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

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

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

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

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

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

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

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

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

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

22 **Abstract:**

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

25 obstacle to the development of vector management products. The purpose of this study
26 was to assess the feasibility, efficacy and selectivity of a water-soluble formulation
27 based on a *S. rubra* wood extract characterized by its content in the larvicidal
28 compounds rubrenolide and rubrynlolide. Using reference and wild strains, the larval
29 mortality, oviposition behavior, hatching response, and fitness of the mosquitoes
30 exposed to the extract were evaluated. Laboratory eco- and cytotoxicity bioassays
31 were concurrently performed. The extract showed larvicide efficacy alongside with no
32 repulsive effect on oviposition, thus supporting its interest for the treatment of breeding
33 sites. The formulation exhibited strong acute toxicity, leading to 100% larval mortality in
34 2 to 4 days either in dry or rainy season at the test dose of 102 µg/ml under semi-
35 operational conditions, with a residual activity maintained during the 5 weeks of test.
36 The formulation allowed for a noticeable gain in selectivity compared with the extract,
37 with lower immobilization rates for *D. magna* and *C. riparius*, and no significant toxicity
38 towards human cells lines (IC₅₀ > 100 µg/ml). These results highlight the potential of a
39 *S. rubra*-based formulation for the management of *Ae. aegypti* larvae, including strains
40 showing resistance to conventional insecticides.

41

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

44

45 **Key Message**

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

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

54

55 **Introduction**

56 Globalization and extensive urbanization have promoted a worldwide expansion of the
57 anthropophilic invasive mosquito *Aedes aegypti* L. (Diptera, Culicidae), notably due to
58 the proliferation of breeding sites that can retain stagnant water (Carvalho and Moreira
59 2017). This species is causing numerous human casualties as the main vector of
60 several arboviruses (Gloria-Soria et al. 2016; Jones et al. 2021). In the absence of
61 clinically approved vaccine or antiviral therapy, except in the case of yellow fever virus,
62 the vector control strategies based on the use of insecticides are of crucial importance.
63 Dengue fever, and Zika or chikungunya viruses are in particular strongly circulating in
64 French Guiana, where vector control strategies have followed one another (Epelboin et
65 al. 2018; Bailly et al. 2021; de Thoisy et al. 2021). Nowadays, this French overseas
66 territory faces a strong resistance of *Ae. aegypti* populations towards pyrethroids as
67 adult vector-control products (Dusfour et al. 2015; Faucon et al. 2017; Guidez et al.
68 2020; Senthil-Nathan 2020; Epelboin et al. 2021). Bti (*Bacillus thuringiensis* var.
69 *israelensis*) is currently used to reduce the density of *Ae. aegypti* larvae, without the
70 apparition of resistance phenomena (Darriet et al. 2010; Kumar et al. 2012). However,
71 the persistence of effect of this non-ecotoxic natural product in breeding sites should be
72 explored (Epelboin et al. 2018). In an epidemiological and legislative context
73 particularly unsteady, it is therefore essential to develop safe, innovative and
74 environmentally friendly methods for vector control (George et al. 2014; Gerwick and
75 Sparks 2014; Benelli 2015; Benelli and Mehlhorn 2016).

76 Due to constant interaction with herbivores, plants synthesize a large variety of
77 molecules as part of their defense system (Agrawal and Weber 2015; Richards et al.
78 2015; Salazar et al. 2018). This makes plants a valuable source for novel insecticide
79 compounds (Miresmailli and Isman 2014; Pavela et al. 2019; Benelli 2020; Silvério et

80 al. 2020). However, even if numerous promising results have been obtained, only few
81 of these findings have made it to commercial applications as new botanical insecticides
82 (Isman and Grieneisen 2014; Isman 2020). One of the major technological challenges
83 for the development of larvicide botanical products is to allow their homogeneous
84 dispersion in water, the natural mosquito-breeding medium, alongside with the increase
85 of field performance through a better persistence of action (Isman 2020). To overcome
86 the applicability limitations, notably linked to the apolar nature of many natural
87 substances or extracts, and without using organic solvents exhibiting toxic profiles,
88 micro- and nanoemulsions stand in good place (de Oliveira et al. 2014; Peng et al.
89 2019). These techniques, which lead to the efficient aqueous suspension or dilution of
90 low water-soluble substances, allow obtaining usable bioactive natural products against
91 mosquito larvae (Kanis et al. 2012; Oliveira et al. 2016; Pavela 2016; Pavela et al.
92 2021). The lower effect on non-target organisms than conventional products also
93 stands among the potential advantages of natural-products-based micro- and
94 nanoemulsions as larvicide products, and low toxicity for humans is notably an
95 important parameter to look after, due to the use of such products in the household
96 area (Oliveira et al. 2016; Pavela et al. 2019; Turchen et al. 2020; Faustino et al. 2020;
97 Rodrigues et al. 2021; Machado et al. 2023).

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

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

116

117 **Materials & method**

118 *Mosquito strains and rearing*

119 Five *Ae. aegypti* (Diptera: Culicidae) strains were used. The laboratory New Orleans
120 (NO) strain is a reference strain susceptible to all insecticides and maintained for
121 decades in an insectary at the Institut Pasteur de la Guyane, Cayenne, French Guiana.
122 The Cayenne (CAY) strain was collected in the eponym city, and larvae of the F1
123 progeny were used for all of the experimentations made in the laboratory and for the
124 semi-field experimentations. This population is resistant to both pyrethroid and
125 organophosphate insecticides. A parent population was obtained from wild-caught
126 larvae from Ile Royale (IR), one of the coastal islands of French Guiana (5.287° N;
127 52.590° W), in 2015, and led to the field strain IR0115. A low insecticide pressure
128 characterizes this area of collection. Two isofemale strains were obtained from this
129 parent population, as previously described after isolation of one mated female
130 (Epelboin et al. 2021). The information concerning the five used strains are
131 summarized in Table 1. Rearing was performed as previously described (Touré et al.
132 2017).

133

134 **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

135 ^a kdr: knock-down resistance

136

137 *Sextonia rubra* wood extract and purified compounds

138 *S. rubra* (Mez.) Van der Werff (Lauraceae) freshly obtained commercial sawmills
139 wastes were collected in French Guiana. Sawdusts were obtained from Montsinéry
140 (4°58'34" N 52°26'45" W; September 2017) and Kourou sawmills (5°08'54" N 52°41'20"
141 W; March 2020) respectively. Mass distribution by particle size was measured as
142 follows: 60% > 500 µm, 33% between 500 and 200 µm, and 6% < 200 µm for
143 Montsinéry sawdust, and 74% > 500 µm, 21% between 500 and 200 µm, and 4% <
144 200 µm for Kourou sawdust. All samples were dried at room temperature (21°C, 65%
145 relative humidity) until equilibrium humidity (H<13%). Any visible bark material was
146 manually removed from crude wood powders, which were then extracted using ethyl
147 acetate (EtOAc, RPE grade, Carlo Erba Reagenti SpA, Rodano, Italy) at a 1/20 ratio
148 (w/v) at room temperature by maceration during 24h under stirring. After extraction, the
149 solution was filtered and the solvent removed by evaporation under reduced pressure
150 with a SpeedVac™ concentrator (Savant SPD121P, Thermo Scientific). The resulting
151 crude extracts (SrM, from Montsinéry sawdust, and SrK from Kourou sawdust) were
152 stored in a freezer at -18°C until assayed. Isolation of rubrenolide (Re) and rubrynlolide
153 (Ry) was performed using HPLC semi-preparative chromatography (Waters, Milford,
154 MA, USA) according to previously described procedures (Fu et al. 2019). Their
155 identification was confirmed and their content evaluated by NMR. To do so, ¹H NMR
156 spectra were recorded at 400 MHz and ¹³C NMR spectra at 100.6 MHz on a Varian

157 400 MR spectrometer (Varian, Palo Alto, CA, USA) equipped with a 5 mm inverse
158 probe (Auto X PGF $^1\text{H}/^{15}\text{N}-^{13}\text{C}$). Samples were dissolved in deuterated chloroform
159 (CDCl_3 , Carlo Erba) in 5 mm tubes for analysis. SrM extract was characterized with 8.4
160 $\pm 0.2\%$ Re and $2.0 \pm 0.0\%$ Ry (w/w), and SrK extract with $10.5 \pm 0.1\%$ Re and $8.9 \pm$
161 0.2% Ry (w/w) (Clervil et al. 2023).

162

163 *Sextonia rubra* wood extract formulation

164 A *S. rubra*-based concentrate was firstly obtained by adding progressively a
165 commercial rapeseed oil methyl ester (RadiaTM 7961, Oleon, Ertvelde, Belgium) to *S.*
166 *rubra* SrK wood extract, containing $10.5 \pm 0.1\%$ and $8.9 \pm 0.2\%$ (w/w) of the larvicidal
167 compounds rubrenolide and rubrymolide, respectively, and which appears as a
168 brownish glutinous molasses. The mixture was left in contact (3 hours) at room
169 temperature ($23 \pm 2^\circ\text{C}$) with regular manual stirring, and then heated (30 minutes,
170 60°C) in a double-walled water bath while stirring manually until the extract is fully
171 solubilized. This process is repeated to blend the concentrate with the emulsifying
172 ingredients, chosen for their specific HLB value and bio-based origin, and selected as
173 an alkylpolyglycoside (APG, SimulsolTM SL 7 G, Seppic, Paris, France) and an
174 ethoxylated rapeseed oil methyl ester (SepigreenTM C 300, Seppic, Paris, France). An
175 antifoaming agent was also added (acetylenic diol in ethylene glycol, SurfynolTM 104E,
176 Evonik Industries AG, Essen, Germany). The finalized formulation was titrated in 10%
177 of *S. rubra* extract. Eventually, 560 g of this formulation were prepared for the various
178 assays. The stability of the final product, which is an emulsifiable concentrate (EC)
179 aiming at obtaining spontaneous oil-in-water micro-emulsion (EW) when added to
180 distilled water, was evaluated by visual observation at room temperature for validation
181 before use. Composition of the matrix (defined as all formulation components without
182 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

201 version 3.2.0 (<https://www.r-project.org/>) as previously described (Falkowski et al.
202 2016).

203

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

206 Assessment of the influence of *S. rubra* wood extract on the oviposition behavior of the
207 mosquito was performed as described in the literature, with some modifications (El
208 Maghrbi and Hosni 2014; Seenivasagan et al. 2019). Holding cages (30 x 30 x 30 cm)
209 were each filled with 40 adult females and 40 adult males (6 days old) of *Ae. aegypti*
210 New Orleans strain (3 replicates) and Cayenne strain (3 replicates). Adult mosquitoes
211 were continuously fed on cotton soaked in 10% sugar until one day before the blood
212 meal. Two days after the female mosquito have been artificially blood fed (beef blood),
213 two breeding sites consisting in laying cups were placed in each cage: a control site
214 (100 ml untreated distilled water), and a site with water (100 ml) treated with SrM
215 extract. Doses for the *S. rubra* treatment were adapted for each population and
216 centered around the two respective 24h-DL₅₀ values (2 µg/ml of SrM *S. rubra* wood
217 extract for New Orleans strain; 70 µg/ml of SrM *S. rubra* wood extract for Cayenne
218 strain). A paper wick was placed in each breeding site on day 3 after the blood meal,
219 and replaced each day during 5 days by a new one, dried, and the number of eggs laid
220 on each paper was counted. The total number of eggs was used as a measure of
221 oviposition response. To obtain a new generation of mosquitoes, each paper wick was
222 put in clear water after 5 days drying, and the rearing performed as previously
223 described (Clervil et al. 2023). The numbers of hatched eggs were counted and 50
224 larvae coming from hatched eggs of each strain and each condition were placed into 5
225 different trays as five replicates. The daily survival and stage development follow-up
226 (average number of individuals having developed to pupal and adult stages from 50
227 larvae according the strain and treatment) lasted until the complete emergence of all
228 individuals. Student t-test was applied to measure the influence of the treated water on

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

232

233 *Simulated field trial*

234 Semi-field trials were conducted on the grounds of the Institut Pasteur de la Guyane
235 (French Guiana Pasteur Institute) in Cayenne. For the bioassays, only the *Ae. aegypti*
236 natural population collected in Cayenne town (CAY) was used for testing the
237 formulated products. The CAY strain was selected to provide a stringent context for an
238 assay as close to the reality of the field as possible. Experiments were carried out in
239 white plastic containers (70 l, Icko Apiculture, Bollène, France) filled with water (50 l)
240 and placed undercover, out of direct sunlight and rainfall, and covered with mosquito
241 nets to prevent oviposition by wild mosquitoes or other insects of the area, as well as
242 falling debris during the experiment. Four test containers were used concurrently for
243 each trial session, alongside with four negatives and four positive controls containers.
244 For the test barrels, *S. rubra* formulation (50 g), titrated at 10 % of wood extract, were
245 added to tap water (50 l), to obtain a final concentration of the active product in the
246 barrel corresponding to twice the extract LD₉₀ against *Ae. aegypti* CAY larvae after 48h
247 of exposure in the laboratory, i.e., a trial dose of 102 µg/ml. Negative control containers
248 were filled with tap water only (50 l), and positive control containers were filled with the
249 formulation matrix (40 g) dissolved in the water. During the experiment, tap water was
250 regularly added in the containers to compensate evaporation and maintain a constant
251 50 l-volume. In each case, tap water was given 24h hours before use for dechlorinating
252 and conditioning. Late third or early fourth-instar larvae from a first generation (F1)
253 reared from wild-caught larvae (F0) were used in the tests. A pool of 100 larvae was
254 initially added to each container at the beginning of the trial, and a new pool of 100 live
255 larvae was released in each container after total emergence of the adults in the control
256 containers (approximately every week). Larval food (yeast powder, 845 mg) was given

257 to the larvae during the test. The larval, and adult survival were recorded every day
258 until the total adult emergence of the controls. This experimentation was realized
259 during both the dry season (August-September 2020; average maximum temperature
260 33.8°C; average temperature 27.8°C, average sunlight 239.5 hours; average rainfall
261 166.5 mm) and the rainy season (January-February 2021; average maximum
262 temperature 30.8°C; average temperature 26.5°C, average sunlight 86.6 hours;
263 average rainfall 507 mm) (infoclimat.fr, 2021, Cayenne). For data analysis, the time (in
264 weeks) is recorded for the inhibition of adult emergence (%IE) to pass below the
265 threshold value of 80%. For each treatment, %IE was estimated according to control
266 containers by the following formula (World Health Organisation 2005; Darriet et al.
267 2010):

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

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

271

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

274 Ecotoxicity assays were adapted from the guidelines of the “Immediate Immobilization
275 Test” (OECD No. 202) for *Daphnia magna* (Straus, 1820) and the “Immediate
276 Immobilization Test” (OECD No. 235) for *Chironomus riparius* (Meigen, 1804). Each
277 product (crude extract, purified compounds rubrenolide and rubrynlide, formulation,
278 and formulation matrix) was tested at doses based on the beforehand established LD₉₀
279 (51.2 µg/ml) and LD₅₀ (18.6 µg/ml) of SrK extract used to obtain the formulation, and
280 measured on the CAY wild strain after 48h of exposure. Formulation and formulation
281 matrix effect on non-target species were compared at the semi-field trial dose of twice
282 the SrK crude extract LD₉₀ (102 µg/ml). The impact of formulation, formulation matrix

283 and the crude extract were concurrently assessed at the reference LD₅₀ of the crude
284 extract. Eventually, the purified compounds rubrenolide and rubrynlolide were tested at
285 2 µg/mL, representing around 10% of the extract LD₅₀ value, as each compound
286 represented in average 10% of the extract (Clervil et al. 2023). Three conditions were
287 tested: water control, water and solvent control, and the dose of analysis, with five
288 replicates per condition. The physicochemical measurements (pH, dissolved oxygen,
289 conductivity) were performed with measuring devices (sensors). The remaining
290 measures (chlorine, nitrites, nitrates, phosphates) were performed in the laboratory
291 using colorimetric detection (spectrophotometer). Photoperiod and temperature were
292 recorded using a “templight” recorder throughout the test period, from clutch incubation
293 until the end of the exposure.

294

295 *Cytotoxicity assays*

296 *Cell culture*

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

305 *Cytotoxic assays*

306 Chemicals were solubilized in DMSO (Sigma, D4540) at a concentration of 20 mM or
307 100 mg/ml (stock solution) and diluted in culture medium to the desired final
308 concentrations. The doses effect cytotoxic assays (IC₅₀ determination) were performed
309 by increasing concentration of each chemical (final well concentrations: 0.3 µM – 0.9
310 µM – 3 µM – 9 µM – 25 µM – 50 µM or 1 µg/ml – 3 µg/ml – 9 µg/ml – 28 µg/ml – 83

311 $\mu\text{g/ml}$ – $250 \mu\text{g/ml}$). Cells were plated in 96 wells (4000 cells/well). Twenty-four hours
312 after seeding, cells were exposed to chemicals. After 48h of treatment, cells were
313 washed in PBS (Sigma D8537) and fixed in cooled 90% ethanol (Sigma 32221) / 5%
314 acetic acid (Sigma A6283) for 20 minutes. Then, the nuclei were stained with Hoechst
315 33342 (B2261 Sigma). Image acquisition and analysis were performed using a
316 Cellomics ArrayScan VTI/HCS Reader (ThermoScientific, Waltham, MA, USA). The
317 survival percentages were calculated as the percentage of cell number after compound
318 treatment over cell number after DMSO treatment. To evaluate the selectivity of the
319 formulation, the selectivity index was used to highlight the results more clearly, as IC_{50}
320 were available in this case for the non-target cells. This value can be defined as the
321 ratio of the toxic concentration of a product (considering in our case the 48h- IC_{50}
322 against human cell lines) against its effective bioactive (in our case, the larvicidal
323 activity defined by the products 48h- LD_{50}) concentration (Indrayanto et al. 2021).

324

325 **Results**

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

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

335

336 **Table 3** Lethal doses ($\mu\text{g/ml}$) for 50% and 90% (LD_{50} and LD_{90} respectively) of the
 337 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)

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

339

340

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

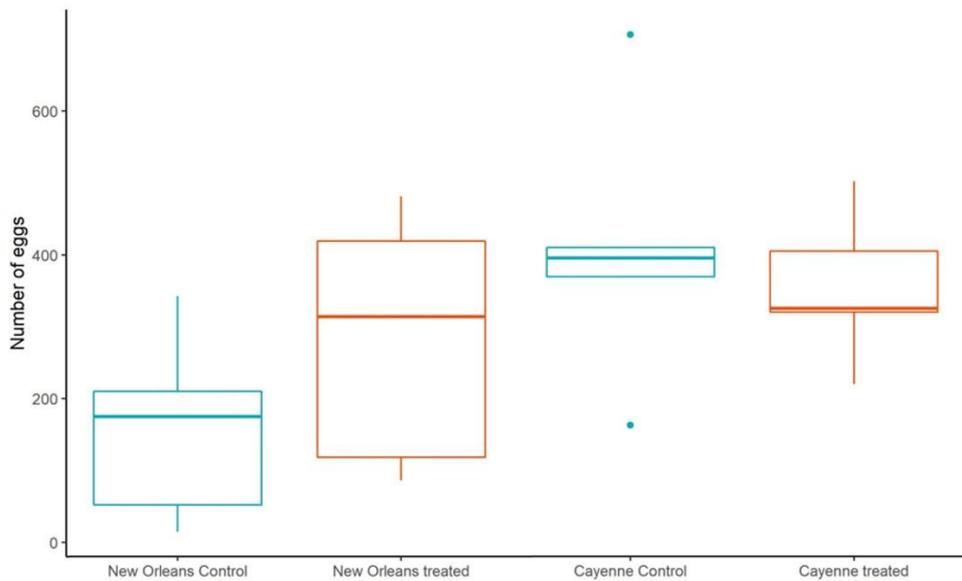
343 Parameters related to the mosquito fitness and life-history were assayed on respective
 344 DL_{50} doses of SrM extract using the reference NO strain and the wild-type CAY strain.

345 Figure 1 represents oviposition preferences of *Ae. aegypti* females in the presence of
 346 two eggs deposition sites (control, and treated with a sub-lethal dose (DL_{50}) of *S. rubra*

347 wood extract). The obtained results show that no statistically significant difference is
 348 observed for the oviposition behavior of *Ae. aegypti* within each strain, and notably the

349 wild CAY strain, between the control breeding site and the treated breeding site (NO
 350 strain Student t-test: $t = -1.2697$, $df = 7.3768$, $p\text{-value} = 0.2428$; CAY strain Student t-

351 test: $t = 0.54894$, $df = 6.1733$, $p\text{-value} = 0.6023$).

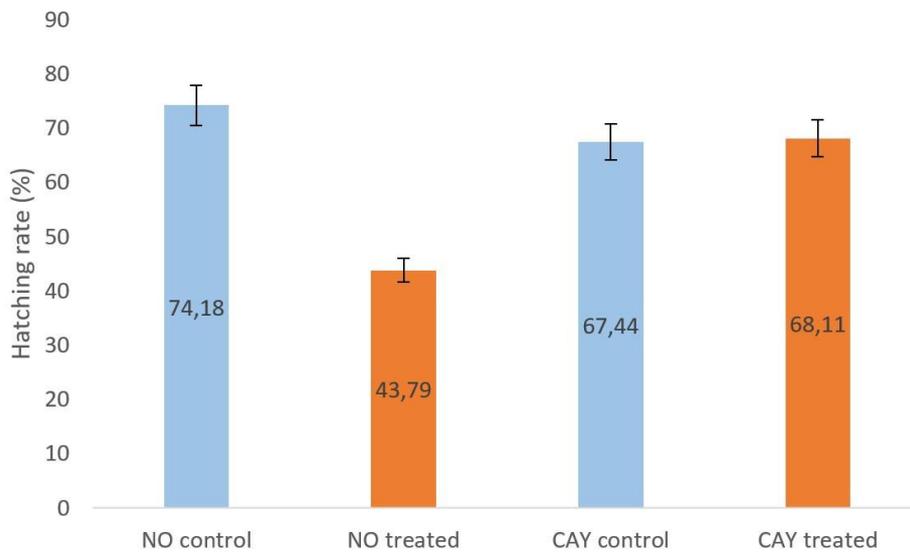


352

353 **Fig. 1** Oviposition responses of gravid females of *Ae. aegypti* to *Sextonia rubra* wood
 354 extract. Values correspond to the daily total number of eggs laid in breeding sites for
 355 each mosquito strain (NO and CAY) and in relation to treatment, with three replicates.
 356 Control: untreated tap water, treated: 2 µg/ml of *S. rubra* wood extract (SrM) for NO
 357 strain and 70 µg/ml of *S. rubra* wood extract (SrM) for CAY strain, corresponding to the
 358 respective DL₅₀ values for each strain

359

360 In a second step, the effects of sub-lethal doses of *S. rubra* extract were assessed on
 361 the fitness of *Ae. aegypti* eggs laid on treated sites from oviposition tests, and on the
 362 mosquitoes originating from these eggs. The first assay aimed at measuring whether
 363 the hatching success of *Ae. aegypti* eggs (NO and CAY strains) is affected by their
 364 previous exposure to *S. rubra* wood extracts (Fig. 2). Statistical difference was
 365 observed concerning the hatching success of the different strains depending on the
 366 treatment of the breeding site (Chi²: X-squared = 4.4986, df = 1, *p*-value = 0.03392).
 367 However, the hatching rate measured for the CAY strain eggs was not affected by the
 368 treatment.



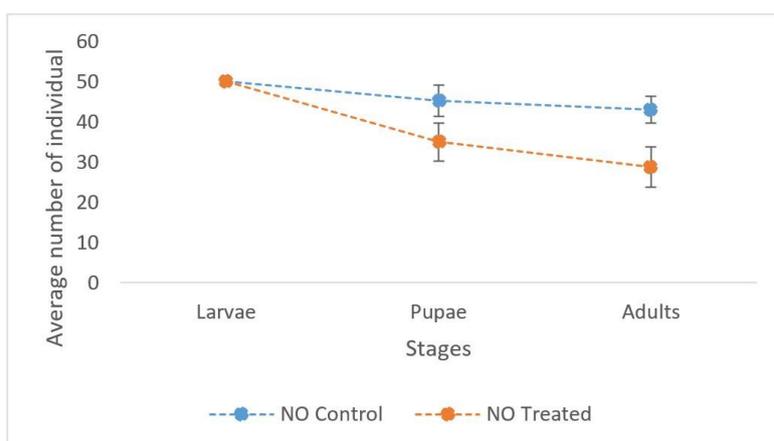
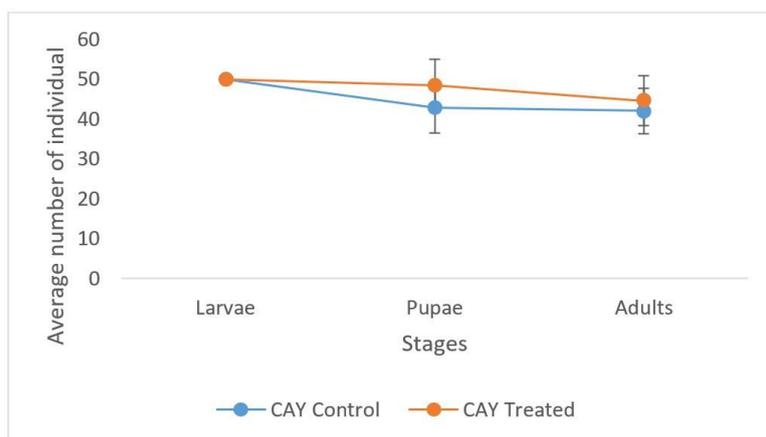
369

370 **Fig. 2** Hatching rate for the New Orleans strain (NO) and the Cayenne strain (CAY)
 371 according to the treatment applied in the breeding site (Control or treated). Control:
 372 untreated tap water, Treated: 2 µg/ml of *S. rubra* wood extract (SrM) for NO strain and
 373 70 µg/ml of *S. rubra* wood extract (SrM) for CAY strain

374

375 To determine whether the maturation of *Ae. aegypti* eggs on a *S. rubra* extract-
 376 impregnated substrate impact the fitness of the mosquitoes at larval, pupal and adult
 377 stage, the average number of individuals having developed at each stage from 50
 378 larvae was counted (Fig. 3). For the NO strain, there is a significant decrease between
 379 the number of adults obtained from eggs laid in treated breeding sites (Student t-test: t
 380 = 5.2774, $df = 6.9332$, p -value = 0.0005935). For the CAY strain, the decrease of the
 381 number of adults obtained from eggs laid in treated breeding sites is not significant
 382 (Student t-test: $t = -0.68374$, $df = 7.8959$, p -value = 0.7431).

383



384

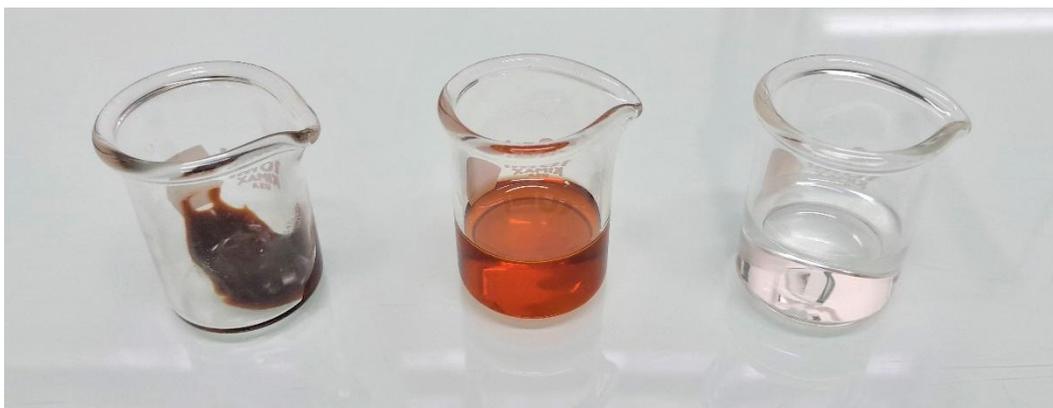
385 **Fig. 3** Average number of individuals having developed to pupal and adult stages from
 386 50 larvae (x five replicates) according the lineage and treatment. CAY: Cayenne wild
 387 strain (upper graph), NO: New Orleans strain (lower graph), Control: individuals from
 388 eggs laid in control breeding site, Treated: individuals from eggs laid in treated
 389 breeding site)

390

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

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

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



402

403 **Fig. 4** *S. rubra*-derived larvicidal products: A) wood ethyl acetate crude extract; B)
404 emulsifiable concentrate (EC); C) micro-emulsion in distilled water (EW), 0.1% solution
405

406 The formulation was developed using the SrK extract (sawdust collected in Kourou
407 sawmill), which was known to exhibit LD₅₀ values of 39.6 and 18.6 µg/mL at 24h and
408 48h, respectively, and LD₉₀ values of 118.2 µg/ml and 51.2 µg/ml at 24h and 48h,
409 respectively, against the CAY strain, and to contain 10.5% and 8.9% (w/w) of the
410 bioactive compounds rubrenolide and rubrynlolide respectively (Clervil et al. 2023).
411 When tested in the laboratory against the CAY strain, the formulation led to LD₅₀ values
412 of 48.0 ± 0.01 and 9.3 ± 0.05 µg/mL at 24h and 48h, respectively, and LD₉₀ values of
413 75.1 ± 0.02 and 39.6 ± 0.04 µg/ml at 24h and 48h, respectively. To ensure that this
414 effect was not due to a toxic activity of the formulation matrix against *Ae. aegypti*
415 larvae, we assessed the matrix and the formulation at 102 µg/ml prior to their
416 evaluation in semi-field conditions. The results obtained for the matrix showed a

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

419

420 *Semi-field bioassays*

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

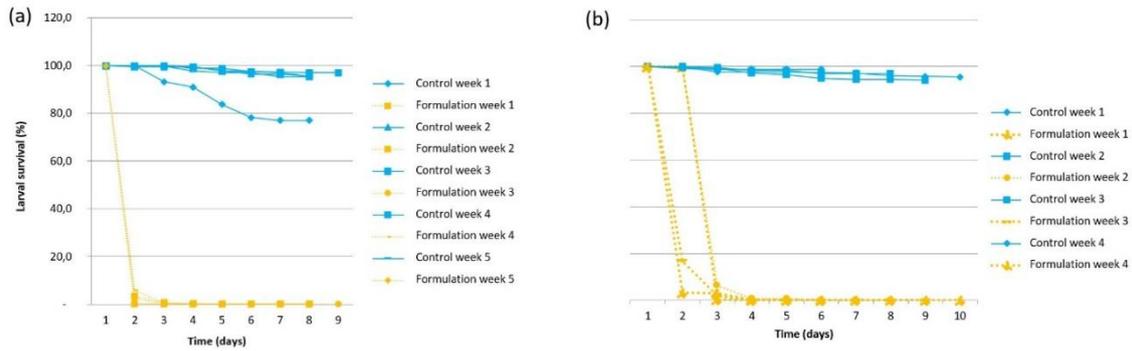


426

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

430

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

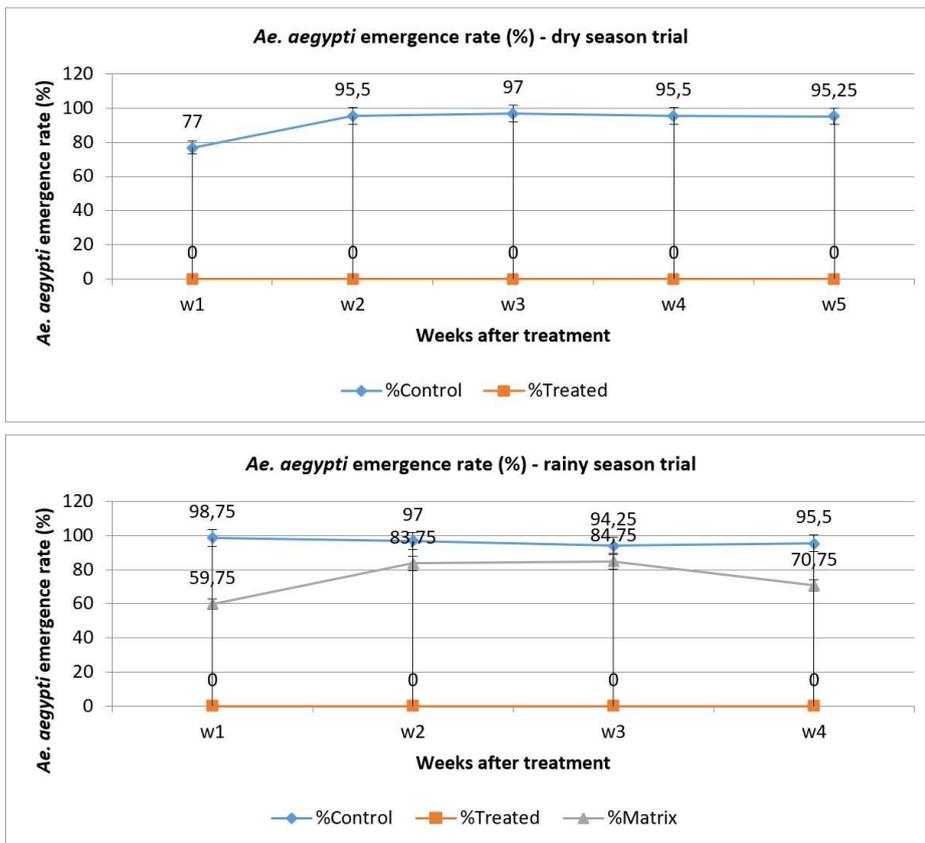


436

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

442

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



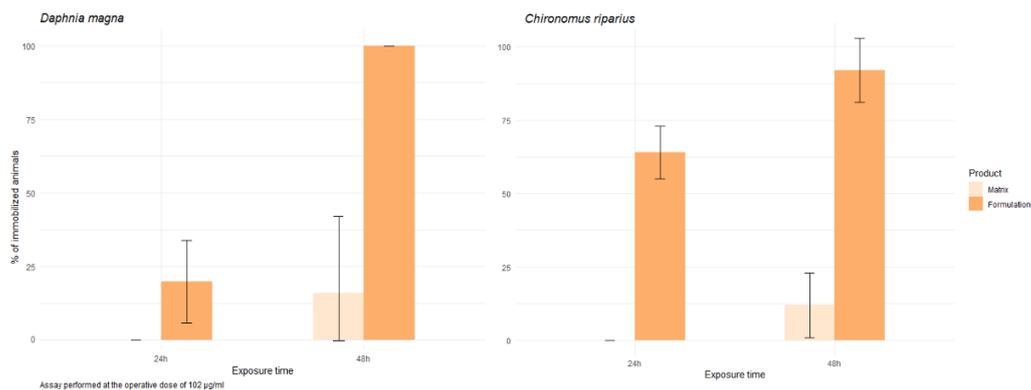
450

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

454

455 *Impact on aquatic non-target organisms and human cell lines*

456 The matrix and the formulation were tested at the dose used for the semi-field trial
 457 (102 μ g/ml). At 24h and 48h, the matrix displayed in our test conditions no (at 24h) to
 458 low toxicity towards both organisms (16 \pm 26% of immobilization for *D. magna*, and 12
 459 \pm 11% immobilization for *C. riparius* at 48h) (Figure 8). For the formulation, significant
 460 immobilization rates were observed after both 24h and 48h of exposure in the case of
 461 *C. riparius*, with 64 \pm 2% and 92 \pm 2% of immobilization respectively. The toxic effect
 462 appeared more slowly towards *D. magna*, with only 20 \pm 3% of immobilization after 24h
 463 of exposure, but 100% of immobilized animals after 48h.



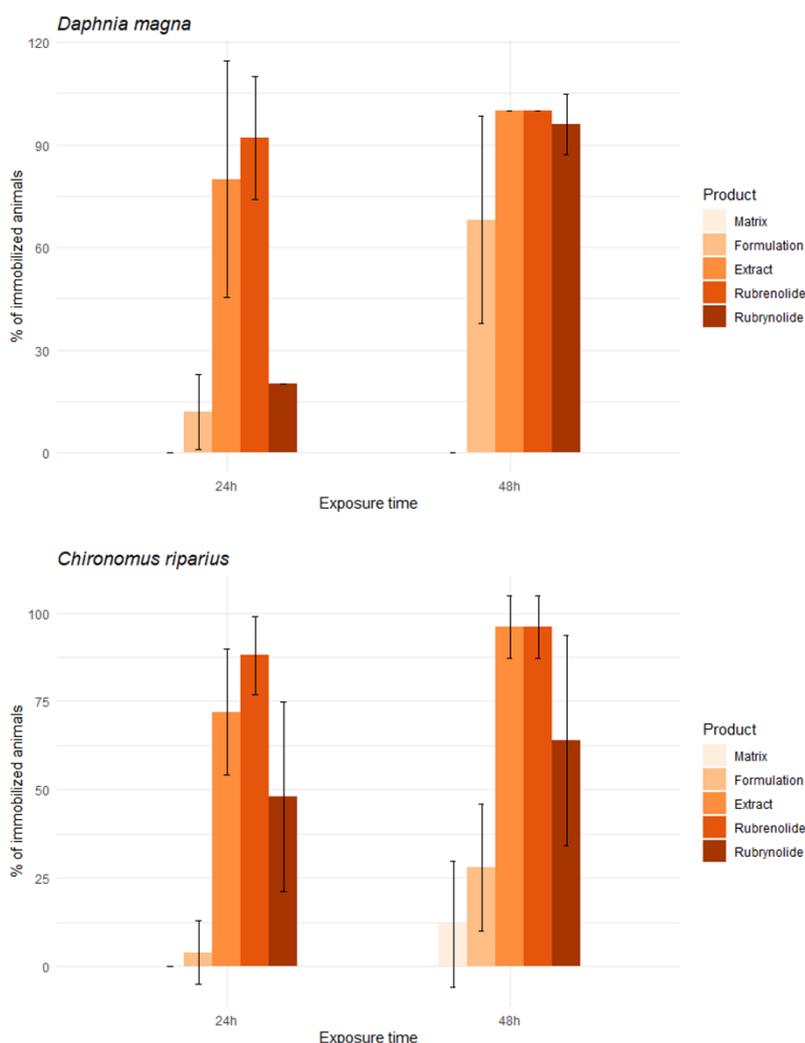
464

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

471

472 The effect of extract, matrix and formulation was then assessed at a lower dose,
 473 chosen as the 48h-LD₅₀ (18.6 µg/ml) of the SrK extract used to prepare the emulsifiable
 474 concentrate. The compounds rubrenolide and rubrynlolide were tested at a
 475 concentration of 2 µg/ml, which corresponds to around 10% of the 48h-LD₅₀ in
 476 consistency with the extract composition, which contains 10.5% of rubrenolide and
 477 8.9% of rubrynlolide (Clervil et al. 2023). The overall obtained results are showed in
 478 Figure 9. No to very low effect was observed for the matrix towards both non-target
 479 organisms. The environmental effect of the formulation could be considered low in the
 480 case of *C. riparius*, with a maximum immobilization rate of only 28 ± 18% after 48h of
 481 exposure at a dose corresponding to the 48-h LD₅₀ of the extract. The effect of the EC
 482 towards *D. magna* was more important, with 68 ± 30% of immobilized animal after 48h.
 483 The results obtained for the pure compounds rubrenolide and rubrynlolide indicated a

484 strong toxicity against non-target organisms, at a dose as low as 2 µg/ml. Rubrenolide
 485 was the most toxic compound, with $92 \pm 18\%$ immobilized *D. magna* and $88 \pm 11\%$
 486 immobilized *C. riparius* as soon as 24h after exposure. At 48 hours, rubrenolide led to
 487 almost complete mortality of the two non-target organisms (100% for *D. magna* and 96
 488 $\pm 9\%$ for *C. riparius*). Rubrynlolide acted more slowly, with immobilization values of $20 \pm$
 489 0% and $48 \pm 27\%$ at 24h, and $96 \pm 9\%$ and $64 \pm 30\%$ at 48h for *D. magna* and *C.*
 490 *riparius* respectively.



491

492 **Fig. 9** Immobilization percentage of non-target aquatic organisms *Daphnia magna*
 493 (upper graph) and *Chironomus riparius* (lower graph) after 24h and 48h of exposure.
 494 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 rubrymolide 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 rubrymolide. 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
Rubrymolide	12	> 50	> 50	> 50	> 50	> 50	> 50

^a For mixtures

^b For pure compounds

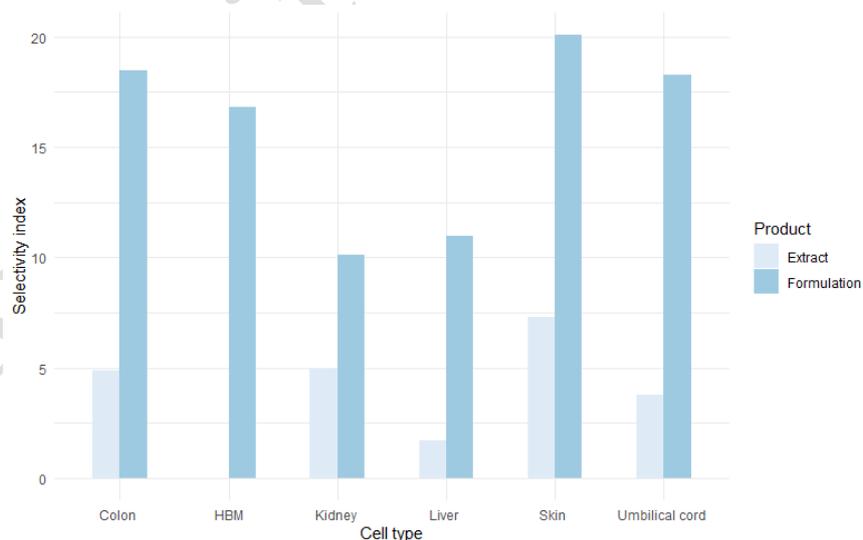
^c EC: emulsifiable concentrate

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

511 $\mu\text{g/ml}$ respectively. The extract however could be considered cytotoxic against liver
 512 HuH7 cells (human hepatocellular carcinoma, IC_{50} value of $32 \mu\text{g/ml}$), and also led to a
 513 moderate effect against HUVEC (human umbilical vein endothelial cells), kidney and
 514 colon Caco2 (human colorectal adenocarcinoma) cells, with IC_{50} values in the 70-93
 515 $\mu\text{g/ml}$ range. Concerning the pure compounds, rubrynolide did not display any
 516 cytotoxicity except against liver cells (IC_{50} of $12 \mu\text{g/ml}$). It was the only case when this
 517 compound was more toxic than rubrenolide, which exhibited a measurable effect
 518 towards liver, kidney and umbilical cord cells, even if above the $25 \mu\text{M}$ criteria (IC_{50} in
 519 the $31\text{-}35 \mu\text{M}$) range. Notably, no toxicity was observed against any of the products in
 520 the case of skin fibroblasts.

521 A selectivity index was calculated (Figure 10), except for human skin fibroblasts and
 522 human bone marrow neuroblasts, as the obtained IC_{50} were $> 250 \mu\text{g/ml}$. Overall, the
 523 EC exhibited higher selectivity indices than the crude extract for all tested cell lines,
 524 with values from 10 (kidney human carcinoma NCI-H295R cells) to 20 (skin human
 525 immortalized keratinocytes HaCat).



526

527 **Fig. 10** Selectivity indices calculated as $\text{SI} = \text{IC}_{50} (\text{non-target cells}) / \text{LD}_{50} (\text{Ae. aegypti})$
 528 Cayenne strain larvae) after 48 hours of exposure for *Sextonia rubra* extract (light blue)
 529 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*

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

563 The ingredients of the formulation were chosen in order to fulfill the regulation (EU)
564 528/2012 and directive 98/8/EC (Directive Biocide) concerning the making available on
565 the market and use of biocidal products, or the regulation (EC) 1107/2009 concerning
566 the placing of plant protection products on the market. Regulatory approvals represent
567 an important barrier to the industrial implementation of botanical insecticides, and
568 taking them into account in an early stage is therefore crucial (Villaverde et al. 2014;
569 Isman 2020). Notably, natural-oil based esters, including methyl esters, have been
570 recorded and patented for their noticeable solvency properties when used to solubilize
571 various insecticides and obtain oil-in-water (EW) emulsions (Taranta et al. 2002;
572 Salimon et al. 2012; Tan et al. 2013). Surfactants were chosen among products
573 obtained from renewable raw material, with non-ecotoxic characters in environmental
574 conditions as well as high biodegradability properties (Rybinski and Hill 1998;
575 Johansson and Svensson 2001; Renkin et al. 2005; Zhang et al. 2011; Geetha and
576 Tyagi 2012; Peng et al. 2019).

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

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

592 The efficiency of the tested formulation as a mosquito larvicide was demonstrated by
593 our results, especially towards the CAY *Ae. aegypti* strain, exhibiting a strong
594 resistance to conventional insecticides. The milky appearance of the aqueous media
595 treated with the emulsifiable concentrate observed in semi-operational conditions can
596 be due to the composition of the tap water used for the assay, although it was let 24h
597 for dechlorination before use, or to the addition of larval food. Indeed, the presence
598 calcium, sodium or chloride ions can modify the spontaneous formation of a micro-
599 emulsion, and lead to a basic emulsion, while changing the translucent aspect for a
600 cloudy one (Egito et al. 2020). The strong and rapid acute toxicity can represent a
601 technical advantage in the field.(Darriet et al. 2010) The lasting residual activity
602 suggests an effective potential of the formulation for vector population inhibition during
603 dengue transmission periods, which is a key parameter for disease control (Kanis et al.
604 2013, 2018; Maia et al. 2019). Botanical products often have to face the problem of
605 residual activity, due to a high biodegradability through photodegradation, abiotic
606 oxidation or volatilization (Amer and Mehlhorn 2006; Isman 2020). In our case, this
607 relatively long residual activity was obtained for a simple emulsion, without using slow-
608 release systems. This can be put in perspective with the previously demonstrated
609 stability of the crude *S. rubra* extract in ambient conditions over time (Clervil et al.
610 2023).

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

625 However, when testing the effect of extract, matrix and formulation at the 48h-LD₅₀
626 (18.6 µg/ml) of the SrK extract used to prepare the emulsifiable concentrate, the
627 formulation led to 1.5 less toxicity towards *D. magna*, and 3.4 less toxic effect towards
628 *C. riparius*, when compared to the extract. Besides solving technological problems due
629 to the solubilization of *S. rubra* lipophilic crude extract in water, the use of the
630 formulation also allows to lower the toxicity of the active ingredient at the same dose,
631 without losing larvicidal efficacy. Complementary assessments should now be
632 performed in cooperation with public health workers to determine the useful dose of
633 formulation in the field, and therefore identify the precise level of selectivity proposed
634 by the *S. rubra* EC. The data obtained for rubrenolide and rubrymolide are consistent
635 with the previous evaluation of these pure compounds against *Ae. aegypti* larvae, with
636 higher toxic activity observed for rubrenolide than rubrymolide both on the laboratory
637 strain Paea and the wild strain Cayenne (Falkowski et al. 2016). Thus, valuing the

638 extract, and even more the formulation, rather than the pure compounds, can add a
639 noticeable value when it comes to the ecotoxicological profile of the larvicidal products.
640 Eventually, except for the extract towards kidney cells, the cytotoxic effect of the tested
641 product was overall moderate to non-significant in an acute toxicity assay. The data
642 obtained for the pure compounds rubrenolide and rubrynlolide are consistent with a
643 previous study, in which the pure compounds exhibited $IC_{50} > 100 \mu\text{g/ml}$ against NIH-
644 3T3 fibroblasts cells (Rodrigues et al. 2010). Even if a noticeable toxicity had been
645 previously reported for rubrenolide (GI_{50} in the 3.3-9.9 $\mu\text{g/ml}$ range i.e., $< 33 \mu\text{M}$)
646 against UACC-62 (human melanoma), MCF-7 (human breast cancer), HT-29 (human
647 colorectal adenocarcinoma) and PC-3 (human prostate cancer) cell lines, most values
648 in our assays remained out of the cytotoxic range (Tófoli et al. 2016). However,
649 complementary cytotoxic evaluation of the products in *in vivo* models would be a
650 valuable asset. The selectivity index data are of high interest, as a $SI \geq 10$ was
651 previously cited in the literature as a valuable criterion for selective samples
652 (Awouafack et al. 2013; Houël et al. 2015; Peña-Morán et al. 2016). These results
653 therefore reinforce those obtained when comparatively assessing the ecotoxicity of the
654 crude extracts towards the formulated products, as in both cases (eco- and
655 cytotoxicity), a clear advantage is obtained when using the *S. rubra* EC.

656

657 **Conclusion**

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

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

678

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916

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927

928 **Competing interests**

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

930

931 **Author Contribution Statement**

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

939

940 **Data availability**

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

943

944 **Ethical statements**

945 Wood material was accessed according to French regulations concerning genetic
946 resources access and benefit-sharing (French Ministry of Ecological Transition and
947 Solidarity permit n° TREL1820249A/80), and obtained the required certificate of
948 compliance delivered by the international Access and Benefit-Sharing Clearing House
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