. 2012). However, the persistence of effect of this non-ecotoxic natural product in breeding sites should be explored (Epelboin et al. 2018). In an epidemiological and legislative context particularly unsteady, it is therefore essential to develop safe, innovative and environmentally friendly methods for vector control (George et al. 2014; Gerwick and Sparks 2014; Benelli 2015; Benelli and Mehlhorn 2016).

Due to constant interaction with herbivores, plants synthesize a large variety of molecules as part of their defense system (Agrawal and Weber 2015; Richards et al. 2015; Salazar et al. 2018). This makes plants a valuable source for novel insecticide compounds (Miresmailli and Isman 2014; Pavela et al. 2019; Benelli 2020; Silvério et

al. 2020). However, even if numerous promising results have been obtained, only few of these findings have made it to commercial applications as new botanical insecticides (Isman and Grieneisen 2014; Isman 2020). One of the major technological challenges for the development of larvicide botanical products is to allow their homogeneous dispersion in water, the natural mosquito-breeding medium, alongside with the increase of field performance through a better persistence of action (Isman 2020). To overcome the applicability limitations, notably linked to the apolar nature of many natural substances or extracts, and without using organic solvents exhibiting toxic profiles, micro- and nanoemulsions stand in good place (de Oliveira et al. 2014; Peng et al. 2019). These techniques, which lead to the efficient aqueous suspension or dilution of low water-soluble substances, allow obtaining usable bioactive natural products against mosquito larvae (Kanis et al. 2012; Oliveira et al. 2016; Pavela 2016; Pavela et al. 2021). The lower effect on non-target organisms than conventional products also stands among the potential advantages of natural-products-based micro- and nanoemulsions as larvicide products, and low toxicity for humans is notably an important parameter to look after, due to the use of such products in the household area (Oliveira et al. 2016; Pavela et al. 2019; Turchen et al. 2020; Faustino et al. 2020; Rodrigues et al. 2021; Machado et al. 2023).

S. rubra (Mez.) Van der Werff is a common Lauraceae tree in the Guianas, Venezuela and in Amazonian Brazil. This species of high economic importance is used in the wood industry in French Guiana, where it is known as “grignon franc” (Soulères 2021). This industry however produces sizeable quantities of wastes, including sawdust during logs transformation. Moreover, previous studies performed by our group demonstrated that the crude wood extract and the purified compounds rubrenolide and rubrynlolide displayed strong activity towards *Ae. aegypti* larvae, and that commercial sawmill residues of *S. rubra* can be considered as a valuable resource for the large-scale production of these larvicidal extracts (Falkowski et al. 2016, 2020; Clervil et al. 2023). We therefore embarked upon characterizing the larvicidal activity of the *S. rubra* wood

extract on *Ae. aegypti* iso-female strains and their parent wild strain collected in French Guiana. We also evaluated the fitness of mosquitoes exposed to the extract, while concurrently assessing an aqueous-based formulation containing *S. rubra* wood extract in semi-field conditions. Eco- and cytotoxicity were investigated, in the objective of ascertaining the selectivity of the products towards aquatic species and human cells, and display a better picture of the potential of *S. rubra* material to develop novel botanical products displaying larvicidal activity against insecticide-resistant *Ae. aegypti* mosquitoes.

Materials & method

Mosquito strains and rearing

Five *Ae. aegypti* (Diptera: Culicidae) strains were used. The laboratory New Orleans (NO) strain is a reference strain susceptible to all insecticides and maintained for decades in an insectary at the Institut Pasteur de la Guyane, Cayenne, French Guiana. The Cayenne (CAY) strain was collected in the eponym city, and larvae of the F1 progeny were used for all of the experimentations made in the laboratory and for the semi-field experimentations. This population is resistant to both pyrethroid and organophosphate insecticides. A parent population was obtained from wild-caught larvae from Ile Royale (IR), one of the coastal islands of French Guiana (5.287° N; 52.590° W), in 2015, and led to the field strain IR0115. A low insecticide pressure characterizes this area of collection. Two isofemale strains were obtained from this parent population, as previously described after isolation of one mated female (Epelboin et al. 2021). The information concerning the five used strains are summarized in Table 1. Rearing was performed as previously described (Touré et al. 2017).

Table 1 Characteristics of the different *Ae. aegypti* mosquito strains used in the study

Strain	Strain type	Resistance level	Resistance mechanism ^a
New Orleans (NO)	Reference	None	None
Cayenne (CAY)	Wild	Very high	Metabolic & kdr
IR0115	Wild	Low	Metabolic
IR03F26	Isofemale	Middle high	Metabolic
IR05F26	Isofemale	High	Metabolic & kdr

^a kdr: knock-down resistance

Sextonia rubra wood extract and purified compounds

S. rubra (Mez.) Van der Werff (Lauraceae) freshly obtained commercial sawmills wastes were collected in French Guiana. Sawdusts were obtained from Montsinéry (4°58'34" N 52°26'45" W; September 2017) and Kourou sawmills (5°08'54" N 52°41'20" W; March 2020) respectively. Mass distribution by particle size was measured as follows: 60% > 500 µm, 33% between 500 and 200 µm, and 6% < 200 µm for Montsinéry sawdust, and 74% > 500 µm, 21% between 500 and 200 µm, and 4% < 200 µm for Kourou sawdust. All samples were dried at room temperature (21°C, 65% relative humidity) until equilibrium humidity (H<13%). Any visible bark material was manually removed from crude wood powders, which were then extracted using ethyl acetate (EtOAc, RPE grade, Carlo Erba Reagenti SpA, Rodano, Italy) at a 1/20 ratio (w/v) at room temperature by maceration during 24h under stirring. After extraction, the solution was filtered and the solvent removed by evaporation under reduced pressure with a SpeedVac™ concentrator (Savant SPD121P, Thermo Scientific). The resulting crude extracts (SrM, from Montsinéry sawdust, and SrK from Kourou sawdust) were stored in a freezer at -18°C until assayed. Isolation of rubrenolide (Re) and rubrynolide (Ry) was performed using HPLC semi-preparative chromatography (Waters, Milford, MA, USA) according to previously described procedures (Fu et al. 2019). Their identification was confirmed and their content evaluated by NMR. To do so, ¹H NMR spectra were recorded at 400 MHz and ¹³C NMR spectra at 100.6 MHz on a Varian

400 MR spectrometer (Varian, Palo Alto, CA, USA) equipped with a 5 mm inverse probe (Auto X PGF $^1\text{H}/^{15}\text{N}-^{13}\text{C}$). Samples were dissolved in deuterated chloroform (CDCl_3 , Carlo Erba) in 5 mm tubes for analysis. SrM extract was characterized with $8.4 \pm 0.2\%$ Re and $2.0 \pm 0.0\%$ Ry (w/w), and SrK extract with $10.5 \pm 0.1\%$ Re and $8.9 \pm 0.2\%$ Ry (w/w) (Clervil et al. 2023).

Sextonia rubra wood extract formulation

A *S. rubra*-based concentrate was firstly obtained by adding progressively a commercial rapeseed oil methyl ester (RadiaTM 7961, Olean, Ertvelde, Belgium) to *S. rubra* SrK wood extract, containing $10.5 \pm 0.1\%$ and $8.9 \pm 0.2\%$ (w/w) of the larvicidal compounds rubrenolide and rubrynlide, respectively, and which appears as a brownish glutinous molasses. The mixture was left in contact (3 hours) at room temperature ($23 \pm 2^\circ\text{C}$) with regular manual stirring, and then heated (30 minutes, 60°C) in a double-walled water bath while stirring manually until the extract is fully solubilized. This process is repeated to blend the concentrate with the emulsifying ingredients, chosen for their specific HLB value and bio-based origin, and selected as an alkylpolyglycoside (APG, SimulsolTM SL 7 G, Seppic, Paris, France) and an ethoxylated rapeseed oil methyl ester (SepigreenTM C 300, Seppic, Paris, France). An antifoaming agent was also added (acetylenic diol in ethylene glycol, SurfynolTM 104E, Evonik Industries AG, Essen, Germany). The finalized formulation was titrated in 10% of *S. rubra* extract. Eventually, 560 g of this formulation were prepared for the various assays. The stability of the final product, which is an emulsifiable concentrate (EC) aiming at obtaining spontaneous oil-in-water micro-emulsion (EW) when added to distilled water, was evaluated by visual observation at room temperature for validation before use. Composition of the matrix (defined as all formulation components without the extract) and formulation are reported in Table 2.

183

184 **Table 2** Composition (w/w) of formulation matrix and *S. rubra* wood extract emulsifiable

Ingredient	Function	Matrix	Emulsifiable concentrate
Rapeseed oil methyl ester	Non-VOC solvent	25,0	25,0
Ethoxylated rapeseed oil methyl ester	Emulsifier	34,0	34,0
Alkyl polyglycoside	Emulsifier	29,0	29,0
Acetylenic diol	Anti-foaming agent	2,0	2,0
<i>S. rubra</i> wood extract	Active substance	-	10,0
Water		10,0	-

185 concentrate (EC)

186

187

188 *Laboratory evaluation of the larvicidal activity of S. rubra wood extract and formulation*

189 *S. rubra* extract (SrM) was investigated using the WHO cup assay for testing mosquito
190 larvicides (World Health Organisation 2005). Late third or early fourth-instar larvae
191 were used in the test. For each bioassay, 25 larvae of each strain were transferred to
192 cups containing distilled water (99 ml) and the tested product (1 ml) diluted in ethanol
193 (100%, Sigma-Aldrich, Saint-Louis, MO, USA), at a suitable concentration. Eight cups
194 per concentration (8 x 25 larvae) were used, and the concentrations adapted to obtain
195 from 0% to 100% mortality, thus allowing for LD₅₀ and LD₉₀ calculation (from 0.001 to 8
196 µg/ml for NO strain and from 3 to 120 or 160 µg/ml for all other strains). Control
197 treatments were performed for each test with ethanol only (1 ml in 99 ml of distilled
198 water), and larval mortality was recorded at 24 and 48 h after exposure. Test was
199 invalidated if mortality in control was >20%. Lethal doses were obtained by a log-Probit
200 regression under a general linearized model using the MASS package in R software

version 3.2.0 (<https://www.r-project.org/>) as previously described (Falkowski et al. 2016).

Influence of S. rubra wood extract on the oviposition behavior of Ae. aegypti, hatching response, and assessment of mosquito fitness

Assessment of the influence of *S. rubra* wood extract on the oviposition behavior of the mosquito was performed as described in the literature, with some modifications (El Maghrbi and Hosni 2014; Seenivasagan et al. 2019). Holding cages (30 x 30 x 30 cm) were each filled with 40 adult females and 40 adult males (6 days old) of *Ae. aegypti* New Orleans strain (3 replicates) and Cayenne strain (3 replicates). Adult mosquitoes were continuously fed on cotton soaked in 10% sugar until one day before the blood meal. Two days after the female mosquito have been artificially blood fed (beef blood), two breeding sites consisting in laying cups were placed in each cage: a control site (100 ml untreated distilled water), and a site with water (100 ml) treated with SrM extract. Doses for the *S. rubra* treatment were adapted for each population and centered around the two respective 24h-DL₅₀ values (2 µg/ml of SrM *S. rubra* wood extract for New Orleans strain; 70 µg/ml of SrM *S. rubra* wood extract for Cayenne strain). A paper wick was placed in each breeding site on day 3 after the blood meal, and replaced each day during 5 days by a new one, dried, and the number of eggs laid on each paper was counted. The total number of eggs was used as a measure of oviposition response. To obtain a new generation of mosquitoes, each paper wick was put in clear water after 5 days drying, and the rearing performed as previously described (Clervil et al. 2023). The numbers of hatched eggs were counted and 50 larvae coming from hatched eggs of each strain and each condition were placed into 5 different trays as five replicates. The daily survival and stage development follow-up (average number of individuals having developed to pupal and adult stages from 50 larvae according the strain and treatment) lasted until the complete emergence of all individuals. Student t-test was applied to measure the influence of the treated water on

the oviposition behavior and on mosquitoes' fitness (hatching rate and mosquito development). In all analyses, a p -value < 0.05 was taken to indicate statistical significance.

Simulated field trial

Semi-field trials were conducted on the grounds of the Institut Pasteur de la Guyane (French Guiana Pasteur Institute) in Cayenne. For the bioassays, only the *Ae. aegypti* natural population collected in Cayenne town (CAY) was used for testing the formulated products. The CAY strain was selected to provide a stringent context for an assay as close to the reality of the field as possible. Experiments were carried out in white plastic containers (70 l, Icko Apiculture, Bollène, France) filled with water (50 l) and placed undercover, out of direct sunlight and rainfall, and covered with mosquito nets to prevent oviposition by wild mosquitoes or other insects of the area, as well as falling debris during the experiment. Four test containers were used concurrently for each trial session, alongside with four negatives and four positive controls containers. For the test barrels, *S. rubra* formulation (50 g), titrated at 10 % of wood extract, were added to tap water (50 l), to obtain a final concentration of the active product in the barrel corresponding to twice the extract LD₉₀ against *Ae. aegypti* CAY larvae after 48h of exposure in the laboratory, i.e., a trial dose of 102 µg/ml. Negative control containers were filled with tap water only (50 l), and positive control containers were filled with the formulation matrix (40 g) dissolved in the water. During the experiment, tap water was regularly added in the containers to compensate evaporation and maintain a constant 50 l-volume. In each case, tap water was given 24h hours before use for dechlorinating and conditioning. Late third or early fourth-instar larvae from a first generation (F1) reared from wild-caught larvae (F0) were used in the tests. A pool of 100 larvae was initially added to each container at the beginning of the trial, and a new pool of 100 live larvae was released in each container after total emergence of the adults in the control containers (approximately every week). Larval food (yeast powder, 845 mg) was given

to the larvae during the test. The larval, and adult survival were recorded every day until the total adult emergence of the controls. This experimentation was realized during both the dry season (August-September 2020; average maximum temperature 33.8°C; average temperature 27.8°C, average sunlight 239.5 hours; average rainfall 166.5 mm) and the rainy season (January-February 2021; average maximum temperature 30.8°C; average temperature 26.5°C, average sunlight 86.6 hours; average rainfall 507 mm) (infoclimat.fr, 2021, Cayenne). For data analysis, the time (in weeks) is recorded for the inhibition of adult emergence (%IE) to pass below the threshold value of 80%. For each treatment, %IE was estimated according to control containers by the following formula (World Health Organisation 2005; Darriet et al. 2010):

$$\%IE = \frac{C - T}{C} \times 100$$

Where C represents the average percentage emergence in the untreated (control) containers at a particular time and T represents the average percentage emergence in the treated containers in the same period.

Ecotoxicological bioassays on non-target species: Daphnia magna and Chironomus riparius

Ecotoxicity assays were adapted from the guidelines of the “Immediate Immobilization Test” (OECD No. 202) for *Daphnia magna* (Straus, 1820) and the “Immediate Immobilization Test” (OECD No. 235) for *Chironomus riparius* (Meigen, 1804). Each product (crude extract, purified compounds rubrenolide and rubrynlide, formulation, and formulation matrix) was tested at doses based on the beforehand established LD₉₀ (51.2 µg/ml) and LD₅₀ (18.6 µg/ml) of SrK extract used to obtain the formulation, and measured on the CAY wild strain after 48h of exposure. Formulation and formulation matrix effect on non-target species were compared at the semi-field trial dose of twice the SrK crude extract LD₉₀ (102 µg/ml). The impact of formulation, formulation matrix

and the crude extract were concurrently assessed at the reference LD₅₀ of the crude extract. Eventually, the purified compounds rubrenolide and rubrynlolide were tested at 2 µg/mL, representing around 10% of the extract LD₅₀ value, as each compound represented in average 10% of the extract (Clervil et al. 2023). Three conditions were tested: water control, water and solvent control, and the dose of analysis, with five replicates per condition. The physicochemical measurements (pH, dissolved oxygen, conductivity) were performed with measuring devices (sensors). The remaining measures (chlorine, nitrites, nitrates, phosphates) were performed in the laboratory using colorimetric detection (spectrophotometer). Photoperiod and temperature were recorded using a “templight” recorder throughout the test period, from clutch incubation until the end of the exposure.

Cytotoxicity assays

Cell culture

Skin normal fibroblastic cells and HUVEC-Umbil Vein (umbilical cord cells) were purchased from Lonza (Basel, Switzerland), Caco-2 (colon), SHY5Y (human bone marrow neuroblasts), NCI-H295R (adrenal gland), HaCaT (skin keratinocytes) and HuH7D12 (liver) cancer cells lines were obtained from the ECACC collection (Porton, OK, USA). Cells were grown according to ECACC recommendations at 37°C and 5% CO₂. All cultures were added with 10 % FBS (HyClone FetalClone III; Cytiva, H30109.03), penicillin-1% streptomycin (Sigma, P4333) and 2mM glutamine (Sigma, G7513).

Cytotoxic assays

Chemicals were solubilized in DMSO (Sigma, D4540) at a concentration of 20 mM or 100 mg/ml (stock solution) and diluted in culture medium to the desired final concentrations. The doses effect cytotoxic assays (IC₅₀ determination) were performed by increasing concentration of each chemical (final well concentrations: 0.3 µM – 0.9 µM – 3 µM – 9 µM – 25 µM – 50 µM or 1 µg/ml – 3 µg/ml – 9 µg/ml – 28 µg/ml – 83

µg/ml – 250 µg/ml). Cells were plated in 96 wells (4000 cells/well). Twenty-four hours after seeding, cells were exposed to chemicals. After 48h of treatment, cells were washed in PBS (Sigma D8537) and fixed in cooled 90% ethanol (Sigma 32221) / 5% acetic acid (Sigma A6283) for 20 minutes. Then, the nuclei were stained with Hoechst 33342 (B2261 Sigma). Image acquisition and analysis were performed using a Cellomics ArrayScan VTI/HCS Reader (ThermoScientific, Waltham, MA, USA). The survival percentages were calculated as the percentage of cell number after compound treatment over cell number after DMSO treatment. To evaluate the selectivity of the formulation, the selectivity index was used to highlight the results more clearly, as IC₅₀ were available in this case for the non-target cells. This value can be defined as the ratio of the toxic concentration of a product (considering in our case the 48h- IC₅₀ against human cell lines) against its effective bioactive (in our case, the larvicidal activity defined by the products 48h-LD₅₀) concentration (Indrayanto et al. 2021).

Results

Activity of S. rubra wood extract on Ae. aegypti strains displaying various degrees and mechanisms of resistance to deltamethrin

The investigation of the possibility of cross-resistance of *S. rubra* extract with pyrethroids was performed using the SrM extract (*S. rubra* sawdust collected in Montsinéry sawmill). Susceptibility tests on *Ae. aegypti* larvae indicated the lethal concentrations presented in Table 3. A strong larvicidal activity of *S. rubra* wood extracts against the susceptible *Ae. aegypti* strain NO was observed. Characterized resistant strains showed higher LD values than the susceptible strain. The insecticide-selected isofemale strains showed, as CAY wild strain, the highest LD values.

Table 3 Lethal doses ($\mu\text{g/ml}$) for 50% and 90% (LD_{50} and LD_{90} respectively) of the population of *Aedes aegypti* strains, obtained with SrM extract, with associated

Strain	Lethal doses ($\mu\text{g/ml}$) at 24 h		Lethal doses ($\mu\text{g/ml}$) at 48 h	
	LD_{50} (CI)	LD_{90} (CI)	LD_{50} (CI)	LD_{90} (CI)
NO	2.2 (1.7-2.7)	5.3 (4.2-6.5)	0.7 (-3.8-5.1)	1.2 (-7.8-10.3)
CAY	62.9 (59.9-68.9)	96.3 (91.8-100.8)	40.1 (36.9-43.3)	62.0 (56.6-67.4)
IR0115	38.8 (33.1-44.5)	72.7 (62.4-83.1)	26.3 (16.3-36.1)	46.7 (28.4-64.9)
IR03F26	58.7 (54.8-62.5)	93.4 (87.3-99.4)	52.1 (48.0-56.1)	83.8 (77.4-90.3)
IR05F26	48.0 (41.7-54.2)	85.6 (74.9-96.2)	34.2 (29.2-39.3)	63.0 (53.6-72.3)

confidence interval (CI), after 24 and 48h of exposure

Influence of S. rubra wood extract on the oviposition behavior of Ae. aegypti, hatching response, and assessment of mosquito fitness

Parameters related to the mosquito fitness and life-history were assayed on respective DL_{50} doses of SrM extract using the reference NO strain and the wild-type CAY strain. Figure 1 represents oviposition preferences of *Ae. aegypti* females in the presence of two eggs deposition sites (control, and treated with a sub-lethal dose (DL_{50}) of *S. rubra* wood extract). The obtained results show that no statistically significant difference is observed for the oviposition behavior of *Ae. aegypti* within each strain, and notably the wild CAY strain, between the control breeding site and the treated breeding site (NO strain Student t-test: $t = -1.2697$, $df = 7.3768$, $p\text{-value} = 0.2428$; CAY strain Student t-test: $t = 0.54894$, $df = 6.1733$, $p\text{-value} = 0.6023$).

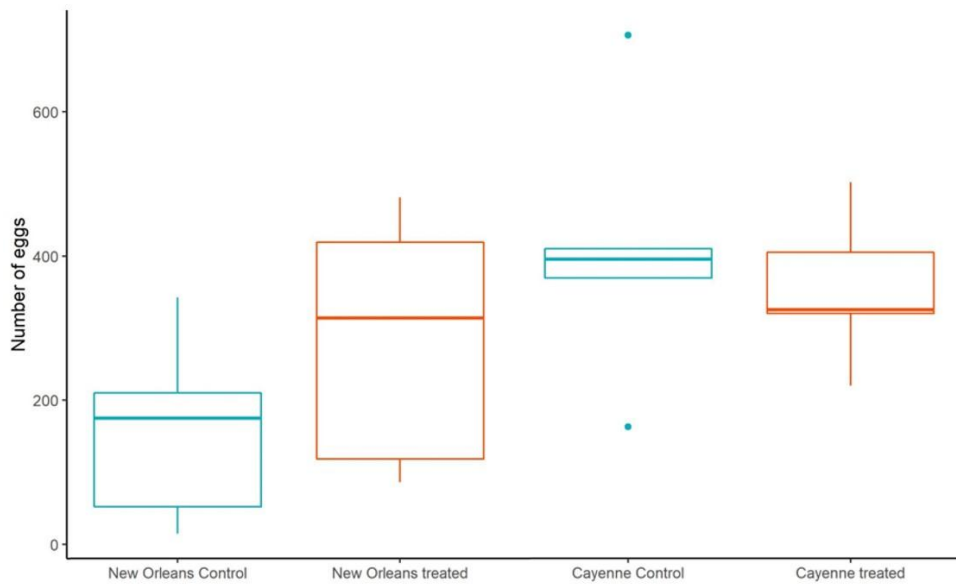


Fig. 1 Oviposition responses of gravid females of *Ae. aegypti* to *Sextonia rubra* wood extract. Values correspond to the daily total number of eggs laid in breeding sites for each mosquito strain (NO and CAY) and in relation to treatment, with three replicates. Control: untreated tap water, treated: 2 µg/ml of *S. rubra* wood extract (SrM) for NO strain and 70 µg/ml of *S. rubra* wood extract (SrM) for CAY strain, corresponding to the respective DL_{50} values for each strain

In a second step, the effects of sub-lethal doses of *S. rubra* extract were assessed on the fitness of *Ae. aegypti* eggs laid on treated sites from oviposition tests, and on the mosquitoes originating from these eggs. The first assay aimed at measuring whether the hatching success of *Ae. aegypti* eggs (NO and CAY strains) is affected by their previous exposure to *S. rubra* wood extracts (Fig. 2). Statistical difference was observed concerning the hatching success of the different strains depending on the treatment of the breeding site (χ^2 : X-squared = 4.4986, df = 1, p -value = 0.03392). However, the hatching rate measured for the CAY strain eggs was not affected by the treatment.

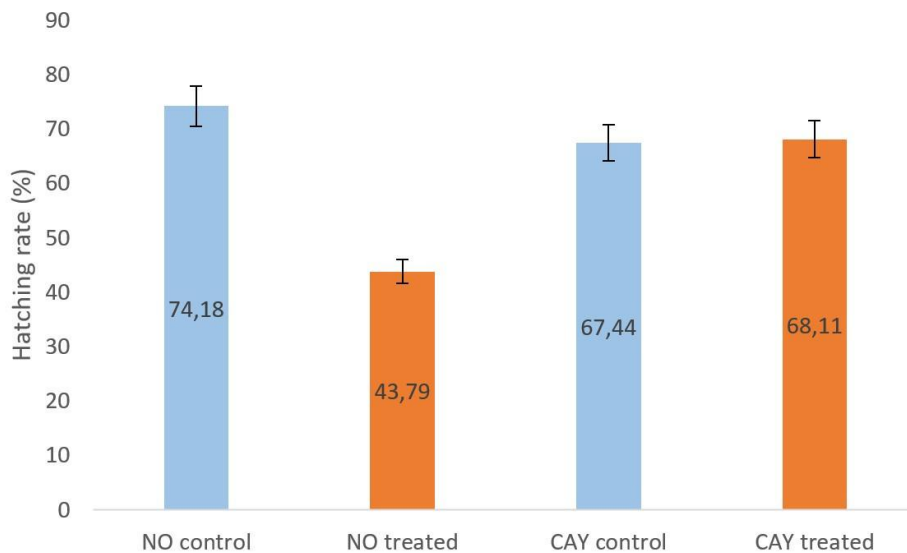


Fig. 2 Hatching rate for the New Orleans strain (NO) and the Cayenne strain (CAY) according to the treatment applied in the breeding site (Control or treated). Control: untreated tap water, Treated: 2 µg/ml of *S. rubra* wood extract (SrM) for NO strain and 70 µg/ml of *S. rubra* wood extract (SrM) for CAY strain

To determine whether the maturation of *Ae. aegypti* eggs on a *S. rubra* extract-impregnated substrate impact the fitness of the mosquitoes at larval, pupal and adult stage, the average number of individuals having developed at each stage from 50 larvae was counted (Fig. 3). For the NO strain, there is a significant decrease between the number of adults obtained from eggs laid in treated breeding sites (Student t-test: $t = 5.2774$, $df = 6.9332$, $p\text{-value} = 0.0005935$). For the CAY strain, the decrease of the number of adults obtained from eggs laid in treated breeding sites is not significant (Student t-test: $t = -0.68374$, $df = 7.8959$, $p\text{-value} = 0.7431$).

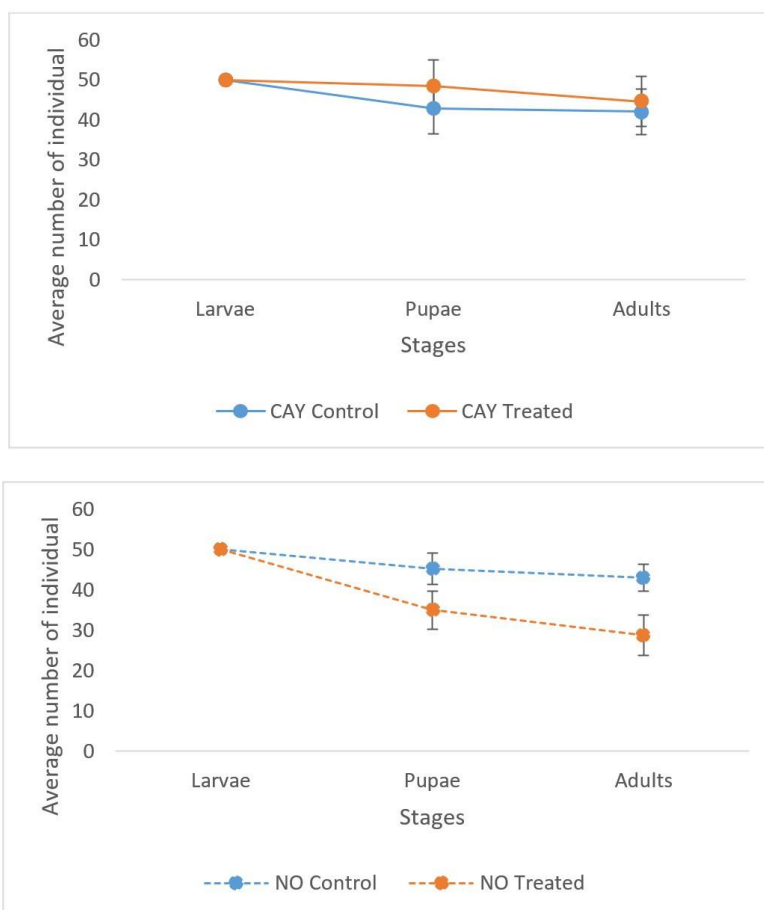


Fig. 3 Average number of individuals having developed to pupal and adult stages from 50 larvae (x five replicates) according the lineage and treatment. CAY: Cayenne wild strain (upper graph), NO: New Orleans strain (lower graph), Control: individuals from eggs laid in control breeding site, Treated: individuals from eggs laid in treated breeding site)

Preparation and preliminary evaluation of a S. rubra-based formulation

A prototype emulsifiable concentrate (EC) was developed to enhance the crude extract water solubility. The EC was titrated in 10% of *S. rubra* extract alongside with stabilizing and dispersing ingredients originating from green chemistry, as described in Table 2. The obtained product was visually stable, the clear brown viscous aspect obtained directly after mixing the ingredients and the extract was maintained during the

two weeks of observation, without the apparition of a cloudy louche or insoluble. This concentrate was also easily dispersed in distilled water in 0.10% solution to reach a concentration of 100 µg/ml of *S. rubra* extract, leading to a homogeneous clear mixture even after 10 days. The visual aspect of the extract, emulsifiable concentrate (EC) and emulsion in distilled water (EW) are represented in Figure 4.

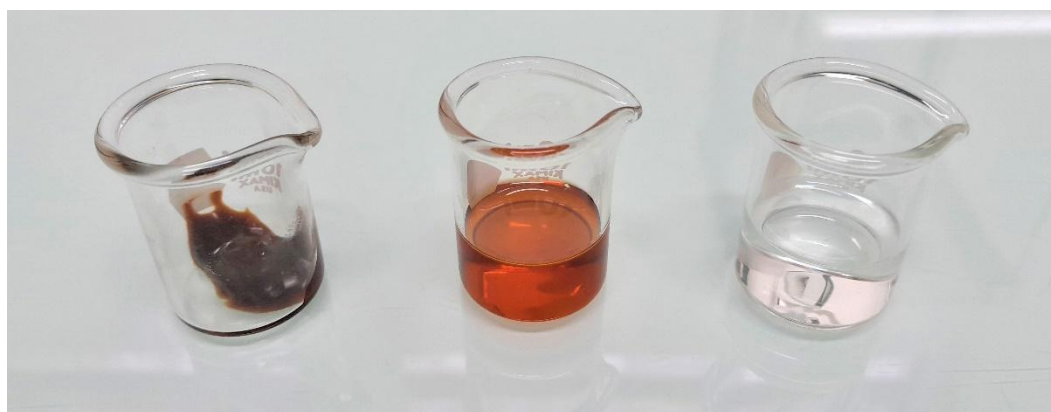


Fig. 4 *S. rubra*-derived larvicidal products: A) wood ethyl acetate crude extract; B) emulsifiable concentrate (EC); C) micro-emulsion in distilled water (EW), 0.1% solution

The formulation was developed using the SrK extract (sawdust collected in Kourou sawmill), which was known to exhibit LD₅₀ values of 39.6 and 18.6 µg/mL at 24h and 48h, respectively, and LD₉₀ values of 118.2 µg/ml and 51.2 µg/ml at 24h and 48h, respectively, against the CAY strain, and to contain 10.5% and 8.9% (w/w) of the bioactive compounds rubrenolide and rubrynlolide respectively (Clervil et al. 2023). When tested in the laboratory against the CAY strain, the formulation led to LD₅₀ values of 48.0 ± 0.01 and 9.3 ± 0.05 µg/mL at 24h and 48h, respectively, and LD₉₀ values of 75.1 ± 0.02 and 39.6 ± 0.04 µg/ml at 24h and 48h, respectively. To ensure that this effect was not due to a toxic activity of the formulation matrix against *Ae. aegypti* larvae, we assessed the matrix and the formulation at 102 µg/ml prior to their evaluation in semi-field conditions. The results obtained for the matrix showed a

mortality rate of $9.5 \pm 4.8\%$ after 24h of exposure, and $14 \pm 2.1\%$ after 48h, whereas the formulation led to 100% of larval mortality in each case.

Semi-field bioassays

The formulation was tested at the trial dose of 102 $\mu\text{g/ml}$ for the final product, corresponding to 2xDL_{90} of the crude extract. The obtained dispersion was homogeneous, without the formation of any oily layer under the water surface, residue, or formation of distinct phases during the whole duration of the assay, despite a certain milky appearance (Fig. 5).



Fig. 5 Comparison of the visual aspects of control (left) and treated (right) container when testing a *S. rubra* wood extract-based formulation against *Ae. aegypti* larvae in semi-field conditions

The overall results of semi-field bioassays are presented in Figures 6 and 7. Figure 6 shows the larval survival of the 3rd-instar larvae of the CAY strain from *Ae. aegypti* mosquitoes. The formulation killed larvae mosquito in less than three days during the dry season, and in less than four days during the rainy season, without any loss of activity during the nine to ten days of observation.

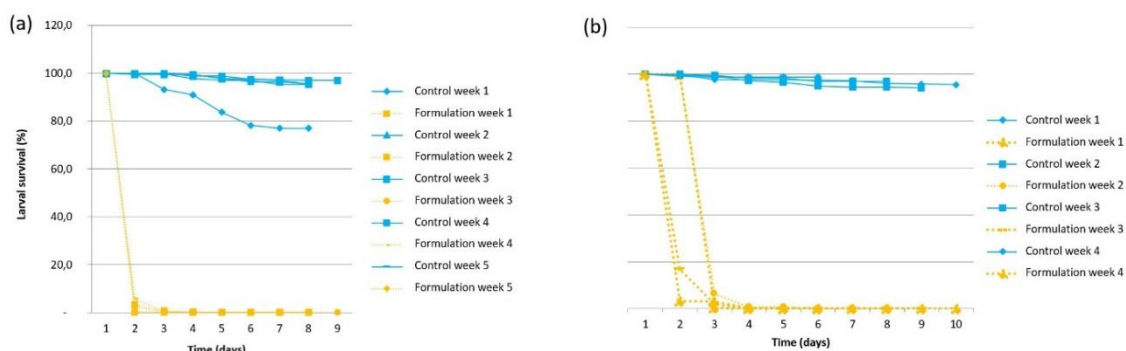


Fig. 6 Survival of *Ae. aegypti* mosquito larvae exposed or not to a formulation based on *S. rubra* wood extract under semi-operational test conditions, during (a) the dry season (August-September 2020) and (b) the rainy season (January-February 2021). Each curve corresponds to one pool of 100 larvae (average value of the four replicates). Control = untreated water, Formulation = EC at a trial dose of 102 µg/ml

Figure 7 shows that the emergence rate of adult *Ae. aegypti* stayed above 80% during all the experimentation (5 weeks i.e., 35 days) for control containers, with a mean emergence rate of $92 \pm 8\%$ in the dry season and $96 \pm 2\%$ in the rainy season. On the contrary, no emergence was observed for the treated containers over all the 5 weeks duration of the experiment. The matrix was tested only once, in the rainy season, exhibiting a slight inhibition of the emergence rate of approximately 15-40%, depending on the time of measurement (Fig. 7).

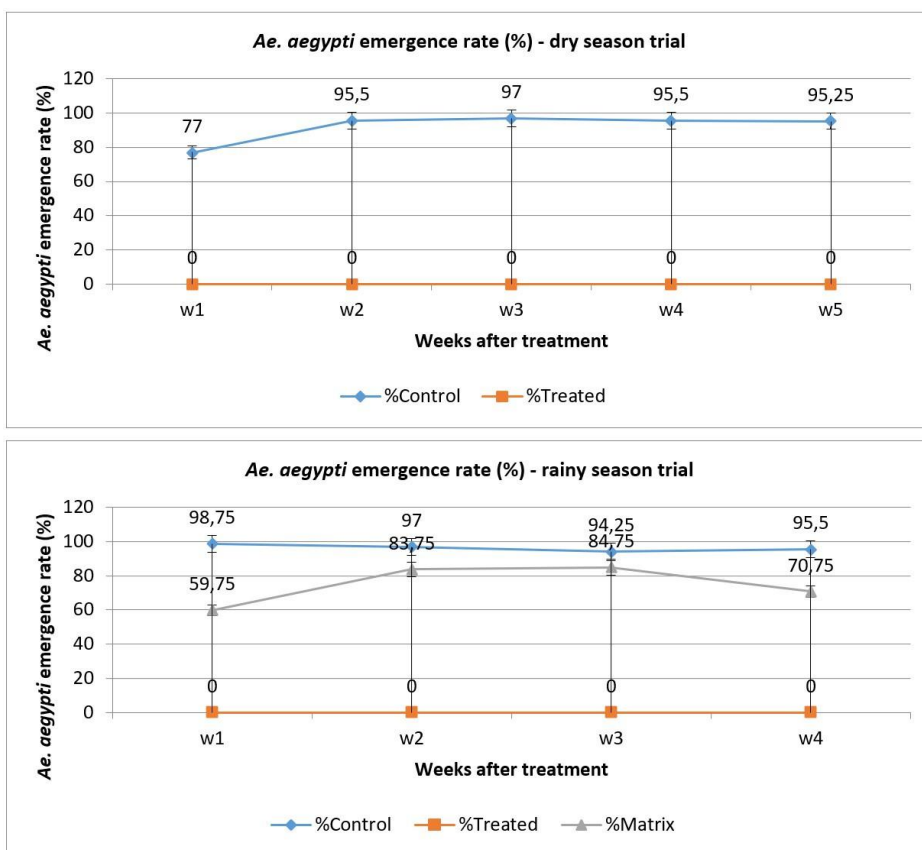


Fig. 7 Simulated field trial performed during dry (upper graph) and rainy (lower graph) seasons respectively: residual activity of *S. rubra* wood extract formulation on the emergence of *Ae. aegypti* (\pm s.e.)

Impact on aquatic non-target organisms and human cell lines

The matrix and the formulation were tested at the dose used for the semi-field trial (102 μ g/ml). At 24h and 48h, the matrix displayed in our test conditions no (at 24h) to low toxicity towards both organisms ($16 \pm 26\%$ of immobilization for *D. magna*, and $12 \pm 11\%$ immobilization for *C. riparius* at 48h) (Figure 8). For the formulation, significant immobilization rates were observed after both 24h and 48h of exposure in the case of *C. riparius*, with $64 \pm 2\%$ and $92 \pm 2\%$ of immobilization respectively. The toxic effect appeared more slowly towards *D. magna*, with only $20 \pm 3\%$ of immobilization after 24h of exposure, but 100% of immobilized animals after 48h.

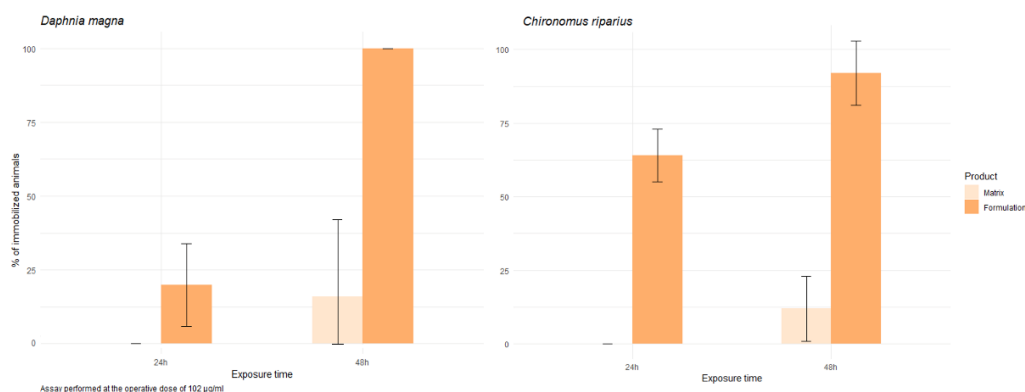


Fig. 8 Comparative assessment of the immobilization percentage (and standard error) of non-target aquatic organisms *Daphnia magna* (left) and *Chironomus riparius* (right) after 24h and 48h of exposure to the formulation matrix (light orange) and full formulation (*S. rubra* extract emulsifiable concentrate, dark orange) at the operative dose of 102 µg/ml. Ecotoxicity assays were performed using 5 animals per condition according to OECD guidelines, hence the high standard deviations observed

The effect of extract, matrix and formulation was then assessed at a lower dose, chosen as the 48h-LD₅₀ (18.6 µg/ml) of the SrK extract used to prepare the emulsifiable concentrate. The compounds rubrenolide and rubrynlolide were tested at a concentration of 2 µg/ml, which corresponds to around 10% of the 48h-LD₅₀ in consistency with the extract composition, which contains 10.5% of rubrenolide and 8.9% of rubrynlolide (Clervil et al. 2023). The overall obtained results are showed in Figure 9. No to very low effect was observed for the matrix towards both non-target organisms. The environmental effect of the formulation could be considered low in the case of *C. riparius*, with a maximum immobilization rate of only 28 ± 18% after 48h of exposure at a dose corresponding to the 48-h LD₅₀ of the extract. The effect of the EC towards *D. magna* was more important, with 68 ± 30% of immobilized animal after 48h. The results obtained for the pure compounds rubrenolide and rubrynlolide indicated a

strong toxicity against non-target organisms, at a dose as low as 2 µg/ml. Rubrenolide was the most toxic compound, with 92 ± 18% immobilized *D. magna* and 88 ± 11% immobilized *C. riparius* as soon as 24h after exposure. At 48 hours, rubrenolide led to almost complete mortality of the two non-target organisms (100% for *D. magna* and 96 ± 9% for *C. riparius*). Rubrynlolide acted more slowly, with immobilization values of 20 ± 0% and 48 ± 27% at 24h, and 96 ± 9% and 64 ± 30% at 48h for *D. magna* and *C. riparius* respectively.

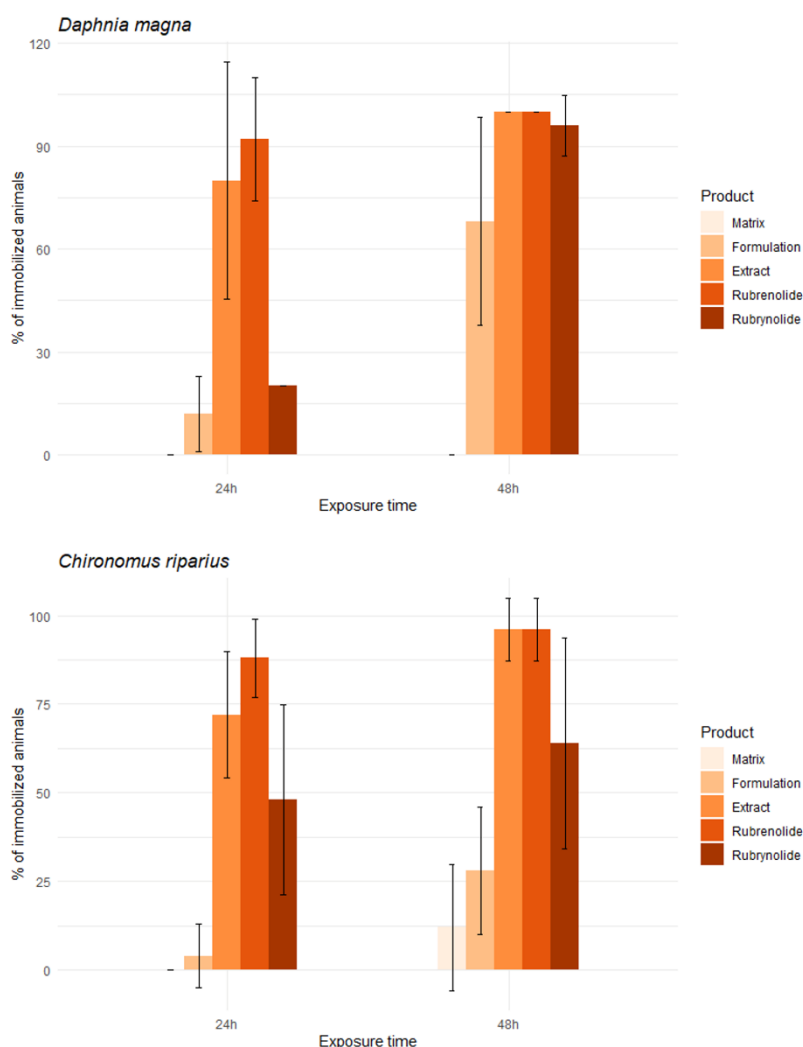


Fig. 9 Immobilization percentage of non-target aquatic organisms *Daphnia magna* (upper graph) and *Chironomus riparius* (lower graph) after 24h and 48h of exposure. Formulation matrix, full formulation (*S. rubra* extract emulsifiable concentrate) and *S.*

495 *rubra* crude extract were tested at 18.6 µg/ml (*S. rubra* SrK extract 48-h LD₅₀ against
496 *Ae. aegypti*), and the pure compounds rubrenolide and rubrynlolide at 2 µg/ml

497

498 Eventually, the cytotoxicity of all products was concurrently assessed towards a panel
499 of human cell lines (Table 4).

500

501 **Table 4** Cytotoxic effect towards human cell lines after 48h of exposure to the
502 formulation matrix, full formulation (*S. rubra* emulsifiable concentrate), *S. rubra* crude
503 extract, and pure compounds rubrenolide and rubrynlolide. IC₅₀ are expressed in µg/ml
504 for mixtures, and in µM for pure compounds

IC ₅₀ (µg/ml ^a or µM ^b)	Liver (HuH7)	Kidney (NCI- H295R)	Colon (Caco2)	Skin fibroblasts	Skin keratinocytes (HaCaT)	Bone marrow neuroblasts (SHY5YSY)	Umbilical cord (HUVEC)
Matrix	143	87	120	> 250	184	> 250	214
Formulation (EC) ^c	102	94	172	> 250	187	156	170
<i>S. rubra</i> extract	32	93	91	> 250	135	> 250	70
Rubrenolide	35	31	> 50	> 50	> 50	> 50	34
Rubrynlolide	12	> 50	> 50	> 50	> 50	> 50	> 50

^a For mixtures

^b For pure compounds

^c EC: emulsifiable concentrate

505

506 According to the standards proposed in the literature, the matrix, formulation and crude
507 extracts could be considered non-toxic when exhibiting IC₅₀ values > 100 µg/ml, this
508 endpoint being set at 25 µM in the case of pure compounds (Cos et al. 2006). In this
509 perspective, both the matrix and the formulation only displayed slight cytotoxic effect
510 against kidney NCI-H295R cells (human carcinoma), with IC₅₀ values of 87 and 94

µg/ml respectively. The extract however could be considered cytotoxic against liver HuH7 cells (human hepatocellular carcinoma, IC₅₀ value of 32 µg/ml), and also led to a moderate effect against HUVEC (human umbilical vein endothelial cells), kidney and colon Caco2 (human colorectal adenocarcinoma) cells, with IC₅₀ values in the 70-93 µg/ml range. Concerning the pure compounds, rubrynlide did not display any cytotoxicity except against liver cells (IC₅₀ of 12 µg/ml). It was the only case when this compound was more toxic than rubrenolide, which exhibited a measurable effect towards liver, kidney and umbilical cord cells, even if above the 25 µM criteria (IC₅₀ in the 31-35 µM) range. Notably, no toxicity was observed against any of the products in the case of skin fibroblasts.

A selectivity index was calculated (Figure 10), except for human skin fibroblasts and human bone marrow neuroblasts, as the obtained IC₅₀ were > 250 µg/ml. Overall, the EC exhibited higher selectivity indices than the crude extract for all tested cell lines, with values from 10 (kidney human carcinoma NCI-H295R cells) to 20 (skin human immortalized keratinocytes HaCat).

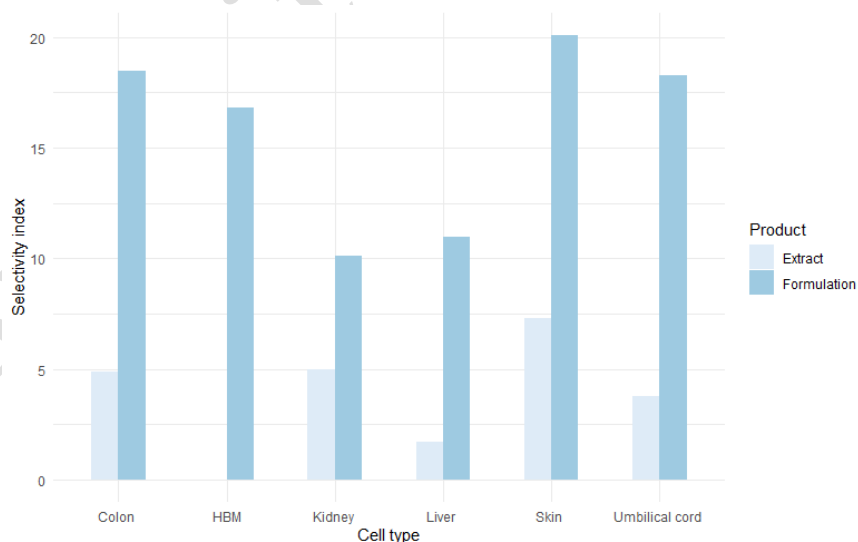


Fig. 10 Selectivity indices calculated as $SI = IC_{50} \text{ (non-target cells)} / LD_{50} \text{ (Ae. aegypti Cayenne strain larvae)}$ after 48 hours of exposure for *Sextonia rubra* extract (light blue) and formulation (dark blue). HBM: human bone marrow

530

531 Discussion

532 In a first step, the activity of *S. rubra* wood extract was assayed against *Ae. aegypti*
533 strains displaying various degrees and mechanisms of resistance to deltamethrin.
534 These new results corroborated the previous efficacy measured against the susceptible
535 Paea strain and the preliminary data obtained about the wild strain Cayenne (Falkowski
536 et al. 2016, 2020). The overall results are in accordance to resistance pattern towards
537 pyrethroids described in French Guiana (Dusfour et al. 2015; Faucon et al. 2017;
538 Guidez et al. 2020; Senthil-Nathan 2020; Epelboin et al. 2021). Besides lethality, the
539 impact of active products on oviposition deterrence and repellence, as well as
540 development, longevity or vitality of the target insect are also of great importance in the
541 field of vector control (Borges et al. 2019; Turchen et al. 2020; Pavela et al. 2021).
542 Plants and plant-derived substances are often known to possibly interfere with
543 oviposition effect, leading to either repellent or attractant effect, notably at
544 concentrations greater than 50 µg/ml (Kanis et al. 2013; El Maghrbi and Hosni 2014;
545 Satho et al. 2015; Seenivasagan et al. 2019). In our case, the absence of repulsive
546 effect concerning *Ae. aegypti* oviposition behavior is of importance when it comes to
547 evaluating the potential of *S. rubra* extract as a larvicide used in stagnant water. A
548 repulsive effect regarding oviposition would indeed lead the mosquitoes to lay eggs in
549 other, untreated sites, and therefore at once make such treatment irrelevant and favor
550 the dispersion of the vector (Kanis et al. 2013). Concurrently, we observed that larval
551 hatching response measured for the CAY strain eggs did not vary significantly
552 depending on the breeding site medium type. Such a result had previously been
553 observed for *Culex molestus* eggs, collected in standing water pools in Australia, and
554 placed in breeding sites with different treatments (Methoprene and QN50, a
555 sesquiterpene alcohol derived from Australian sandalwood *Santalum spicatum* A. DC.)
556 (Spafford et al. 2007). Moreover, the chance to obtain pupae and adult *Ae. aegypti*

mosquitoes is lowered when eggs are laid in treated sites in the case of the NO strain only, as no statistical difference was obtained in the case of the Cayenne wild strain. It would thus be interesting to assess such an effect on the formulation and on other wild-type strains collected in French Guiana, in order to evaluate the existence of a potential secondary beneficial effect of *S. rubra* extract and formulation in case of sublethal exposition of the larvae (Silva et al. 2019).

The ingredients of the formulation were chosen in order to fulfill the regulation (EU) 528/2012 and directive 98/8/EC (Directive Biocide) concerning the making available on the market and use of biocidal products, or the regulation (EC) 1107/2009 concerning the placing of plant protection products on the market. Regulatory approvals represent an important barrier to the industrial implementation of botanical insecticides, and taking them into account in an early stage is therefore crucial (Villaverde et al. 2014; Isman 2020). Notably, natural-oil based esters, including methyl esters, have been recorded and patented for their noticeable solvency properties when used to solubilize various insecticides and obtain oil-in-water (EW) emulsions (Taranta et al. 2002; Salimon et al. 2012; Tan et al. 2013). Surfactants were chosen among products obtained from renewable raw material, with non-ecotoxic characters in environmental conditions as well as high biodegradability properties (Rybinski and Hill 1998; Johansson and Svensson 2001; Renkin et al. 2005; Zhang et al. 2011; Geetha and Tyagi 2012; Peng et al. 2019).

The LD₅₀ and LD₉₀ values obtained in the laboratory for the formulation can lead to its categorization as a significant larvicidal agent (Machado et al. 2023). The micro- and nano-encapsulation of active ingredients, notably botanical products, may lead to somewhat to noticeable increase of lethal concentrations value for the formulated products (Kanis et al. 2012; Pavela et al. 2021). Such phenomena did not appear in our hands, with on the contrary the same range of activity for both the crude extract and the emulsifiable concentrate, and thus a gain of effect for the active ingredient, as the

EC is titrated in 10% of *S. rubra* extract. This could be due to an increased bioavailability because of the presence of tensioactive compounds in the EC, such as previously observed for controlled-release systems and nano-emulsions of essential oils (Maia et al. 2019; Rodrigues et al. 2021). Moreover, the mortality rates obtained for both the matrix and the formulation, comparatively, confirmed the relative innocuousness of the matrix against *Ae. aegypti*, and ensured that the semi-field bioassay would give us information on the *S. rubra* extract in the form of an EC as the only active product.

The efficiency of the tested formulation as a mosquito larvicide was demonstrated by our results, especially towards the CAY *Ae. aegypti* strain, exhibiting a strong resistance to conventional insecticides. The milky appearance of the aqueous media treated with the emulsifiable concentrate observed in semi-operational conditions can be due to the composition of the tap water used for the assay, although it was let 24h for dechlorination before use, or to the addition of larval food. Indeed, the presence calcium, sodium or chloride ions can modify the spontaneous formation of a micro-emulsion, and lead to a basic emulsion, while changing the translucent aspect for a cloudy one (Egito et al. 2020). The strong and rapid acute toxicity can represent a technical advantage in the field.(Darriet et al. 2010) The lasting residual activity suggests an effective potential of the formulation for vector population inhibition during dengue transmission periods, which is a key parameter for disease control (Kanis et al. 2013, 2018; Maia et al. 2019). Botanical products often have to face the problem of residual activity, due to a high biodegradability through photodegradation, abiotic oxidation or volatilization (Amer and Mehlhorn 2006; Isman 2020). In our case, this relatively long residual activity was obtained for a simple emulsion, without using slow-release systems. This can be put in perspective with the previously demonstrated stability of the crude *S. rubra* extract in ambient conditions over time (Clervil et al. 2023).

Concerning the ecotoxicity of the matrix and the formulation under our experimental conditions, *D. magna* were overall more sensitive to the tested products than *C. riparius*, with high standard deviations observed, due to the protocol involving a low number of animals. For the matrix and the formulation at the dose used for the semi-field trial (102 µg/ml), our results highlighted the harmlessness of the green formulation basis against non-targeted organisms, as observed towards *Ae. aegypti* larvae. These results are also consistent with suppliers, regulatory and literature data concerning the ecotoxicological assessment of the formulation matrix components (Renkin et al. 2005; Szwach and Lukosek 2017; ECHA 2022a, b). At this dose, an *Ae. aegypti* larval mortality of 100% was obtained as soon as 24h when assessing the formulation in the laboratory. At 102 µg/ml, the formulation thus only showed selectivity towards both non-target organisms at 24h (5 times less mortality for *C. riparius* than for *Ae. aegypti* larvae at the same dose, and around 1.5 times less mortality for *D. magna*), this advantage being lost after 48h of exposure.

However, when testing the effect of extract, matrix and formulation at the 48h-LD₅₀ (18.6 µg/ml) of the SrK extract used to prepare the emulsifiable concentrate, the formulation led to 1.5 less toxicity towards *D. magna*, and 3.4 less toxic effect towards *C. riparius*, when compared to the extract. Besides solving technological problems due to the solubilization of *S. rubra* lipophilic crude extract in water, the use of the formulation also allows to lower the toxicity of the active ingredient at the same dose, without losing larvicidal efficacy. Complementary assessments should now be performed in cooperation with public health workers to determine the useful dose of formulation in the field, and therefore identify the precise level of selectivity proposed by the *S. rubra* EC. The data obtained for rubrenolide and rubrymolide are consistent with the previous evaluation of these pure compounds against *Ae. aegypti* larvae, with higher toxic activity observed for rubrenolide than rubrymolide both on the laboratory strain Paea and the wild strain Cayenne (Falkowski et al. 2016). Thus, valuing the

extract, and even more the formulation, rather than the pure compounds, can add a noticeable value when it comes to the ecotoxicological profile of the larvicidal products. Eventually, except for the extract towards kidney cells, the cytotoxic effect of the tested product was overall moderate to non-significant in an acute toxicity assay. The data obtained for the pure compounds rubrenolide and rubrynlide are consistent with a previous study, in which the pure compounds exhibited $IC_{50} > 100 \mu\text{g/ml}$ against NIH-3T3 fibroblasts cells (Rodrigues et al. 2010). Even if a noticeable toxicity had been previously reported for rubrenolide (GI_{50} in the 3.3-9.9 $\mu\text{g/ml}$ range i.e., $< 33 \mu\text{M}$) against UACC-62 (human melanoma), MCF-7 (human breast cancer), HT-29 (human colorectal adenocarcinoma) and PC-3 (human prostate cancer) cell lines, most values in our assays remained out of the cytotoxic range (Tófoli et al. 2016). However, complementary cytotoxic evaluation of the products in *in vivo* models would be a valuable asset. The selectivity index data are of high interest, as a $SI \geq 10$ was previously cited in the literature as a valuable criterion for selective samples (Awouafack et al. 2013; Houël et al. 2015; Peña-Morán et al. 2016). These results therefore reinforce those obtained when comparatively assessing the ecotoxicity of the crude extracts towards the formulated products, as in both cases (eco- and cytotoxicity), a clear advantage is obtained when using the *S. rubra* EC.

Conclusion

This study aimed at the evaluation of *S. rubra* wood extract towards *Ae. aegypti* reference, laboratory and wild strains, concurrently to the assessment of a *S. rubra*-based formulated product for application in aqueous media. We demonstrated the efficiency of the wood extracts on *Ae. aegypti* larvae, notably resistant wild strains, and highlighted the absence of repulsive effect of the wood extract on the mosquitoes' oviposition behavior. A simple, stable, easy-to-produce and easy-to-use formulation, in

the form of an emulsifiable concentrate titrated in 10% of *S. rubra* extract, was successfully obtained. The choice of the ingredients, derived from green chemistry, allowed both the application of a lipophilic botanical extract in water and the compliance with European standards and regulations. This formulation demonstrated strong larvicidal activity (acute toxicity and residual activity) through semi-field assays alongside with better selectivity towards non-target aquatic organisms and human cell lines than the crude wood extract. Following previous promising results on the larvicidal activity of *S. rubra* wood extract and the useability of sawmill residues for a large-scale production of the extract, in a biorefinery perspective included in circular economy objectives, this work aims to contribute to the development of novel solutions for the management of *Ae. aegypti* mosquitoes and the control of vector-borne diseases such as dengue fever and Zika or chikungunya viruses, while taking into account environmental and human health issues associated with the use of insecticide products.

References

- Agrawal AA, Weber MG (2015) On the study of plant defence and herbivory using comparative approaches: How important are secondary plant compounds. *Ecol Lett* 18:985–991. <https://doi.org/10.1111/ele.12482>
- Amer A, Mehlhorn H (2006) Persistency of larvicidal effects of plant oil extracts under different storage conditions. *Parasitol Res* 99:473–477. <https://doi.org/10.1007/s00436-006-0183-2>
- Awouafack MD, McGaw LJ, Gottfried S et al (2013) Antimicrobial activity and cytotoxicity of the ethanol extract, fractions and eight compounds isolated from *Eriosema robustum* (Fabaceae). *BMC Complement Altern Med* 13:289–297. <https://doi.org/10.1186/1472-6882-13-289>

- Bailly S, Rousset D, Fritzell C et al (2021) Spatial distribution and burden of emerging arboviruses in French Guiana. *Viruses* 13:1299. <https://doi.org/10.3390/v13071299>
- Benelli G (2015) Research in mosquito control: current challenges for a brighter future. *Parasitol Res* 114:2801–2805. <https://doi.org/10.1007/s00436-015-4586-9>
- Benelli G (2020) On a magical mystery tour of green insecticide research: Current issues and challenges. *Molecules* 25:5014. <https://doi.org/10.3390/molecules25215014>
- Benelli G, Mehlhorn H (2016) Declining malaria, rising of dengue and Zika virus: insights for mosquito vector control. *Parasitol Res* 115:1747–1754. <https://doi.org/10.1007/s00436-016-4971-z>
- Borges JCM, Haddi K, Oliveira EE et al (2019) Mosquitocidal and repellent potential of formulations containing wood residue extracts of a Neotropical plant, *Tabebuia heptaphylla*. *Ind Crops Prod* 129:424–433. <https://doi.org/10.1016/j.indcrop.2018.12.022>
- Carvalho FD, Moreira LA (2017) Why is *Aedes aegypti* Linnaeus so successful as a species? *Neotrop Entomol* 46:243–255. <https://doi.org/10.1007/s13744-017-0520-4>
- Clervil E, Bossu J, Duchemin JB et al (2023) *Sextonia rubra* (Mez.) van der Werff sawmills residues as a valuable resource for the production of larvicidal extracts against *Ae. aegypti* Linnaeus (Diptera: Culicidae). *Ind Crops Prod* 193: 116162. <https://doi.org/10.1016/j.indcrop.2022.116162>
- Cos P, Vlietinck AJ, Berghe D Vanden, Maes L (2006) Anti-infective potential of natural products: How to develop a stronger *in vitro* “proof-of-concept.” *J Ethnopharmacol* 106:290–302. <https://doi.org/10.1016/j.jep.2006.04.003>

- Darriet F, Marcombe S, Etienne M et al (2010) Field evaluation of pyriproxyfen and spinosad mixture for the control of insecticide resistant *Aedes aegypti* in Martinique (French West Indies). *Parasit Vectors* 3:1–8. <https://doi.org/10.1186/1756-3305-3-88>
- de Oliveira JL, Campos EVR, Bakshi M, et al (2014) Application of nanotechnology for the encapsulation of botanical insecticides for sustainable agriculture: Prospects and promises. *Biotechnol Adv* 32:1550–1561. <https://doi.org/10.1016/j.biotechadv.2014.10.010>
- de Thoisy B, Duron O, Epelboin L et al (2021) Ecology, evolution, and epidemiology of zoonotic and vector-borne infectious diseases in French Guiana: Transdisciplinarity does matter to tackle new emerging threats. *Infection, Genetics and Evolution* 93:104916. <https://doi.org/10.1016/j.meegid.2021.104916>
- Dusfour I, Zorrilla P, Guidez A et al (2015) Deltamethrin resistance mechanisms in *Aedes aegypti* populations from three French overseas territories worldwide. *PLoS Negl Trop Dis* 9:1–17. <https://doi.org/10.1371/journal.pntd.0004226>
- ECHA (2022a) Fatty acids, rape-oil, Me esters. <https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/16753>. Accessed 11 Mar 2022
- ECHA (2022b) Alkyl Polyglycosides (APG). <https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14407/12>. Accessed 11 Mar 2022
- Egito E, Amaral-Machado L, Alencar E, Oliveira A (2020) Microemulsion systems: from the design and architecture to the building of a new delivery system for multiple - route drug delivery. *Drug Deliv Transl Res*. <https://doi.org/10.1007/s13346-020-00872-8>

- El Maghrbi AA, Hosni MM (2014) Influence of some plant extracts on the ovi-position behavior of *Aedes fluviatilis* and *Culex quinquefasciatus*. Int J Vet Sci Med 2:95–98. <https://doi.org/10.1016/j.ijvsm.2014.04.003>
- Epelboin Y, Chaney SC, Guidez A et al (2018) Successes and failures of sixty years of vector control in French Guiana: What is the next step? Mem Inst Oswaldo Cruz 113: e170398. <https://doi.org/10.1590/0074-02760170398>
- Epelboin Y, Wang L, Gianetto QG, et al (2021) CYP450 core involvement in multiple resistance strains of *Aedes aegypti* from French Guiana highlighted by proteomics, molecular and biochemical studies. PLoS One 16:1–22. <https://doi.org/10.1371/journal.pone.0243992>
- Falkowski M, Jahn-Oyac A, Ferrero E et al (2016) Assessment of a simple compound-saving method to study insecticidal activity of natural extracts and pure compounds against mosquito larvae. J Am Mosq Control Assoc 32:337–340. <https://doi.org/10.2987/16-6613.1>
- Falkowski M, Jahn-Oyac A, Odonne G et al (2020) Towards the optimization of botanical insecticides research: *Aedes aegypti* larvicidal natural products in French Guiana. Acta Trop 201:105179. <https://doi.org/10.1016/j.actatropica.2019.105179>
- Faucon F, Gaude T, Dusfour I et al (2017) In the hunt for genomic markers of metabolic resistance to pyrethroids in the mosquito *Aedes aegypti*: An integrated next-generation sequencing approach. PLoS Negl Trop Dis 11:1–20. <https://doi.org/10.1371/journal.pntd.0005526>
- Faustino CG, de Medeiros FA, Galardo AKR et al (2020) Larvicide activity on *Aedes aegypti* of essential oil nanoemulsion from the *Protium heptaphyllum* resin. Molecules 25: 5333. <https://doi.org/10.3390/MOLECULES25225333>

- 763 Fu T, Houël E, Amusant N et al (2019) Biosynthetic investigation of γ -lactones in
764 *Sextonia rubra* wood using in situ TOF-SIMS MS/MS imaging to localize and
765 characterize biosynthetic intermediates. Sci Rep 9:1928.
766 <https://doi.org/10.1038/s41598-018-37577-5>
- 767 Geetha D, Tyagi R (2012) Alkyl Poly Glucosides (APGs) surfactants and their
768 properties: A review. Tenside, Surfactants, Deterg 49:417–427.
769 <https://doi.org/10.3139/113.110212>
- 770 George DR, Finn RD, Graham KM, Sparagano OA (2014) Present and future potential
771 of plant-derived products to control arthropods of veterinary and medical
772 significance. Parasit Vectors 7:1–12. <https://doi.org/10.1186/1756-3305-7-28>
- 773 Gerwick BC, Sparks TC (2014) Natural products for pest control: An analysis of their
774 role, value and future. Pest Manag Sci 70:1169–1185.
775 <https://doi.org/10.1002/ps.3744>
- 776 Gloria-Soria A, Kellner DA, Brown JE et al (2016) Temporal genetic stability of
777 *Stegomyia aegypti* (= *Aedes aegypti*) populations. Med Vet Entomol 30:235–240.
778 <https://doi.org/10.1111/mve.12153>
- 779 Guidez A, Pocquet N, Restrepo J, et al (2020) Spatiotemporal multiple insecticide
780 resistance in *Aedes aegypti* populations in French Guiana: Need for alternative
781 vector control. Mem Inst Oswaldo Cruz 115:1–9. [https://doi.org/10.1590/0074-](https://doi.org/10.1590/0074-02760200313)
782 [02760200313](https://doi.org/10.1590/0074-02760200313)
- 783 Houël E, Gonzalez G, Bessière J-M, et al (2015) Therapeutic switching: From
784 antidermatophytic essential oils to new leishmanicidal products. Mem Inst
785 Oswaldo Cruz 110:106-113. <https://doi.org/10.1590/0074-02760140332>

786 Indrayanto G, Putra GS, Suhud F (2021) Validation of *in-vitro* bioassay methods:
 787 Application in herbal drug research. Profiles Drug Subst Excip Relat Methodol
 788 46:273–307. <https://doi.org/10.1016/bs.podrm.2020.07.005>

789 Isman MB (2020) Botanical insecticides in the twenty-first century-fulfilling their
 790 promise? Annu Rev Entomol 65:233–249. [https://doi.org/10.1146/annurev-ento-](https://doi.org/10.1146/annurev-ento-011019-025010)
 791 011019-025010

792 Isman MB, Grieneisen ML (2014) Botanical insecticide research: Many publications,
 793 limited useful data. Trends Plant Sci 19:140–145.
 794 <https://doi.org/10.1016/j.tplants.2013.11.005>

795 Johansson I, Svensson M (2001) Surfactants based on fatty acids and other natural
 796 hydrophobes. Curr Opin Colloid Interface Sci 6:178–188.
 797 [https://doi.org/10.1016/S1359-0294\(01\)00076-0](https://doi.org/10.1016/S1359-0294(01)00076-0)

798 Jones RT, Ant TH, Cameron MM, Logan JG (2021) Novel control strategies for
 799 mosquito-borne diseases. Philosophical Transactions of the Royal Society B:
 800 Biological Sciences 376:20190802. <https://doi.org/10.1098/rstb.2019.0802>

801 Kanis LA, Prophiro JS, Da Silva Vieira E et al (2012) Larvicidal activity of *Copaifera* sp.
 802 (Leguminosae) oleoresin microcapsules against *Aedes aegypti* (Diptera:
 803 Culicidae) larvae. Parasitol Res 110:1173–1178. [https://doi.org/10.1007/s00436-](https://doi.org/10.1007/s00436-011-2610-2)
 804 011-2610-2

805 Kanis LA, Rabelo BD, Moterle D et al (2013) Standardized extract of *Piper ovatum*
 806 (Piperaceae) to control *Aedes aegypti* larvae (Diptera: Culicidae). Ind Crops Prod
 807 50:816–820. <https://doi.org/10.1016/j.indcrop.2013.08.034>

808 Kanis LA, Rabelo BD, Moterle D et al (2018) *Piper ovatum* (Piperaceae) extract/starch-
 809 cellulose films to control *Aedes aegypti* (Diptera: Culicidae) larvae. Ind Crops Prod
 810 122:148–155. <https://doi.org/10.1016/j.indcrop.2018.05.055>

- Kumar PM, Murugan K, Kovendan K et al (2012) Mosquito larvicidal and pupicidal efficacy of *Solanum xanthocarpum* (Family: Solanaceae) leaf extract and bacterial insecticide, *Bacillus thuringiensis*, against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol Res* 110:2541–2550. <https://doi.org/10.1007/s00436-011-2797-2>
- Machado FP, Folly D, Salas Enriquez JJ et al (2023) Nanoemulsion of *Ocotea indecora* (Shott) Mez essential oil: Larvicidal effects against *Aedes aegypti*. *Ind Crops Prod* 192: 116031. <https://doi.org/10.1016/j.indcrop.2022.116031>
- Maia JD, La Corte R, Martinez J, et al (2019) Improved activity of thyme essential oil (*Thymus vulgaris*) against *Aedes aegypti* larvae using a biodegradable controlled release system. *Ind Crops Prod* 136:110–120. <https://doi.org/10.1016/j.indcrop.2019.03.040>
- Miresmailli S, Isman MB (2014) Botanical insecticides inspired by plant-herbivore chemical interactions. *Trends Plant Sci* 19:29–35. <https://doi.org/10.1016/j.tplants.2013.10.002>
- Oliveira AEMFM, Duarte JL, Amado JRR et al (2016) Development of α larvicidal nanoemulsion with *Pterodon emarginatus* Vogel oil. *PLoS One* 11:1–16. <https://doi.org/10.1371/journal.pone.0145835>
- Pavela R (2016) Encapsulation – A convenient way to extend the persistence of the effect of eco-friendly mosquito larvicides. *Curr Org Chem* 20:2674–2680. <https://doi.org/10.2174/1385272820666151026231851>
- Pavela R, Maggi F, Iannarelli R, Benelli G (2019) Plant extracts for developing mosquito larvicides: From laboratory to the field, with insights on the modes of action. *Acta Trop* 193:236–271. <https://doi.org/10.1016/j.actatropica.2019.01.019>

- Pavela R, Pavoni L, Bonacucina G et al (2021) Encapsulation of *Carlina acaulis* essential oil and carlina oxide to develop long - lasting mosquito larvicides: microemulsions versus nanoemulsions. J Pest Sci (2004) 94:899–915
- Peña-Morán OA, Villarreal ML, Álvarez-Berber L et al (2016) Cytotoxicity, post-treatment recovery, and selectivity analysis of naturally occurring podophyllotoxins from *Bursera fagaroides* var. *fagaroides* on breast cancer cell lines. Molecules 21:1013–1024. <https://doi.org/10.3390/molecules21081013>
- Peng Y, Xiao D, Yu G, et al (2019) Effect of an eco-friendly o/w emulsion stabilized with amphiphilic sodium alginate derivatives on lambda-cyhalothrin adsorption–desorption on natural soil minerals. J Environ Sci 78:230–238. <https://doi.org/10.1016/j.jes.2018.09.025>
- Renkin M, Fleurackers S, Szwach I, Hreczuch W (2005) Rapeseed methyl ester ethoxylates: A new class of surfactants of environmental and commercial interest. Tenside, Surfactants, Deterg 42:280–287. <https://doi.org/10.3139/113.100269>
- Richards LA, Dyer LA, Forister ML et al (2015) Phytochemical diversity drives plant–insect community diversity. Proc Natl Acad Sci USA 112:10973–10978. <https://doi.org/10.1073/pnas.1504977112>
- Rodrigues A, Theodoro PNET, Eparvier V et al (2010) Search for antifungal compounds from the wood of durable tropical trees. J Nat Prod 73:1706–1707. <https://doi.org/10.1021/np1001412>
- Rodrigues ABL, Martins RL, Rabelo É de M et al (2021) Development of nano-emulsions based on *Ayapana triplinervis* essential oil for the control of *Aedes aegypti* larvae. PLoS One 16:1–23. <https://doi.org/10.1371/journal.pone.0254225>
- Rybinski W Von, Hill K (1998) Alkyl Polyglycosides—Properties and Applications of a new Class of Surfactants. Angew Chem Int Ed Engl 37:1328–1345.

860 [https://doi.org/10.1002/\(SICI\)1521-3773\(19980605\)37:10<1328::AID-](https://doi.org/10.1002/(SICI)1521-3773(19980605)37:10<1328::AID-)
861 [ANIE1328>3.0.CO;2-9](https://doi.org/10.1002/(SICI)1521-3773(19980605)37:10<1328::AID-)

862 Salazar D, Lokvam J, Mesones I et al (2018) Origin and maintenance of chemical
863 diversity in a species-rich tropical tree lineage. *Nat Ecol Evol* 2:983–990.
864 <https://doi.org/10.1038/s41559-018-0552-0>

865 Salimon J, Salih N, Yousif E (2012) Industrial development and applications of plant
866 oils and their biobased oleochemicals. *Arab J Chem* 5:135–145.
867 <https://doi.org/10.1016/j.arabjc.2010.08.007>

868 Satho T, Dieng H, Ahmad MHI et al (2015) Coffee and its waste repel gravid *Aedes*
869 *albopictus* females and inhibit the development of their embryos. *Parasit Vectors*
870 8:272–286. <https://doi.org/10.1186/s13071-015-0874-6>

871 Seenivasagan T, Sharma A, Yadav R et al (2019) Plant infusions mediate oviposition
872 of malaria, dengue and filariasis vectors: Push-pull approach for vector
873 surveillance and control. *J Biopestic* 12:95–103

874 Senthil-Nathan S (2020) A Review of resistance mechanisms of synthetic insecticides
875 and botanicals, phytochemicals, and essential oils as alternative larvicidal agents
876 against mosquitoes. *Front Physiol* 10:1591.
877 <https://doi.org/10.3389/fphys.2019.01591>

878 Silva LL de S, Fernandes KM, Miranda FR et al (2019) Exposure of mosquito (*Aedes*
879 *aegypti*) larvae to the water extract and lectin-rich fraction of *Moringa oleifera*
880 seeds impairs their development and future fecundity. *Ecotoxicol Environ Saf* 183:
881 109583. <https://doi.org/10.1016/j.ecoenv.2019.109583>

882 Silvério MRS, Espindola LS, Lopes NP, Vieira PC (2020) Plant natural products for the
883 control of *Aedes aegypti*: The main vector of important arboviruses. *Molecules*
884 25:3484. <https://doi.org/10.3390/molecules25153484>

885 Soulères O (2021) L'aménagement des forêts de Guyane française. In: Lanly JP and
 886 Soulères O (eds) Travailler au service des forêts tropicales, regards croisés de
 887 forestiers, L'Harmattan, Paris, France, pp. 37-57

888 Spafford H, Jardine A, Carver S et al (2007) Laboratory determination of efficacy of a
 889 *Santalum spicatum* extract for mosquito control. J Am Mosq Control Assoc
 890 23:304–311. [https://doi.org/10.2987/8756-971X\(2007\)23\[304:LDOEOA\]2.0.CO;2](https://doi.org/10.2987/8756-971X(2007)23[304:LDOEOA]2.0.CO;2)

891 Szwach I, Lukosek M (2017) Comparative evaluation of environmental impact of
 892 selected ethoxylate fatty alcohols and ethoxylate fatty acid methyl esters as
 893 nonionic surfactants. Pol J Environ Stud 26:1245–1250.
 894 <https://doi.org/10.15244/pjoes/67748>

895 Tan S, Saad J, Wong L et al (2013) A formulated solvent composition for pesticide. WO
 896 2013/05

897 Taranta C, Mansour P, Bourgogne M, Henriët M (2002) Oil-in-water emulsion
 898 formulation of insecticides. US 2002/00

899 Tófoli D, Martins L, Matos M, et al (2016) Antiproliferative Butyrolactones from
 900 *Mezilaurus crassiramea*. Planta Med Lett 3:e14–e16. [https://doi.org/10.1055/s-](https://doi.org/10.1055/s-0035-1568355)
 901 [0035-1568355](https://doi.org/10.1055/s-0035-1568355)

902 Touré S, Nirma C, Falkowski M et al (2017) *Aedes aegypti* larvicidal sesquiterpene
 903 alkaloids from *Maytenus oblongata*. J Nat Prod 80:384–390.
 904 <https://doi.org/10.1021/acs.jnatprod.6b00850>

905 Turchen LM, Cosme-Júnior L, Guedes RNC (2020) Plant-derived insecticides under
 906 meta-analyses: Status, biases, and knowledge gaps. Insects 11:1–13.
 907 <https://doi.org/10.3390/insects11080532>

Villaverde JJ, Sevilla-Morán B, Sandín-España P et al (2014) Biopesticides in the framework of the European Pesticide Regulation (EC) No. 1107/2009. *Pest Manag Sci* 70:2–5. <https://doi.org/10.1002/ps.3663>

World Health Organisation W (2005) Guidelines for laboratory and field testing of mosquito larvicides

Zhang F, Gu W, Xu P et al (2011) Effects of alkyl polyglycoside (APG) on composting of agricultural wastes. *Waste Management* 31:1333–1338. <https://doi.org/10.1016/j.wasman.2011.02.002>

Funding

The SextoniAedes project (Valorization of molecules from *Sextonia rubra* wood wastes for the formulation of larvicidal products against the *Aedes aegypti* mosquito: effectiveness and selectivity) was funded by the European Regional Development Funds (FEDER) for French Guiana (ERDF OP, agreement n° FEDER/2017/N°65). This work benefited from an “Investissement d’Avenir” grant managed by Agence Nationale de la Recherche (Infrastructure Nationale en Biologie Santé “ANAEE-France” ANR-11-INBS-0001) through the use of the U3E INRA1036 PEARL platform. This work has benefited from an “Investissement d’Avenir” grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-25-01).

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contribution Statement

EH, ID and JBD conceived and designed research. EH and NA collected the wood samples and conducted the extractions and chemical analysis. EC conducted the laboratory and semi-field larvicidal assays and the mosquito fitness study. EW conceived the formulation. DA, MC and DH designed and conducted the ecotoxicity assays. RLG and HS designed and conducted the cytotoxicity assays. EH, EC, ID and JBD analyzed the data and wrote the manuscript. All authors read and approved the manuscript.

Data availability

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

Ethical statements

Wood material was accessed according to French regulations concerning genetic resources access and benefit-sharing (French Ministry of Ecological Transition and Solidarity permit n° TREL1820249A/80), and obtained the required certificate of compliance delivered by the international Access and Benefit-Sharing Clearing House (UID: ABSCH-IRCC-FR-246958-1).