Chronic exposure of mosquito larvae to pesticide residues endangers a new generation of agrochemicals repurposed for malaria prevention

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Abstract

Agrochemicals have been successfully repurposed to control mosquitoes worldwide, but pesticides used in agriculture challenge their effectiveness by contaminating surface waters and driving insecticide resistance in larval populations. Here we implemented a new experimental approach to predict the efficacy of agricultural pesticides newly repurposed for malaria vector control. We mimicked insecticide resistance selection as it occurs in contaminated aquatic habitats by rearing field-collected mosquito larvae in water containing a dose of insecticide at which 100% of individuals from a susceptible strain died within 24 h. We then simultaneously monitored short-term lethal toxicity within 24 h and sublethal effects for 7 days. We found that due to chronic exposure to agricultural pesticides some mosquito populations are currently predisposed to rapidly adapt to neonicotinoids if they were used in vector control. Larvae collected from rural and agricultural areas where neonicotinoid formulations are intensively used for insect pest management were able to survive, grow,

pupate and emerge in lethal doses of acetamiprid, imidacloprid and clothianidin. These results emphasize the importance of addressing prior exposure of larvae to formulations used in agriculture before applying agrochemicals against malaria vectors.

Keywords: pesticide, neonicotinoids, *Anopheles*, mosquito, urbanization.

1. Introduction

Vector control for malaria prevention relies heavily on repurposing agrochemicals, which provides a rapid and cost-effective approach to identify new active ingredients [1,2]. In order to effectively control *Anopheles* mosquitoes that have become resistant to several classes of insecticides, dozens of agrochemicals have recently been tested against adult populations leading to the identification of some promising candidates [1,3–6]. However, concomitant use of the same chemicals in agricultural pest management can provide sufficient selection pressure to engineer resistant mosquito populations well before any application of the agrochemical in public health [7–9]. For example, prior to the scale up of pyrethroid-impregnated bed nets across the continent, resistance driven by crop protection spraying was already reported in West Africa [10]. There is a concern that history may repeat itself with the new generation of agrochemicals that are being adopted for malaria vector control [11].

Lax regulations allow the use of hundreds of pesticide formulations for agricultural pest management in some African countries [12–16]. A large part of this application occurs in small farms held by non-professional operators who are not trained and not supervised for safe and effective use of pesticides. As a result, too frequent and untargeted application provides suitable conditions for chronic exposure of mosquito larvae to pesticide residues. In tropical regions, rain and human activities create ponds in farmlands, which become ideal breeding sites of the most dangerous malaria mosquito species such as *Anopheles gambiae*

and *An. coluzzii* in agricultural areas [17–19]. When these breeding sites are contaminated with pesticide residues, they turn into culture media where chronic exposure contributes to selecting larval populations with increased adaptation to synthetic chemicals [9]. One of the consequences of this unwanted experiment is the emergence of resistance to a number of active ingredients used in agriculture, which in turn undermines their suitability for public health application.

Neonicotinoids are among the most widely used pesticides in agriculture worldwide [20,21]. They are highly water-soluble and environmentally persistent, and thus may leach into surface waters and become a risk for aquatic insects [22–24]. Two new formulations of clothianidin, a neonicotinoid, were recently approved for indoor residual spraying, but have yet to be applied in endemic countries [11,25–27]. Additionally, clothianidin is normally not a registered neonicotinoids for crop protection in most African countries [13,14]. So far, agricultural spraying of other registered compounds such as acetamiprid, imidacloprid, thiamethoxam and thiacloprid remain the main source of selection pressure that could drive resistance to clothianidin in *Anopheles* mosquitoes. This hypothesis has yet to be supported by empirical evidence, but observational studies have described suboptimal levels of neonicotinoid susceptibility in adults of at least two *Anopheles* species, populations from agricultural areas being the most tolerant [28–30]. Importantly, neonicotinoids used for crop protection were also suspected to confer cross-resistance to the new insecticide clothianidin, raising concerns about the efficacy the new generation of agrochemicals repurposed for malaria prevention [29–31].

Large African cities are the venue of dynamic coevolution between humans and disease vectors, which has sometimes reveled textbook examples of rapid adaptation of mosquitoes to

74 synthetic chemicals [32–34]. In Yaoundé the capital of Cameroon, agricultural activities
75 associated with intensive use of pesticides are more common in suburban and rural settings in
76 the outskirts of the city. The center of the city provides an island where surface waters are less
77 exposed to pesticide contamination and aquatic species are therefore less likely to develop
78 resistance. In the neighboring rural areas by contrast, mixtures containing neonicotinoids such
79 as acetamiprid, imidacloprid, and thiamethoxam, as well as pyrethroids and fungicides are
80 frequently sprayed on diverse crops, creating ideal conditions for contamination of surface
81 waters and resistance selection [13,15]. Indeed, a survey of susceptibility in adult mosquitoes
82 using standard testing procedures confirmed this prediction. *An. coluzzii*, the only species
83 present in urbanized areas of Yaoundé remains susceptible to neonicotinoids while its sibling
84 species *An. gambiae*, which occurs in the countryside, has developed resistance [29–31]. This
85 geographic area thus provides a unique setting to dissect the environmental drivers of
86 neonicotinoid resistance in *Anopheles* mosquitoes.
87
88 It has been known for nearly a half century that exposure to agricultural pesticides can lead to
89 the spread of resistance to public health insecticides in *Anopheles* mosquitoes [7,8]. However,
90 we still lack a holistic approach that could enable us to simultaneously decipher the lethal
91 (mortality) and sub-lethal effects (survival, growth, emergence, reproduction, swimming
92 behavior, mobility etc.) which contribute to resistance selection in agricultural settings. To fill
93 this gap, we implemented a bioassay protocol where we mimicked pesticide exposure in
94 agricultural settings by rearing filed-collected *Anopheles* larvae in water containing lethal
95 doses of agricultural neonicotinoids. Using larval populations collected from urban and rural
96 areas of Yaoundé, we compared mortality in 24 h as well as some life table parameters in
97 order to infer the role of exposure to neonicotinoid contaminants in resistance selection. Our
98 findings indicated that resistance selection in *An. gambiae* due to chronic exposure to

neonicotinoids in rural and agricultural settings translates into low mortality but also high survival, growth, pupation and emergence in lethal concentrations of pesticides. The present study highlights concerns regarding the efficacy of some agrochemicals repurposed for malaria vector control, especially active ingredients that are concomitantly used in agricultural pest management.

2. Material and methods

(a) Study sites

The study was carried out in Yaoundé, the capital of Cameroon, and some of its neighboring rural areas. Approval to conduct a study in the Center region (N°: 1-140/L/MINSANTE/SG/RDPH-Ce), ethical clearance (N°: 1-141/CRERSH/2020) and research permit (N°: 000133/MINRESI/B00/C00/C10/C13) were granted by the ministry of public health and the ministry of scientific research and innovation of Cameroon. We surveyed four sites which included a farming area located in the suburbs (Nkolondom, 3°56'43" N, 11°3'01" E), two densely urbanized neighborhoods (Etoa Meki, 3°52'53" N, 11°31'40" E and Combattant, 3°52'53" N, 11°31'40" E) and another suburban site (Nkolnkoumou, 3°52'29" N, 11°23'2" E) (Figure 1). The average distance between sites was 4-5 km. The most abundant malaria vector populations in this area belong to the species complex *An. gambiae sensu lato (s.l.)* [19,33,35]. Previous surveys have revealed that the nominal species *An. gambiae sensu stricto* (hereafter *An. gambiae*) is the only member of the complex present in the agricultural site Nkolondom while its sibling species *An. coluzzii* prefers the most urbanized areas of Yaoundé [33,36,37]. Larvae from the semi-rural neighborhood, Nkolnkoumou, are a mixture of ~ 80% *An. gambiae* and 20% *An. coluzzii* [29–31]. In rural and urban settings, larvae thrive in rain-dependent puddles, which may contain high concentrations of organic pollutants in some densely urbanized areas of the city [19]. In the agricultural site Nkolondom, *An. gambiae* larvae occur in waters that accumulate between

124 furrows and ridges in farms due to rain and/or irrigation and are likely contaminated with
125 pesticide residues (Figure 1).

126 **(b) Larval collection and insecticides**

127 Typical *Anopheles gambiae s.l.* breeding sites were inspected during rainy seasons in 2022 to
128 collect larvae using the standard dipping method [38]. Larvae were transported in plastic
129 containers to the insectary where they were immediately tested in a controlled room (27°C,
130 80% relative humidity, light:dark = 12:12 h).

131 Three neonicotinoids were tested in this study: in addition to acetamiprid and imidacloprid
132 that are used in local farming activities, we also tested clothianidin, which remains an exotic
133 insecticide in Cameroon. Deltamethrin, a first-generation malaria-control insecticide, was
134 used to compare the efficacy of clothianidin. The following commercial formulations were
135 tested: acetamiprid (Aceplant 40EC, 40g/L, emulsifiable concentrate, JACO, Yaoundé,
136 Cameroon), imidacloprid (Plantima 30SC, 30g/L, concentrated suspension, JACO, Yaoundé,
137 Cameroon), clothianidin (Pestanal^{TM}, analytical standard, Sigma-Aldrich) and deltamethrin
138 (Decis 25EC, 25 g/L, emulsifiable concentrate, Bayer Cropscience SL, Valencia, Spain).
139 Stock solutions were prepared by diluting the formulation in absolute ethanol or distilled
140 water.

141

142 **(c) Larval bioassays**

143 In order to assess the lethal and sublethal effects of each insecticide, we reared larvae
144 collected from the field in water containing a dose of the insecticide that was lethal to larvae
145 of a susceptible strain and we measured mortality as well as some life history parameters. The
146 mosquito life cycle comprises four larval stages that precede pupation and emergence of a
147 male or female adult [38]. The four stages include the 1st (L1), 2nd (L2), 3rd (L3) and 4th (L4)
148 instars and can last between one and two weeks depending on the mosquito species, the

149 feeding regime and the environmental conditions. We monitored mortality and life table
150 parameters starting from 3rd instars until emergence. 3rd instar larvae of *Anopheles* are
151 abundant in the wild, are easy to identify morphologically and typically complete
152 transformation into L4, pupation and emergence in approximately 7 days.
153 The doses tested for each insecticide were determined as follows: we exposed 3rd instar larvae
154 of the susceptible strain *An. gambiae* Kisumu to increasing concentrations of the insecticide,
155 starting from 0.001 mg/L and we selected the lowest dose at which 100% of individuals died
156 in 24 h. Batches of 25 larvae were placed in 500 mL plastic trays filled with 200 mL borehole
157 water containing the desired concentration of insecticide and covered with a net. Four
158 replicates were tested for each insecticide dose in addition to a control without insecticide (i.e.
159 containing only water). The lowest lethal concentrations were 0.035 mg/L clothianidin, 0.075
160 mg/L imidacloprid, 0.15 mg/L acetamiprid and 1.5 mg/L deltamethrin. According to studies
161 conducted worldwide, the concentrations of neonicotinoids tested fell in the upper limits of
162 average values detected in contaminated waters and soils [22–24]. However, in some
163 agricultural areas, these concentrations can be 100 times higher [24].
164 Larvae were collected from the field in the morning and brought to the insectary. 3rd instars
165 were sorted immediately and rinse in a tray containing borehole before being transferred into
166 test trays. Four replicates of 25 larvae were tested in 500 mL plastic trays that were filled with
167 200 mL borehole water containing the above-determined lethal dose of the insecticide. Water
168 was not changed throughout the experiment, and 10 mg of TetraMin® fish food was added to
169 each tray daily. Every 24 h, the number of L3, L4, pupae and adults was counted in each tray.
170 Larvae were considered dead if they were unable to move when touched with a dropper. Dead
171 larvae were removed from the test containers and were not replaced. Adults were also
172 removed using a mouth aspirator. Survival, growth, pupation and emergence were assessed
173 daily for 7 days, which was sufficient for 3rd instar larvae to reach the adult stage.

174

175 **(d) Data analysis**

176 The lethal endpoint of insecticide exposure was assessed using the mortality rate after 24 h.
177 The sublethal effects of the different insecticides were addressed using survival probability as
178 well as L4 transformation rate, pupation rate and emergence rate. L4 transformation rate was
179 defined as the percentage of L3 larvae transformed into L4. Pupation rate represented the
180 percentage of L3 that reached the pupa stage and emergence rate the percentage of L3 that
181 became adults. Line plots of mean and standard error, computed with the packages *plyr* and
182 *ggplot2* in R, were used to compare L4 transformation rate, pupation rate and emergence rate
183 at 24 h intervals. The ratio between the number of dead larvae and the initial number of larvae
184 was calculated every 24 h and provided an estimate of survival probability. Kaplan Meier
185 survival curves were plotted using the packages *ggplot 2* and *ggfortify* in R [39]. Larvae
186 reaching the adult stage before the end of the test were treated as censored data. Confidence
187 intervals were computed for the four replicates, and a log-rank test was used to determine if
188 survival was significantly different between treatments.

189

190 **3. Results**

191 **(a) Lethal toxicity in 24 h**

192 We first tested a gradient of insecticide doses on the laboratory strain *An. gambiae* Kisumu,
193 which is susceptible to common classes of insecticides used in mosquito control such as
194 pyrethroids, organophosphates, carbamates and organochlorines. The objective was to
195 determine a dose yielding 100% mortality in 24 h. We then used this dose as a discriminating
196 concentration to study the lethal and sublethal effects of neonicotinoids on field collected
197 *Anophleles* larvae. We compared the efficacy of neonicotinoids to that of deltamethrin. As
198 opposed to the susceptible strain, 100% mortality was not achieved with field populations in

199 24 h (Figure 2). Mortality rates were very low in larvae of both *An. gambiae* and *An. coluzzii*
200 reared in water containing deltamethrin. Pyrethroid resistance is widespread in anopheline
201 mosquitoes, and parallel application of deltamethrin in malaria prevention and in agricultural
202 pest management has drastically reduced its efficacy against vectors. 24-h mortality in water
203 containing deltamethrin was typically below 30% and was not significantly different between
204 the four tested populations (Wilcoxon rank sum test, $p>0.05$). In contrast with deltamethrin,
205 susceptibility to neonicotinoids varied between species and geographic areas. Consistent with
206 findings obtained using adult bioassays [29,30], *An. coluzzii* larvae collected from the two
207 urban neighborhoods, Combattant and Etoa meki, were more susceptible, with mortality rates
208 between 70-80%, except clothianidin for which only about 50% of larvae were killed in 24 h.
209 The species present in suburban and rural areas of Yaoundé, *An. gambiae*, has developed
210 strong resistance to neonicotinoids. Particularly, larvae collected from the Nkolondom farm
211 where pesticide spraying has selected for resistance to acetamiprid and imidacloprid and
212 conferred cross-resistance to clothianidin [29,30], displayed less than 10% mortality in water
213 containing a neonicotinoid. Mortality rate of larvae collected from the agricultural site was
214 <10% in the new malaria-control chemical, clothianidin, just as in deltamethrin, a first-
215 generation insecticide, suggesting that both active ingredients currently have similar efficacy
216 against some *An. gambiae* populations.

217 **(b) L4 transformation rate**

218 Some neonicotinoid insecticides act slowly and are also known to have a wide range of
219 sublethal effects in addition to acute toxicity on non-target species [23,40]. Thus, beside lethal
220 toxicity in 24 h, we used life table analysis to monitor some of the sublethal effects of
221 neonicotinoids on *Anopheles* larvae, starting with L4 transformation rate. The three
222 neonicotinoids tested had little effect on the ability of *An. gambiae* L3 larvae to become L4
223 (Figure 3A). The highest transformation rate was observed in populations from the

224 agricultural site, Nkolondom: 90 ± 3% in water containing acetamiprid followed by 75 ± 9%
225 in imidacloprid in two days and 60 ± 3% in clothianidin after 3 days. Similarly, in
226 Nkolnkoumou, a semi-rural site harboring ~ 80% *An. gambiae*, transformation rates varied
227 from 60 ± 6% for acetamiprid, to 35 ± 16% and 23 ± 7% for imidacloprid and clothianidin,
228 respectively after 2 to 4 days. Conversely, even if based on 24-h mortality, *An. coluzzii* larvae
229 showed a degree of tolerance, they were strongly inhibited in water containing neonicotinoids
230 leading to very low transformation rates between 0 and 10% in both Etoa Meki and
231 Combattant populations. In control tests, transformation rates were 100% after 2 to 3 days.
232
233 **(c) Pupation rate**
234 Pupation rate was consistent with L4 transformation and highlighted the low susceptibility of
235 agricultural and semi-rural populations of *An. gambiae* to neonicotinoids (Figure 3B). The
236 agricultural population of *An. gambiae* (Nkolondom) had the highest pupation rate, 77.5 ± 4%
237 in acetamiprid, and 45 ± 11% in imidacloprid reflecting their tolerance to the both
238 neonicotinoid pesticides that are sprayed in local farms. The exotic insecticide, clothianidin, at
239 0.035 mg/L had the most important inhibitory power among neonicotinoids, but 15 to 20% of
240 *An. gambiae* larvae were able to achieve pupation. *Anopheles coluzzii* larvae had low pupation
241 rates with less than 5% pupae obtained in all the neonicotinoids tested. Notably, none of the
242 *An. coluzzii* populations were able to pupate in clothianidin.100% pupae were obtained in all
243 the control tests after 72 h.
244
245 **(d) Emergence rate**
246 100% of *An. gambiae* and *An. coluzzii* pupae emerged into adults in the absence of
247 insecticides between the first and the sixth day in standard laboratory conditions. The total
248 emergence rate of *An. coluzzii* larvae was 0%, 2% and 4% in water containing clothianidin,

249 acetamiprid or imidacloprid, respectively after 7 days (Figure 3C). Meanwhile, at least half of
250 *An. gambiae* L3 larvae tested on average emerged between 2-7 days in acetamiprid and
251 imidacloprid. Only *An. gambiae* larvae from the agricultural site were able to emerge in
252 clothianidin albeit at lower rate (7 ± 3%) compared with the other neonicotinoids. However,
253 emergence rate was also very low in deltamethrin, around 10%, indicating that for any
254 insecticide, the ability to inhibit emergence can be compatible with strong resistance in adult
255 mosquitoes and loss of efficacy of the insecticide. In addition to lethal toxicity in 24 h,
256 emergence rate revealed another similarity between clothianidin, a new insecticide and
257 deltamethrin whose effectiveness in malaria vector control has become very limited.
258
259 **(e) Survival probability**
260 Rearing field-collected larvae in water containing an agricultural pesticide affected their
261 survival probability as revealed by Kaplan Meier survival curves (Figure 4). Larvae that
262 survived lethal toxicity within the first 24 h of the experiment were monitored for seven days.
263 Although *An. coluzzii* showed potential to resist the 24-h lethal effect of some of the
264 agricultural pesticides, larvae of this species had low survival probability in neonicotinoids.
265 By contrast, *An. gambiae* populations from agricultural and semi-rural sites displayed high
266 survival potential in water containing lethal doses of neonicotinoids. A log-rank test revealed
267 that regardless of the neonicotinoid that was added to water, larvae collected from
268 Nkolondom were super-resistant and had significantly higher survival probability compared
269 with any of the three other populations tested ($p < 0.05$). 100% survival was obtained in all
270 control experiments without insecticide.
271
272 **4. Discussion**

273 Here we have used an innovative approach to assess both the lethal and sublethal effects of
274 pesticide exposure in mosquito larvae. We found that in addition to routine quantification of
275 mortality in larvae and adults, monitoring some sublethal effects provide critical information
276 to better evaluate pre-existing levels of adaptation to agrochemicals in malaria mosquitoes.
277 We conducted a laboratory experiment mimicking the selection process, which is promoting
278 the emergence of *Anopheles* populations with increased tolerance to neonicotinoids. While
279 analyzing mortality and life table in wild larvae reared in artificial media containing a lethal
280 dose of the active ingredient, we observed gradients of tolerance reflecting past exposure to
281 neonicotinoids in nature. Control tests supported the validity of our experimental approach as
282 3rd instar larvae unexposed to insecticides showed standard survival, growth and emergence in
283 controlled insectary conditions.

284 Previous studies have suggested that resistance to neonicotinoids observed in anopheline
285 populations from some agricultural areas could be due to chronic exposure of larvae to
286 sublethal doses of agricultural pesticides [28–31]. Our findings provide the first experimental
287 support to this hypothesis. Indeed, we found that *An. gambiae* larvae had exceptional fitness
288 in field-realistic concentrations of acetamiprid and imidacloprid, two neonicotinoids whose
289 formulations are extensively used in farming activities in Cameroon [29]. As anopheline
290 populations have not yet been exposed to clothianidin in sub-Saharan Africa, this exotic
291 neonicotinoid strongly inhibited larval emergence in both *An. gambiae* and *An. coluzzii*.
292 However, likely due to cross-resistance selected by intensive exposure to acetamiprid and
293 imidacloprid, larvae from the agricultural site, Nkolondom, were able to survive in water
294 contaminated with clothianidin, albeit at lower frequency.

295 Exposure of non-target insects to sublethal doses of neonicotinoids affect functions such as
296 motility, behavior, growth, fecundity and survival [23,41,42]. Our study reveals that
297 notwithstanding these sublethal effects whose magnitude remains unknown, *An. gambiae*

298 populations have evolved adaptation to chronic exposure to neonicotinoid residues in
299 contaminated aquatic habitats. *An. gambiae* larvae from rural areas around Yaoundé exhibited
300 high survivorship, L4 transformation, pupation and emergence rate in water containing
301 neonicotinoids contrary to urban *An. coluzzii* mosquitoes whose growth and survival were
302 inhibited. This result corroborates susceptibility profiles of adult populations: *An. coluzzii*
303 from Yaoundé is more susceptible to neonicotinoids while *An. gambiae* is resistant to at least
304 four different active ingredients [29–31]. Larval populations whose adults were resistant to an
305 agrochemical (i.e. mortality against the discriminating dose < 90%) typically displayed less
306 than 50% mortality in 24 h and more than 5% emergence in 6 days in water containing a
307 lethal dose of the active ingredient. Testing a larger number of chemicals on diverse
308 *Anopheles* species and populations will provide more robust guidelines on the interpretation
309 of mortality, growth and emergence of larvae reared in pesticide-laced water. However, based
310 on the current study and complimentary evidence from adults testing [29–31], we could
311 establish that any agrochemical yielding less that 50% mortality after 24 h and more than 5%
312 emergence within 6 days is likely to have limited efficacy against *Anopheles* mosquitoes.

313 The first evidence of insecticide resistance driven by agricultural pesticide application dates
314 back to the 1960s. Based on observations made in El Salvador, it was clearly established that
315 aerial spraying of carbamates and organophosphates led to the development of broad-
316 spectrum resistance to both insecticide classes in the vector *Anopheles albimanus* [7,8]. Since
317 then, a number of observational studies have argued that intensive use of crop protection
318 chemicals is a major driver of resistance to public health insecticides in anopheline
319 mosquitoes [16,43–47]. However, in the majority of cases, it was difficult to determine the
320 guilty part between agricultural chemicals and malaria vector control insecticides. In a review
321 of evidence, Lines (1988) [9] proposed a set of conditions that should be met for a crop
322 protection chemical to be the source of insecticide resistance selection in malaria vectors.

323 Appearance of resistance in mosquitoes to a particular chemical should occur before it has
324 been used for mosquito control; there should be correlation in space and time, e.g. higher
325 levels of resistance in areas with agricultural spraying and seasonality of resistance
326 overlapping with spraying periods; the cross-resistance spectrum should show that the
327 chemicals used in agriculture may confer cross-resistance to public health insecticides and
328 that both adults and larvae can be killed by the agricultural pesticides. Agricultural application
329 of neonicotinoids fits all of the criteria in Sub-Saharan Africa and provides the best example
330 of human activities accidentally engineering insecticide resistance in mosquitoes.
331 In our study, both lethal and sublethal effects point to the existence of levels of resistance that
332 could be a major obstacle to using neonicotinoids in malaria mosquito control. We noted that
333 lethal toxicity in 24 h, pupation rate and emergence rate were very similar in water containing
334 either deltamethrin or clothianidin. This finding suggested that, just like deltamethrin, the
335 efficacy of clothianidin in *An. gambiae* might already be substantially reduced. In line with
336 this prediction, it has been shown that neonicotinoid resistant mosquitoes from Nkolondom
337 were significantly less susceptible to SumiShield® 50WG, a formulation of clothianidin
338 recently approved for indoor residual spraying [29]. Moreover, a laboratory experiment
339 recently demonstrated that exposure of *Anopheles* larvae to sublethal concentrations of a
340 mixture containing several herbicides, pesticides and fungicides resulted in ~ 2.5 increase in
341 tolerance to clothianidin and Fludora® Fusion, a formulation combining clothianidin and
342 deltamethrin [48]. These studies and ours highlight the vulnerability of some agrochemicals
343 repurposed for malaria vector control, as resistance driven by agricultural activities could
344 become a major thread to their efficacy.
345 Although our findings are based on only two *Anopheles* species sampled from a relatively
346 small geographic area, these results confirm concerns about the efficacy of neonicotinoids
347 against malaria vectors [1,28–30,49]. Repurposing agrochemicals has thus far provided a

rapid mechanism to identifying new candidate insecticides for malaria prevention. However, the case of neonicotinoids emphasizes the crucial role of evaluating prior exposure and cross-resistance in areas where mosquito larval populations are likely to be in contact with agricultural pesticides.

Author Contributions

MA: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft; CF: Conceptualization, Formal analysis, Investigation, Methodology; FA: Investigation, Methodology; VP-B, Resources, Supervision. CK: Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Writing – review & editing.

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Informed Consent Statement

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Data Availability Statement

The data for this study have been presented within this article.

Conflicts of Interest

The authors declare that they have no competing interests.

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Figure legends

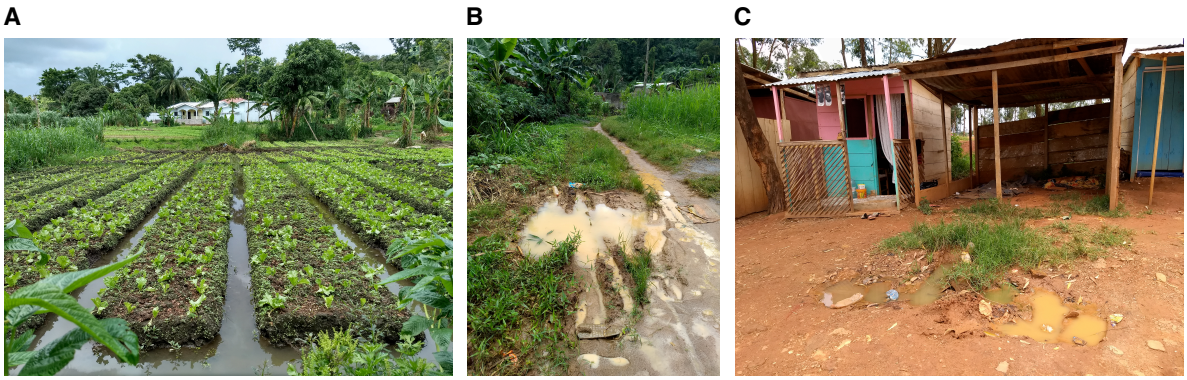


Figure 1: Examples of breeding sites where wild mosquito larvae were sampled. *An. gambiae* larvae were collected from furrows between ridges in the agricultural site (A) or from puddles created by human activities in Nkolnkoumou (suburban area) (B). *An. coluzzii* larvae were sampled from human-made puddles in densely urbanized settings (C).

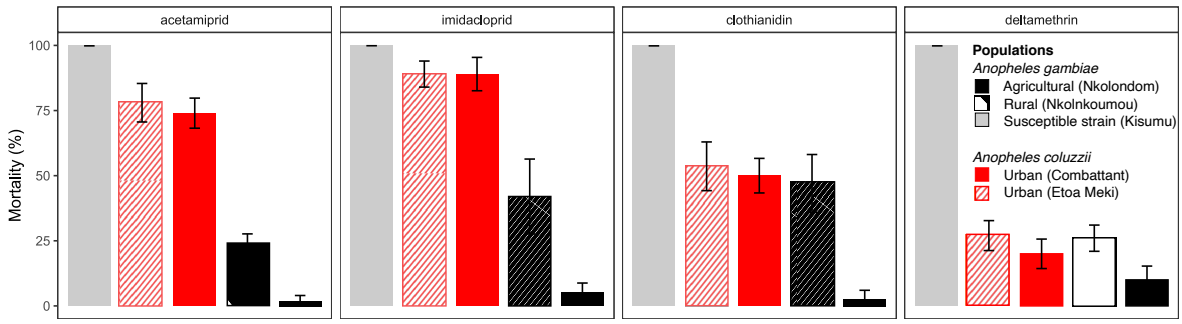
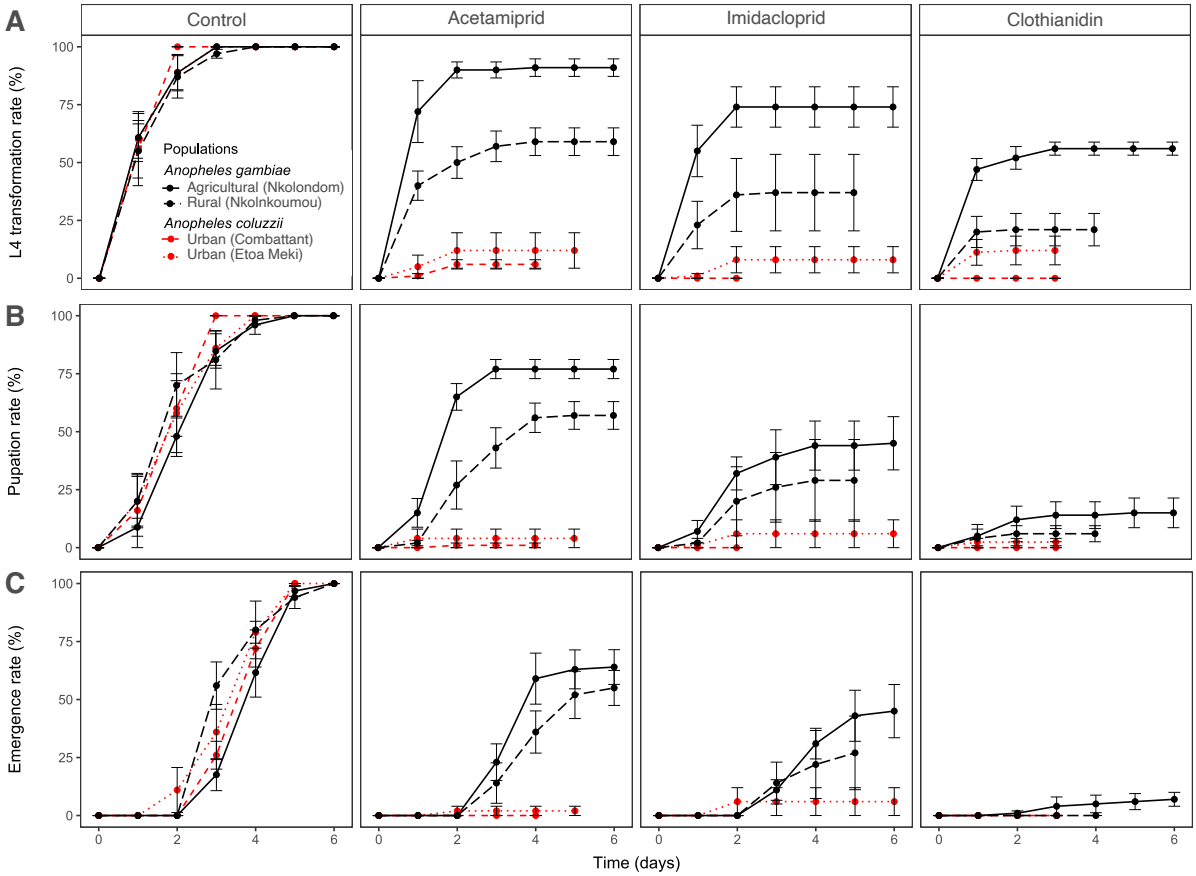


Figure 2: 24-h mortality of mosquito larvae reared in water containing a lethal dose of agrochemical. 100% of larvae of the susceptible strain *An. gambiae* Kisumu were killed in 24 h. Wild populations showed varying levels of tolerance to four insecticides. Mortality values were lowest in larvae collected from the agricultural area (Nkolondom). Error bars represent the standard error of the mean.



549 **Figure 3:** Sublethal effects of three neonicotinoids on life table parameters of *Anopheles*

550 larvae. Rate of transformation of 3rd instars (L3) into 4th instars (L4) (A), pupation rate (B)

551 and emergence rate (C). Larvae that survived 24-h lethal toxicity were monitored for 7 days

552 under standard laboratory conditions. Controls were larvae reared in water without

553 insecticide. Vertical bars represent the standard error of the mean.

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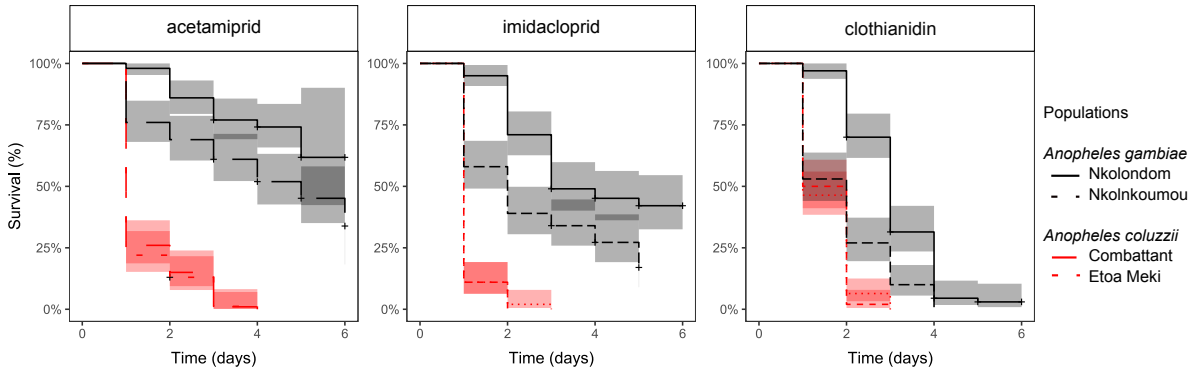

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556 **Figure 4:** Kaplan Meier survival curves (lines) with 95% confidence intervals (colored bands)

557 of 3rd instar larvae reared in lethal doses of three neonicotinoids. Survival probabilities of four

558 wild populations were compared under controlled laboratory conditions. (+) indicates

559 emergence that occurred before the end of the experiment and was treated as censored data.