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4 Efficacy and selectivity of *Sextonia rubra* wood extracts and bio-based 5 formulation for the management of insecticide resistant *Aedes aegypti* in French 6 Guiana

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25 Abstract:

26 Sextonia rubra is an Amazonian tree known for the larvicide activity of its wood extracts 27 against Aedes aegypti. However, the lipophilic character of this extract represents an 28 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 29 30 based on a S. rubra wood extract characterized by its content in the larvicidal 31 compounds rubrenolide and rubrynolide. Using reference and wild strains, the larval mortality, oviposition behavior, hatching response, and fitness of the mosquitoes 32 exposed to the extract were evaluated. Laboratory eco- and cytotoxicity bioassays 33 34 were concurrently performed. The extract showed larvicide efficacy alongside with no 35 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 36 37 2 to 4 days either in dry or rainy season at the test dose of 102 µg/ml under semioperational conditions, with a residual activity maintained during the 5 weeks of test. 38 39 The formulation allowed for a noticeable gain in selectivity compared with the extract, 40 with lower immobilization rates for D. magna and C. riparius, and no significant toxicity towards human cells lines (IC₅₀ > 100 μ g/ml). These results highlight the potential of a 41 42 S. rubra-based formulation for the management of Ae. aegypti larvae, including strains 43 showing resistance to conventional insecticides.

44

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

47

48 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

55

• S. rubra crude extract has no effect on Ae. aegypti oviposition behaviour

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

57

58 Introduction

Globalization and extensive urbanization have promoted a worldwide expansion of the 59 anthropophilic invasive mosquito Aedes aegypti L. (Diptera, Culicidae), notably due to 60 the proliferation of breeding sites that can retain stagnant water (Carvalho and Moreira 61 62 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 63 64 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. 65 66 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 67 al. 2018; Bailly et al. 2021; de Thoisy et al. 2021). Nowadays, this French overseas 68 69 territory faces a strong resistance of Ae. aegypti populations towards pyrethroids as 70 adult vector-control products (Dusfour et al. 2015; Faucon et al. 2017; Guidez et al. 71 2020; Senthil-Nathan 2020; Epelboin et al. 2021). Bti (Bacillus thuringiensis var. 72 israelensis) is currently used to reduce the density of Ae. aegypti larvae, without the 73 apparition of resistance phenomena (Darriet et al. 2010; Kumar et al. 2012). However, 74 the persistence of effect of this non-ecotoxic natural product in breeding sites should be 75 explored (Epelboin et al. 2018). In an epidemiological and legislative context particularly unsteady, it is therefore essential to develop safe, innovative and 76 77 environmentally friendly methods for vector control (George et al. 2014; Gerwick and 78 Sparks 2014; Benelli 2015; Benelli and Mehlhorn 2016).

79 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. 80 81 2015; Salazar et al. 2018). This makes plants a valuable source for novel insecticide 82 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 83 84 of these findings have made it to commercial applications as new botanical insecticides 85 (Isman and Grieneisen 2014; Isman 2020). One of the major technological challenges 86 for the development of larvicide botanical products is to allow their homogeneous dispersion in water, the natural mosquito-breeding medium, alongside with the increase 87 of field performance through a better persistence of action (Isman 2020). To overcome 88 89 the applicability limitations, notably linked to the apolar nature of many natural substances or extracts, and without using organic solvents exhibiting toxic profiles, 90 91 micro- and nanoemulsions stand in good place (de Oliveira et al. 2014; Peng et al. 92 2019). These techniques, which lead to the efficient aqueous suspension or dilution of 93 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. 94 95 2021). The lower effect on non-target organisms than conventional products also 96 stands among the potential advantages of natural-products-based micro- and 97 nanoemulsions as larvicide products, and low toxicity for humans is notably an 98 important parameter to look after, due to the use of such products in the household 99 area (Oliveira et al. 2016; Pavela et al. 2019; Turchen et al. 2020; Faustino et al. 2020; 100 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 rubrynolide

107 displayed strong activity towards Ae. aegypti larvae, and that commercial sawmill 108 residues of S. rubra can be considered as a valuable resource for the large-scale 109 production of these larvicidal extracts (Falkowski et al. 2016, 2020; Clervil et al. 2023). 110 We therefore embarked upon characterizing the larvicidal activity of the S. rubra wood 111 extract on Ae. aegypti iso-female strains and their parent wild strain collected in French 112 Guiana. We also evaluated the fitness of mosquitoes exposed to the extract, while 113 concurrently assessing an aqueous-based formulation containing S. rubra wood extract 114 in semi-field conditions. Eco- and cytotoxicity were investigated, in the objective of 115 ascertaining the selectivity of the products towards aquatic species and human cells, 116 and display a better picture of the potential of S. rubra material to develop novel 117 botanical products displaying larvicidal activity against insecticide-resistant Ae. aegypti 118 mosquitoes.

119

120 Materials & method

121 Mosquito strains and rearing

122 Five Ae. aegypti (Diptera: Culicidae) strains were used. The laboratory New Orleans 123 (NO) strain is a reference strain susceptible to all insecticides and maintained for 124 decades in an insectary at the Institut Pasteur de la Guyane, Cayenne, French Guiana. 125 The Cayenne (CAY) strain was collected in the eponym city, and larvae of the F1 126 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 127 128 organophosphate insecticides. A parent population was obtained from wild-caught 129 larvae from Ile Royale (IR), one of the coastal islands of French Guiana (5.287° N; 130 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 131 parent population, as previously described after isolation of one mated female 132 (Epelboin et al. 2021). The information concerning the five used strains are 133

134 summarized in Table 1. Rearing was performed as previously described (Touré et al.

135 2017).

136

137 **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

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^a kdr: knock-down resistance

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140 Sextonia rubra wood extract and purified compounds

141 S. rubra (Mez.) Van der Werff (Lauraceae) freshly obtained commercial sawmills 142 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" 143 144 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 145 Montsinéry sawdust, and 74% > 500 µm, 21% between 500 and 200 µm, and 4% < 146 200 µm for Kourou sawdust. All samples were dried at room temperature (21°C, 65% 147 148 relative humidity) until equilibrium humidity (H<13%). Any visible bark material was 149 manually removed from crude wood powders, which were then extracted using ethyl 150 acetate (EtOAc, RPE grade, Carlo Erba Reagenti SpA, Rodano, Italy) at a 1/20 ratio 151 (w/v) at room temperature by maceration during 24h under stirring. After extraction, the 152 solution was filtered and the solvent removed by evaporation under reduced pressure with a SpeedVac[™] concentrator (Savant SPD121P, Thermo Scientific). The resulting 153 154 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 155

(Ry) was performed using HPLC semi-preparative chromatography (Waters, Milford, 156 MA, USA) according to previously described procedures (Fu et al. 2019). Their 157 identification was confirmed and their content evaluated by NMR. To do so, ¹H NMR 158 spectra were recorded at 400 MHz and ¹³C NMR spectra at 100.6 MHz on a Varian 159 160 400 MR spectrometer (Varian, Palo Alto, CA, USA) equipped with a 5 mm inverse probe (Auto X PGF ${}^{1}H/{}^{15}N-{}^{13}C$). Samples were dissolved in deuterated chloroform 161 (CDCl₃, Carlo Erba) in 5 mm tubes for analysis. SrM extract was characterized with 8.4 162 \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 163 0.2% Ry (w/w) (Clervil et al. 2023). 164

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166 Sextonia rubra wood extract formulation

A S. rubra-based concentrate was firstly obtained by adding progressively a 167 commercial rapeseed oil methyl ester (RadiaTM 7961, Oleon, Ertvelde, Belgium) to S. 168 rubra SrK wood extract, containing $10.5 \pm 0.1\%$ and $8.9 \pm 0.2\%$ (w/w) of the larvicidal 169 compounds rubrenolide and rubrynolide, respectively, and which appears as a 170 171 brownish glutinous molasses. The mixture was left in contact (3 hours) at room temperature $(23 \pm 2^{\circ}C)$ with regular manual stirring, and then heated (30 minutes, 172 173 60°C) in a double-walled water bath while stirring manually until the extract is fully 174 solubilized. This process is repeated to blend the concentrate with the emulsifying 175 ingredients, chosen for their specific HLB value and bio-based origin, and selected as an alkypolyglycoside (APG, SimulsolTM SL 7 G, Seppic, Paris, France) and an 176 ethoxylated rapeseed oil methyl ester (Sepigreen[™] C 300, Seppic, Paris, France). An 177 antifoaming agent was also added (acetylenic diol in ethylene glycol, Surfynol[™] 104E, 178 179 Evonik Industries AG, Essen, Germany). The finalized formulation was titrated in 10% 180 of S. rubra extract. Eventually, 560 g of this formulation were prepared for the various 181 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 182

183 distilled water, was evaluated by visual observation at room temperature for validation

184 before use. Composition of the matrix (defined as all formulation components without

185 the extract) and formulation are reported in Table 2.

- 186
- 187 **Table 2** Composition (w/w) of formulation matrix and *S. rubra* wood extract emulsifiable

Ingredient	Function	Matrix	Emulsifiable
ingredient	Function	Matrix	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
S. rubra wood extract	Active substance	-	10,0
Water	2	10,0	-

189

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190

Laboratory evaluation of the larvicidal activity of S. rubra wood extract and formulation 191 192 S. rubra extract (SrM) was investigated using the WHO cup assay for testing mosquito 193 larvicides (World Health Organisation 2005). Late third or early fourth-instar larvae 194 were used in the test. For each bioassay, 25 larvae of each strain were transferred to 195 cups containing distilled water (99 ml) and the tested product (1 ml) diluted in ethanol 196 (100%, Sigma-Aldrich, Saint-Louis, MO, USA), at a suitable concentration. Eight cups per concentration (8 x 25 larvae) were used, and the concentrations adapted to obtain 197 198 from 0% to 100% mortality, thus allowing for LD₅₀ and LD₉₀ calculation (from 0.001 to 8 199 µg/ml for NO strain and from 3 to 120 or 160 µg/ml for all other strains). Control treatments were performed for each test with ethanol only (1 ml in 99 ml of distilled 200

water), and larval mortality was recorded at 24 and 48 h after exposure. Test was invalidated if mortality in control was >20%. Lethal doses were obtained by a log-Probit 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. 205 2016).

206

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

209 Assessment of the influence of S. rubra wood extract on the oviposition behavior of the 210 mosquito was performed as described in the literature, with some modifications (El 211 Maghrbi and Hosni 2014; Seenivasagan et al. 2019). Holding cages (30 x 30 x 30 cm) 212 were each filled with 40 adult females and 40 adult males (6 days old) of Ae. aegypti 213 New Orleans strain (3 replicates) and Cayenne strain (3 replicates). Adult mosquitoes 214 were continuously fed on cotton soaked in 10% sugar until one day before the blood 215 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 216 217 (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 218 219 centered around the two respective 24h-DL₅₀ values (2 µg/ml of SrM S. rubra wood 220 extract for New Orleans strain; 70 µg/ml of SrM S. rubra wood extract for Cayenne 221 strain). A paper wick was placed in each breeding site on day 3 after the blood meal, 222 and replaced each day during 5 days by a new one, dried, and the number of eggs laid 223 on each paper was counted. The total number of eggs was used as a measure of 224 oviposition response. To obtain a new generation of mosquitoes, each paper wick was 225 put in clear water after 5 days drying, and the rearing performed as previously 226 described (Clervil et al. 2023). The numbers of hatched eggs were counted and 50 227 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 228

229 (average number of individuals having developed to pupal and adult stages from 50 230 larvae according the strain and treatment) lasted until the complete emergence of all 231 individuals. Student t-test was applied to measure the influence of the treated water on 232 the oviposition behavior and on mosquitoes' fitness (hatching rate and mosquito 233 development). In all analyses, a *p*-value < 0.05 was taken to indicate statistical 234 significance.

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236 Simulated field trial

Semi-field trials were conducted on the grounds of the Institut Pasteur de la Guyane 237 (French Guiana Pasteur Institute) in Cayenne. For the bioassays, only the Ae. aegypti 238 239 natural population collected in Cayenne town (CAY) was used for testing the 240 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 241 white plastic containers (70 I, Icko Apiculture, Bollène, France) filled with water (50 I) 242 243 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 244 falling debris during the experiment. Four test containers were used concurrently for 245 246 each trial session, alongside with four negatives and four positive controls containers. 247 For the test barrels, S. rubra formulation (50 g), titrated at 10 % of wood extract, were 248 added to tap water (50 I), to obtain a final concentration of the active product in the 249 barrel corresponding to twice the extract LD₉₀ against Ae. aegypti CAY larvae after 48h 250 of exposure in the laboratory, i.e., a trial dose of 102 µg/ml. Negative control containers 251 were filled with tap water only (50 l), and positive control containers were filled with the 252 formulation matrix (40 g) dissolved in the water. During the experiment, tap water was 253 regularly added in the containers to compensate evaporation and maintain a constant 254 50 I-volume. In each case, tap water was given 24h hours before use for dechlorinating 255 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 256

257 initially added to each container at the beginning of the trial, and a new pool of 100 live 258 larvae was released in each container after total emergence of the adults in the control 259 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 260 261 until the total adult emergence of the controls. This experimentation was realized during both the dry season (August-September 2020; average maximum temperature 262 263 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 264 temperature 30.8°C; average temperature 26.5°C, average sunlight 86.6 hours; 265 average rainfall 507 mm) (infoclimat.fr, 2021, Cayenne). For data analysis, the time (in 266 267 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 268 containers by the following formula (World Health Organisation 2005; Darriet et al. 269 270 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.

274

275 Ecotoxicological bioassays on non-target species: Daphnia magna and Chironomus
276 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 rubrynolide, formulation, and formulation matrix) was tested at doses based on the beforehand established LD_{90} (51.2 µg/ml) and LD_{50} (18.6 µg/ml) of SrK extract used to obtain the formulation, and 283 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 284 285 the SrK crude extract LD₉₀ (102 µg/ml). The impact of formulation, formulation matrix 286 and the crude extract were concurrently assessed at the reference LD₅₀ of the crude extract. Eventually, the purified compounds rubrenolide and rubrynolide were tested at 287 288 2 µg/mL, representing around 10% of the extract LD₅₀ value, as each compound 289 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 290 291 replicates per condition. The physicochemical measurements (pH, dissolved oxygen, conductivity) were performed with measuring devices (sensors). The remaining 292 293 measures (chlorine, nitrites, nitrates, phosphates) were performed in the laboratory 294 using colorimetric detection (spectrophotometer). Photoperiod and temperature were recorded using a "templight" recorder throughout the test period, from clutch incubation 295 296 until the end of the exposure. Ker

297

Cytotoxicity assays 298

299 Cell culture

Skin normal fibroblastic cells and HUVEC-Umbil Vein (umbilical cord cells) were 300 301 purchased from Lonza (Basel, Switzerland), Caco-2 (colon), SHY5Y (human bone 302 marrow neuroblasts), NCI-H295R (adrenal gland), HaCaT (skin keratinocytes) and 303 HuH7D12 (liver) cancer cells lines were obtained from the ECACC collection (Porton, 304 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, 305 H30109.03), penicillin-1% streptomycin (Sigma, P4333) and 2mM glutamine (Sigma, 306 G7513). 307

308 Cytotoxic assays

309 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 310

311 concentrations. The doses effect cytotoxic assays (IC₅₀ determination) were performed 312 by increasing concentration of each chemical (final well concentrations: 0.3 µM - 0.9 313 μ M - 3 μ M - 9 μ M - 25 μ M - 50 μ M or 1 μ g/ml - 3 μ g/ml - 9 μ g/ml - 28 μ g/ml - 83 314 µg/ml – 250 µg/ml). Cells were plated in 96 wells (4000 cells/well). Twenty-four hours 315 after seeding, cells were exposed to chemicals. After 48h of treatment, cells were 316 washed in PBS (Sigma D8537) and fixed in cooled 90% ethanol (Sigma 32221) / 5% 317 acetic acid (Sigma A6283) for 20 minutes. Then, the nuclei were stained with Hoechst 318 33342 (B2261 Sigma). Image acquisition and analysis were performed using a 319 Cellomics ArrayScan VTI/HCS Reader (ThermoScientific, Waltham, MA, USA). The 320 survival percentages were calculated as the percentage of cell number after compound 321 treatment over cell number after DMSO treatment. To evaluate the selectivity of the 322 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 323 ratio of the toxic concentration of a product (considering in our case the 48h- IC₅₀ 324 325 against human cell lines) against its effective bioactive (in our case, the larvicidal 326 activity defined by the products 48h-LD₅₀) concentration (Indravanto et al. 2021).

327

328 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 insecticideselected isofemale strains showed, as CAY wild strain, the highest LD values.

Table 3 Lethal doses (μ g/ml) for 50% and 90% (LD₅₀ and LD₉₀ respectively) of the

340	population	of	Aedes	aegypti	strains,	obtained	with	SrM	extract,	with	associated	

	Lethal doses	(µg/ml) at 24 h	Lethal doses (µg/ml) at 48 h			
Strain	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)		

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

342

343

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

346 Parameters related to the mosquito fitness and life-history were assayed on respective 347 DL₅₀ doses of SrM extract using the reference NO strain and the wild-type CAY strain. 348 Figure 1 represents oviposition preferences of Ae. aegypti females in the presence of 349 two eggs deposition sites (control, and treated with a sub-lethal dose (DL₅₀) of S. rubra 350 wood extract). The obtained results show that no statistically significant difference is 351 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 352 353 strain Student t-test: t = -1.2697, df = 7.3768, p-value = 0.2428; CAY strain Student ttest: t = 0.54894, df = 6.1733, *p*-value = 0.6023). 354

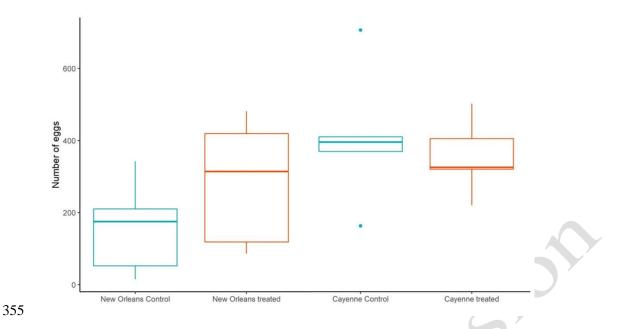


Fig. 1 Oviposition responses of gravid females of *Ae. aegypti* to *Sextonia rubra* wood extract. Values correspond to the daily total number of eggs laid in breeding sites for each mosquito strain (NO and CAY) and in relation to treatment, with three replicates. Control: untreated tap water, treated: 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 DL₅₀ values for each strain

In a second step, the effects of sub-lethal doses of S. rubra extract were assessed on 363 364 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 365 the hatching success of Ae. aegypti eggs (NO and CAY strains) is affected by their 366 367 previous exposure to S. rubra wood extracts (Fig. 2). Statistical difference was 368 observed concerning the hatching success of the different strains depending on the 369 treatment of the breeding site (Chi²: X-squared = 4.4986, df = 1, p-value = 0.03392). 370 However, the hatching rate measured for the CAY strain eggs was not affected by the 371 treatment.

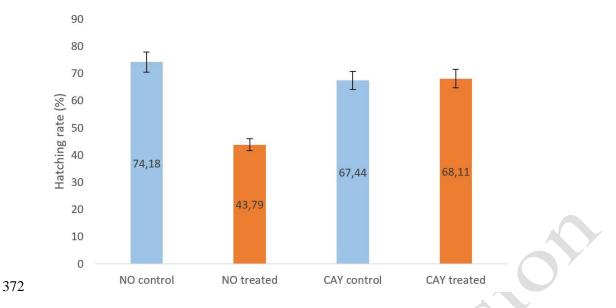


Fig. 2 Hatching rate for the New Orleans strain (NO) and the Cayenne strain (CAY)
according to the treatment applied in the breeding site (Control or treated). Control:
untreated tap water, Treated: 2 µg/ml of *S. rubra* wood extract (SrM) for NO strain and
70 µg/ml of *S. rubra* wood extract (SrM) for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

378 To determine whether the maturation of Ae. aegypti eggs on a S. rubra extractimpregnated substrate impact the fitness of the mosquitoes at larval, pupal and adult 379 stage, the average number of individuals having developed at each stage from 50 380 381 larvae was counted (Fig. 3). For the NO strain, there is a significant decrease between 382 the number of adults obtained from eggs laid in treated breeding sites (Student t-test: t 383 = 5.2774, df = 6.9332, *p*-value = 0.0005935). For the CAY strain, the decrease of the 384 number of adults obtained from eggs laid in treated breeding sites is not significant (Student t-test: t = - 0.68374, df = 7.8959, p-value = 0.7431). 385

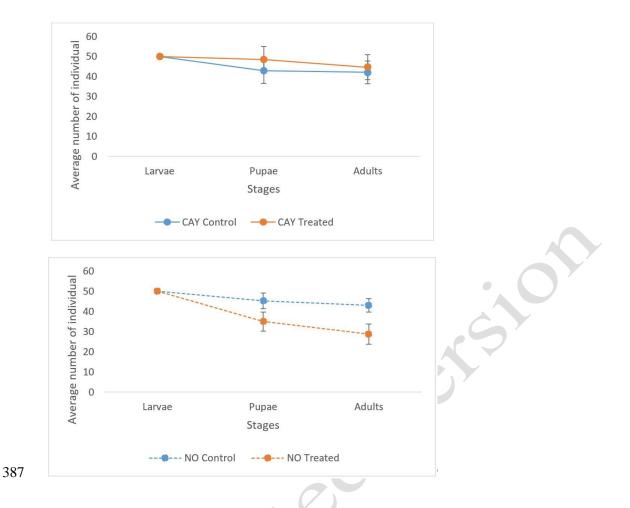
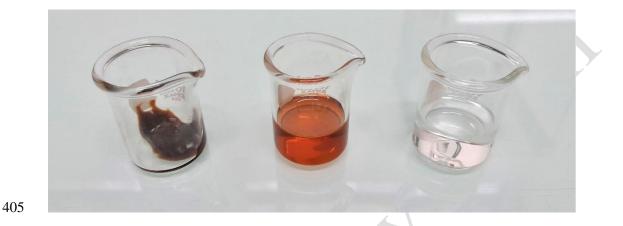


Fig. 3 Average number of individuals having developed to pupal and adult stages from 50 larvae (x five replicates) according the lineage and treatment. CAY: Cayenne wild strain (upper graph), NO: New Orleans strain (lower graph), Control: individuals from eggs laid in control breeding site, Treated: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

393

394 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 400 two weeks of observation, without the apparition of a cloudy louche or insoluble. This 401 concentrate was also easily dispersed in distilled water in 0.10% solution to reach a 402 concentration of 100 μ g/ml of *S. rubra* extract, leading to a homogeneous clear mixture 403 even after 10 days. The visual aspect of the extract, emulsifiable concentrate (EC) and 404 emulsion in distilled water (EW) are represented in Figure 4.



406 Fig. 4 S. rubra-derived larvicidal products: A) wood ethyl acetate crude extract; B)
407 emulsifiable concentrate (EC); C) micro-emulsion in distilled water (EW), 0.1% solution
408

The formulation was developed using the SrK extract (sawdust collected in Kourou 409 410 sawmill), which was known to exhibit LD₅₀ values of 39.6 and 18.6 µg/mL at 24h and 411 48h, respectively, and LD_{90} values of 118.2 µg/ml and 51.2 µg/ml at 24h and 48h, 412 respectively, against the CAY strain, and to contain 10.5% and 8.9% (w/w) of the 413 bioactive compounds rubrenolide and rubrynolide respectively (Clervil et al. 2023). 414 When tested in the laboratory against the CAY strain, the formulation led to LD_{50} values 415 of 48.0 \pm 0.01 and 9.3 \pm 0.05 µg/mL at 24h and 48h, respectively, and LD₉₀ values of 416 75.1 \pm 0.02 and 39.6 \pm 0.04 µg/ml at 24h and 48h, respectively. To ensure that this 417 effect was not due to a toxic activity of the formulation matrix against Ae. aegypti 418 larvae, we assessed the matrix and the formulation at 102 µg/ml prior to their 419 evaluation in semi-field conditions. The results obtained for the matrix showed a

420 mortality rate of $9.5 \pm 4.8\%$ after 24h of exposure, and $14 \pm 2.1\%$ after 48h, whereas 421 the formulation led to 100% of larval mortality in each case.

422

423 Semi-field bioassays

The formulation was tested at the trial dose of 102 μ g/ml for the final product, corresponding to 2xDL₉₀ 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).



429

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

433

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.

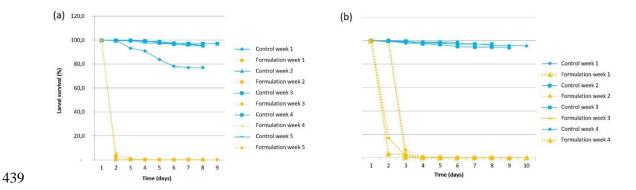


Fig. 6 Survival of *Ae. aegypti* mosquito larvae exposed or not to a formulation based on *S. rubra* wood extract under semi-operational test conditions, during (a) the dry season
(August-September 2020) and (b) the rainy season (January-February 2021). Each
curve corresponds to one pool of 100 larvae (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).

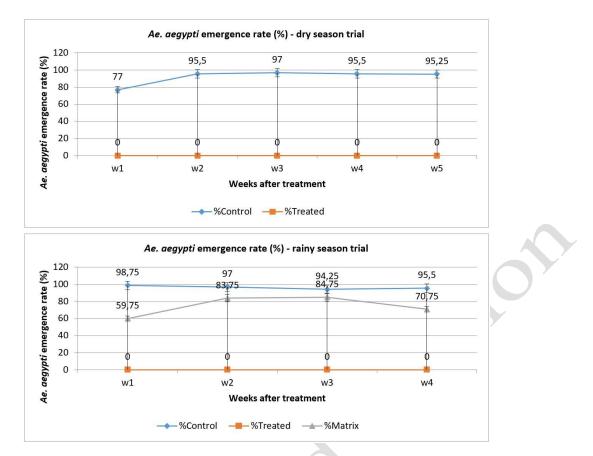




Fig. 7 Simulated field trial performed during dry (upper graph) and rainy (lower graph)
seasons respectively: residual activity of *S. rubra* wood extract formulation on the
emergence of *Ae. aegypti* (± s.e.)

458 Impact on aquatic non-target organisms and human cell lines

459 The matrix and the formulation were tested at the dose used for the semi-field trial 460 (102 µg/ml). At 24h and 48h, the matrix displayed in our test conditions no (at 24h) to 461 low toxicity towards both organisms ($16 \pm 26\%$ of immobilization for *D. magna*, and 12 ± 11% immobilization for *C. riparius* at 48h) (Figure 8). For the formulation, significant 462 463 immobilization rates were observed after both 24h and 48h of exposure in the case of 464 C. riparius, with 64 \pm 2% and 92 \pm 2% of immobilization respectively. The toxic effect 465 appeared more slowly towards *D. magna*, with only $20 \pm 3\%$ of immobilization after 24h 466 of exposure, but 100% of immobilized animals after 48h.

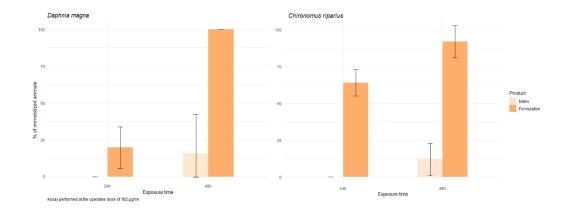
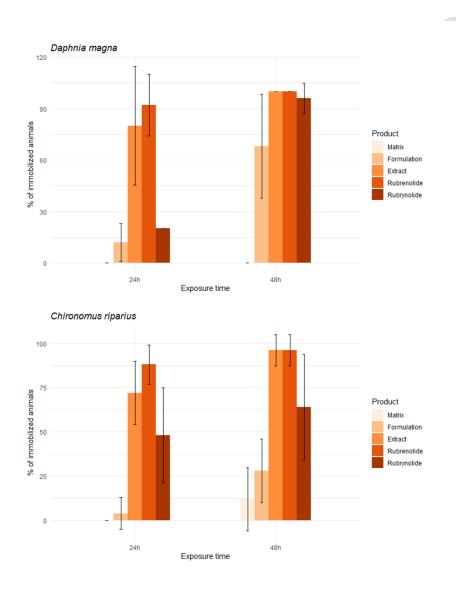


Fig. 8 Comparative assessment of the immobilization percentage (and standard error) of non-target aquatic organisms *Daphnia magna* (left) and *Chironomus riparius* (right) after 24h and 48h of exposure to the formulation matrix (light orange) and full formulation (*S. rubra* extract emulsifiable concentrate, 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

474

475 The effect of extract, matrix and formulation was then assessed at a lower dose, 476 chosen as the 48h-LD₅₀ (18.6 µg/ml) of the SrK extract used to prepare the emulsifiable 477 concentrate. The compounds rubrenolide and rubrynolide were tested at a 478 concentration of 2 µg/ml, which corresponds to around 10% of the 48h-LD₅₀ in 479 consistency with the extract composition, which contains 10.5% of rubrenolide and 480 8.9% of rubrynolide (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 481 482 organisms. The environmental effect of the formulation could be considered low in the 483 case of C. riparius, with a maximum immobilization rate of only 28 ± 18% after 48h of 484 exposure at a dose corresponding to the 48-h LD₅₀ of the extract. The effect of the EC 485 towards *D. magna* was more important, with $68 \pm 30\%$ of immobilized animal after 48h. 486 The results obtained for the pure compounds rubrenolide and rubrynolide indicated a

strong toxicity against non-target organisms, at a dose as low as 2 μ g/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*). Rubrynolide 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.



494

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.*

rubra crude extract were tested at 18.6 µg/ml (S. rubra SrK extract 48-h LD₅₀ against 498

Ae. aegypti), and the pure compounds rubrenolide and rubrynolide at 2 µg/ml 499

500

501 Eventually, the cytotoxicity of all products was concurrently assessed towards a panel 502 of human cell lines (Table 4).

503

Table 4 Cytotoxic effect towards human cell lines after 48h of exposure to the 504 formulation matrix, full formulation (S. rubra emulsifiable concentrate), S. rubra crude 505 506 extract, and pure compounds rubrenolide and rubrynolide. IC50 are expressed in µg/ml 507 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
S. rubra extract	32	93	91	> 250	135	> 250	70
Rubrenolide	35	31	> 50	> 50	> 50	> 50	34
Rubrynolide	12	> 50	> 50	> 50	> 50	> 50	> 50

^a For mixtures ^b For pure compounds

^c EC: emulsifiable concentrate

508

509 According to the standards proposed in the literature, the matrix, formulation and crude extracts could be considered non-toxic when exhibiting IC_{50} values > 100 μ g/ml, this 510 511 endpoint being set at 25 µm in the case of pure compounds (Cos et al. 2006). In this 512 perspective, both the matrix and the formulation only displayed slight cytotoxic effect against kidney NCI-H295R cells (human carcinoma), with IC₅₀ values of 87 and 94 513

514 µg/ml respectively. The extract however could be considered cytotoxic against liver 515 HuH7 cells (human hepatocellular carcinoma, IC_{50} value of 32 µg/ml), and also led to a 516 moderate effect against HUVEC (human umbilical vein endothelial cells), kidney and colon Caco2 (human colorectal adenocarcinoma) cells, with IC₅₀ values in the 70-93 517 518 µg/ml range. Concerning the pure compounds, rubrynolide did not display any 519 cytotoxicity except against liver cells (IC₅₀ of 12 μ g/ml). It was the only case when this 520 compound was more toxic than rubrenolide, which exhibited a measurable effect towards liver, kidney and umbilical cord cells, even if above the 25 μ M criteria (IC₅₀ in 521 522 the 31-35 µM) range. Notably, no toxicity was observed against any of the products in 523 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 IC_{50} 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).

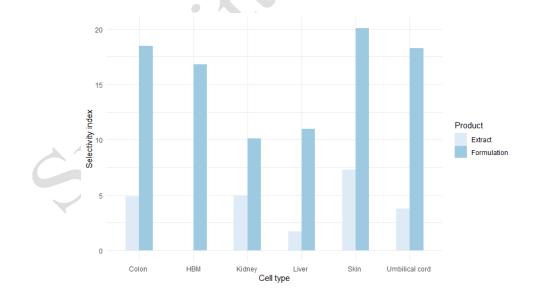


Fig. 10 Selectivity indices calculated as $SI = IC_{50}$ (non-target cells) / LD_{50} (*Ae. aegypti* Cayenne strain larvae) after 48 hours of exposure for *Sextonia rubra* extract (light blue) and formulation (dark blue). HBM: human bone marrow

534 **Discussion**

535 In a first step, the activity of S. rubra wood extract was assayed against Ae. aegypti 536 strains displaying various degrees and mechanisms of resistance to deltamethrin. 537 These new results corroborated the previous efficacy measured against the susceptible 538 Paea strain and the preliminary data obtained about the wild strain Cayenne (Falkowski 539 et al. 2016, 2020). The overall results are in accordance to resistance pattern towards pyrethroids described in French Guiana (Dusfour et al. 2015; Faucon et al. 2017; 540 541 Guidez et al. 2020; Senthil-Nathan 2020; Epelboin et al. 2021). Besides lethality, the 542 impact of active products on oviposition deterrence and repellence, as well as development, longevity or vitality of the target insect are also of great importance in the 543 field of vector control (Borges et al. 2019; Turchen et al. 2020; Pavela et al. 2021). 544 Plants and plant-derived substances are often known to possibly interfere with 545 oviposition effect, leading to either repellent or attractant effect, notably at 546 547 concentrations greater than 50 µg/ml (Kanis et al. 2013; El Maghrbi and Hosni 2014; 548 Satho et al. 2015; Seenivasagan et al. 2019). In our case, the absence of repulsive 549 effect concerning Ae. aegypti oviposition behavior is of importance when it comes to 550 evaluating the potential of S. rubra extract as a larvicide used in stagnant water. A repulsive effect regarding oviposition would indeed lead the mosquitoes to lay eggs in 551 552 other, untreated sites, and therefore at once make such treatment irrelevant and favor 553 the dispersion of the vector (Kanis et al. 2013). Concurrently, we observed that larval 554 hatching response measured for the CAY strain eggs did not vary significantly depending on the breeding site medium type. Such a result had previously been 555 observed for Culex molestus eggs, collected in standing water pools in Australia, and 556 557 placed in breeding sites with different treatments (Methoprene and QN50, a 558 sesquiterpene alcohol derived from Australian sandalwood Santalum spicatum A. DC.) 559 (Spafford et al. 2007). Moreover, the chance to obtain pupae and adult Ae. aegypti 560 mosquitoes is lowered when eggs are laid in treated sites in the case of the NO strain 561 only, as no statistical difference was obtained in the case of the Cayenne wild strain. It 562 would thus be interesting to assess such an effect on the formulation and on other wild-563 type strains collected in French Guiana, in order to evaluate the existence of a potential 564 secondary beneficial effect of *S. rubra* extract and formulation in case of sublethal 565 exposition of the larvae (Silva et al. 2019).

566 The ingredients of the formulation were chosen in order to fulfill the regulation (EU) 567 528/2012 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 568 the placing of plant protection products on the market. Regulatory approvals represent 569 570 an important barrier to the industrial implementation of botanical insecticides, and 571 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 572 573 recorded and patented for their noticeable solvency properties when used to solubilize 574 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 575 576 obtained from renewable raw material, with non-ecotoxic characters in environmental 577 conditions as well as high biodegradability properties (Rybinski and Hill 1998; 578 Johansson and Svensson 2001; Renkin et al. 2005; Zhang et al. 2011; Geetha and Tyagi 2012; Peng et al. 2019). 579

The LD_{50} and LD_{90} 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

587 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 588 589 previously observed for controlled-release systems and nano-emulsions of essential 590 oils (Maia et al. 2019; Rodrigues et al. 2021). Moreover, the mortality rates obtained for 591 both the matrix and the formulation, comparatively, confirmed the relative innocuousness of the matrix against Ae. aegypti, and ensured that the semi-field 592 593 bioassay would give us information on the S. rubra extract in the form of an EC as the 594 only active product.

595 The efficiency of the tested formulation as a mosquito larvicide was demonstrated by 596 our results, especially towards the CAY Ae. aegypti strain, exhibiting a strong 597 resistance to conventional insecticides. The milky appearance of the aqueous media 598 treated with the emulsifiable concentrate observed in semi-operational conditions can 599 be due to the composition of the tap water used for the assay, although it was let 24h 600 for dechlorination before use, or to the addition of larval food. Indeed, the presence 601 calcium, sodium or chloride ions can modify the spontaneous formation of a micro-602 emulsion, and lead to a basic emulsion, while changing the translucent aspect for a 603 cloudy one (Egito et al. 2020). The strong and rapid acute toxicity can represent a 604 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 605 606 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 607 residual activity, due to a high biodegradability through photodegradation, abiotic 608 609 oxidation or volatilization (Amer and Mehlhorn 2006; Isman 2020). In our case, this 610 relatively long residual activity was obtained for a simple emulsion, without using slow-611 release systems. This can be put in perspective with the previously demonstrated 612 stability of the crude S. rubra extract in ambient conditions over time (Clervil et al. 613 2023).

614 Concerning the ecotoxicity of the matrix and the formulation under our experimental 615 conditions, D. magna were overall more sensitive to the tested products than C. 616 riparius, with high standard deviations observed, due to the protocol involving a low 617 number of animals. For the matrix and the formulation at the dose used for the semi-618 field trial (102 µg/ml), our results highlighted the harmlessness of the green formulation 619 basis against non-targeted organisms, as observed towards Ae. aegypti larvae. These 620 results are also consistent with suppliers, regulatory and literature data concerning the 621 ecotoxicological assessment of the formulation matrix components (Renkin et al. 2005; 622 Szwach and Lukosek 2017; ECHA 2022a, b). At this dose, an Ae. aegypti larval 623 mortality of 100% was obtained as soon as 24h when assessing the formulation in the 624 laboratory. At 102 µg/ml, the formulation thus only showed selectivity towards both 625 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 626 627 advantage being lost after 48h of exposure.

However, when testing the effect of extract, matrix and formulation at the 48h-LD₅₀ 628 (18.6 µg/ml) of the SrK extract used to prepare the emulsifiable concentrate, the 629 630 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 631 to the solubilization of S. rubra lipophilic crude extract in water, the use of the 632 633 formulation also allows to lower the toxicity of the active ingredient at the same dose, without losing larvicidal efficacy. Complementary assessments should now be 634 635 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 636 637 by the S. rubra EC. The data obtained for rubrenolide and rubrynolide are consistent 638 with the previous evaluation of these pure compounds against Ae. aegypti larvae, with 639 higher toxic activity observed for rubrenolide than rubrynolide both on the laboratory 640 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 anoticeable value when it comes to the ecotoxicological profile of the larvicidal products.

643 Eventually, except for the extract towards kidney cells, the cytotoxic effect of the tested 644 product was overall moderate to non-significant in an acute toxicity assay. The data 645 obtained for the pure compounds rubrenolide and rubrynolide are consistent with a previous study, in which the pure compounds exhibited $IC_{50} > 100 \mu g/ml$ against NIH-646 647 3T3 fibroblasts cells (Rodrigues et al. 2010). Even if a noticeable toxicity had been 648 previously reported for rubrenolide (GI₅₀ in the 3.3-9.9 μ g/ml range i.e., < 33 μ M) 649 against UACC-62 (human melanoma), MCF-7 (human breast cancer), HT-29 (human 650 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, 651 652 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 653 654 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 655 therefore reinforce those obtained when comparatively assessing the ecotoxicity of the 656 crude extracts towards the formulated products, as in both cases (eco- and 657 658 cytotoxicity), a clear advantage is obtained when using the S. rubra EC.

659

660 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 667 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, 668 669 allowed both the application of a lipophilic botanical extract in water and the compliance with European standards and regulations. This formulation demonstrated 670 671 strong larvicidal activity (acute toxicity and residual activity) through semi-field assays 672 alongside with better selectivity towards non-target aquatic organisms and human cell 673 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 674 production of the extract, in a biorefinery perspective included in circular economy 675 objectives, this work aims to contribute to the development of novel solutions for the 676 677 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 678 environmental and human health issues associated with the use of insecticide 679 680 products.

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931 Competing interests

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

934 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.

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943 Data availability

944 The datasets generated during and analyzed during the current study are available 945 from the corresponding author on reasonable request.

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947 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).