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Alphonse Keller Konkon
[1] Centre de Recherche entomologique de Cotonou (CREC), Cotonou 06 BP 2604, Benin
[2] Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

Germain Gil Padonou
[1] Centre de Recherche entomologique de Cotonou (CREC), Cotonou 06 BP 2604, Benin
[2] Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

Albert Sourou Salako
Centre de Recherche entomologique de Cotonou (CREC), Cotonou 06 BP 2604, Benin

David Mahouton Zoungbédji
[1] Centre de Recherche entomologique de Cotonou (CREC), Cotonou 06 BP 2604, Benin
[2] Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

Juvenal Minassou Ahouandjinou
Centre de Recherche entomologique de Cotonou (CREC), Cotonou 06 BP 2604, Benin

Arsène Jacques YH Fassinou
Centre de Recherche entomologique de Cotonou (CREC), Cotonou 06 BP 2604, Benin

Haziz Sina
Centre de Recherche entomologique de Cotonou (CREC), Cotonou 06 BP 2604, Benin

Lamine Baba-Moussa
[1] Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin
[2] Laboratoire de biologie et de typage moléculaire en microbiologie, Département de biochimie et de biologie cellulaire, Bénin

Martin Akogbéto
Centre de Recherche entomologique de Cotonou (CREC), Cotonou 06 BP 2604, Benin

Corresponding Author:
Alphonse Keller Konkon
[1] Centre de Recherche entomologique de Cotonou (CREC), Cotonou 06 BP 2604, Benin
[2] Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

Association of Culicidae larvae and effectiveness of 0.4% S-methoprene Mosquilarv in reducing *Aedes* density in southern Benin

Alphonse Keller Konkon, Germain Gil Padonou, Albert Sourou Salako, David Mahouton Zoungbédji, Juvenal Minassou Ahouandjinou, Arsène Jacques YH Fassinou, Haziz Sina, Lamine Baba-Moussa and Martin Akogbéto

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Abstract
This study evaluated the efficacy of Mosquilarv containing 0.4% S-methoprene in reducing population densities of *Aedes* spp. Oviposition traps treated with 1.5 mg Mosquilarv granules containing 0.4% S-methoprene and untreated controls were placed in houses where natural breeding sites of *Aedes* spp. are often found traps were collected 7 days later, eggs were counted and kept under rearing conditions until complete emergence of larvae in control traps and death or emergence of larvae in traps treated with 0.4% Mosquilarv. Our results show a predominance of the *Aedes* vector species collected in Porto-Novo and Ifangni. *Ae. aegypti* and *Ae. albopictus* share the same ovitraps. The results revealed a considerable reduction in emergence in treated ovitraps, ranging from 64.42% to 93.53%. Mosquilarv with 0.4% S-methoprene showed interesting results in reducing the density of *Aedes* spp vectors and could be considered as a response and prevention tool for possible dengue epidemics in Benin.

Keywords: S-methoprene, ovitrap, *Aedes*, dengue, sympatry, Benin

1. Introduction

In recent years, several cases of dengue epidemics have been reported in the West African region [1]. Indeed, dengue fever outbreaks have been reported in Ivory Coast [2], Burkina Faso, Senegal, and Nigeria [3]. Several other arboviruses are prevalent in the same African zone. For instance, Cape Verde has recorded the Zika virus, and chikungunya was detected in Senegal in 2015 [4]. Moreover, recent epidemics have involved Rift Valley fever in Niger in 2016, with epidemic foci in Senegal and Mali [5], as well as Lassa fever in Nigeria. Dengue is a disease caused by a Flavivirus mainly transmitted by *Aedes aegypti* and *Aedes albopictus*. This disease has been rapidly expanding worldwide over the past decade. In 2019, 4.2 million cases of dengue were reported, with 125 countries at risk worldwide and 20,000 deaths occurring annually [6,7]. In Benin, cases of dengue have been reported among both native people and French tourists in recent years [8-10]. Children are the most vulnerable to dengue infection, but it should be noted that the incidence increases in older age groups [11]. Several factors, most of them man-made, increase the risk of the disease, such as urbanization, unprecedented population growth in major cities, and poor lifestyle hygiene, which promote the reproduction of the vector population [12]. *Aedes albopictus* has made its way into Benin and is increasingly populating localities in the south of the country. *Aedes aegypti* is found throughout Benin, both in urban and peri-urban areas that are becoming densely populated, confirming the anthropophilic nature of *Aedes aegypti* and *Aedes albopictus*. In fact, since 1950, the density of the urban human population has tripled in Africa. Slums are associated with an increased risk of dengue infection, as the artificial collection of water increases the habitat available to *Aedes* vectors. Control and elimination of dengue vectors remain the most important options in long-term dengue control programmes. Without an effective vaccine and specific treatment, integrated vector management approaches such as source reduction, insecticide use, biological control, public education and awareness, and personal protection offer the most promising results. Insecticide use remains a major component of antivector control strategy, especially during an epidemic. The insecticides frequently used belong to the pyrethroid class, against which vectors have developed resistance. Insect Growth Regulators (IGRs) present themselves as an alternative. Insect growth regulators (IGRs) are a group of chemicals containing substances with growth retarding and inhibiting properties [13]. Generally, insect growth regulators are effective larvicides against mosquito larvae and have low toxicity to mammals and a good safety margin for most other non-target organisms [14]. Based on this foundation, this study was initiated to evaluate the effectiveness of Mosquilarv at 0.4% S-methoprene for controlling dengue vectors in Benin.

2. Materials and methods

2.1 Study site

The study area is in the southeast of Benin and includes the municipalities of Porto-Novo and Ifangni. These municipalities have a subequatorial humid climate with two rainy seasons and two dry seasons. In both study municipalities, two districts and two villages from each of these districts were selected, including one peripheral and one central village. Oviposition traps used for collecting *Aedes* eggs were placed in the districts of Anavie, Tokpota, Dowa, and Gbodjè in the municipality of Porto-Novo, and in the districts of Banigbe, Igolo, Doke, and Baoudjo in the municipality of Ifangni (Figure 1).

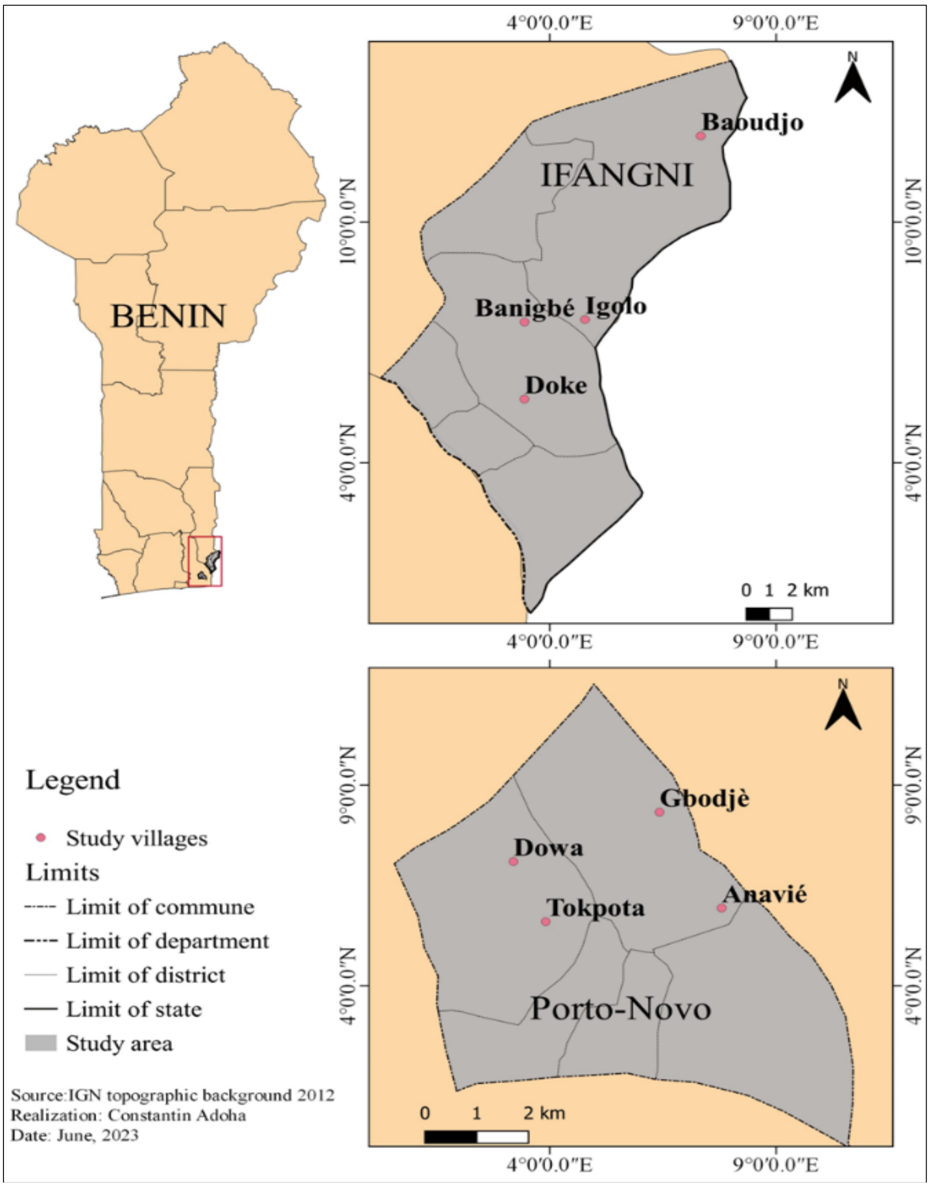

Fig 1: Study sites in the municipalities of Porto-Novo and Ifangni

2.2 Installation and Treatment of Oviposition Traps with 0.4% S-methoprene Mosquilarv

Oviposition traps, or oviposition containers, were made using cut polyethylene bottles with a volume of 80 cl, painted black. They were filled with 50 cl of water, and a 5 cm by 20 cm isorel plaque was placed in the water to serve as a support for the eggs. A total of 24 oviposition traps were set up per village, with 12 control traps and 12 traps treated with 1.5 mg of Mosquilarv granules containing 0.4% S-methoprene. The oviposition traps were spaced approximately 100 meters apart. The traps were placed at a height of 1.5 cm above the ground, suspended by a nail and a metal string, either on a tree or a wall, and left in place for a week in domestic environments (Within the courtyards) or in peri-domestic areas. They are inspected regularly every day by local agents recruited in such a way as not to give the eggs time to hatch. This frequent monitoring ensured that any eggs that might hatch did not have enough time to develop into adults. The isorel supports with the eggs were collected, wrapped in absorbent paper, and brought back to the laboratory for egg counting per trap. After counting, the supports were immersed in water collected from the field traps to facilitate egg hatching. The larvae were fed throughout the trial with a yeast broth at a concentration of 10 mg/l at regular two-day intervals until complete emergence in the control group. After hatching, the emerged adults were counted, identified, and categorized based on whether they originated from the control traps, or the traps treated with 0.4% S-methoprene Mosquilarv.

2.3 Statistical Analysis

The chi-square test of proportion comparison was used to compare the proportions of eggs and the emergence rate in the oviposition traps treated with 0.4% S-methoprene Mosquilarv compared to the control. A descriptive analysis of mosquito diversity was conducted after emergence using proportions. The Reduction Rate (RR) of emergence by Mosquilarv was calculated using the following formula: RR = (Pre-test - Pctrl/Pctrl) * 100, where RR is the reduction rate, Pre-test is the proportion of mosquitoes that emerged from the treated traps, and Pctrl is the proportion of mosquitoes that emerged from the untreated traps. All analyses were performed using version 4.1.3 of the R software.

3. Results

3.1 Species of Culicidae Collected in Oviposition Traps

The species of Culicidae trapped in the oviposition traps were studied. The data analysis reveals the presence of three species in the municipalities of Ifangni and Porto-Novo, including two dengue vector species, *Aedes aegypti* and *Aedes albopictus*, as well as *Culex quinquefasciatus*. In Porto-Novo, *Aedes aegypti* was the most abundant species (89.70%), followed by *Aedes albopictus* (8.02%) and *Culex quinquefasciatus* (2.28%) (Table 1). In Ifangni, a similar trend was observed, with the abundance of *Aedes aegypti* (77.18%) followed by *Aedes albopictus* (19.52%) and *Culex quinquefasciatus* (3.31%).

Table 1: Species of Culicidae Collected in Oviposition Traps

Municipalities	Districts	*Aedes aegypti % (n)*	*Aedes albopictus %(n)*	*Culex quinquefasciatus %(n)*
Porto-Novo	Anavié	60.29 (123)	29.41(60)	10.29 (21)
	Tokpota	92.07 (267)	5.86(17)	2.07 (6)
	Dowa	97.84 (317)	2.16 (7)	0(0)
	Gbodje	96.99 (355)	3.01 (11)	0 (0)
	Total	89.70 (1062)	8.02(95)	2.28 (27)
Ifangni	Banigbe	83.45 (489)	13.65 (80)	2.90(17)
	Igolo	93.66 (133)	6.34(9)	0(0)
	Doke	71.53(201)	24.20(68)	4.27 (12)
	Baoudjo	69.33 (391)	26.60(150)	4.08 (23)
	Total	77.18 (1214)	19.52 (307)	3.31 (52)

n: count; %: percentage

Association or non-association of Culicidae species in the oviposition traps and collection sites

In the districts of Anavié, Tokpota, Dowa, and Gbodje in the municipality of Porto-Novo, as well as in Banigbe, Igolo, Doke, and Baoudjo in the municipality of Ifangni (Table 2), *Ae. aegypti* and *Ae. albopictus* share the same oviposition traps. The two species are described as sympatric in the oviposition traps. This is also the case for *Ae. aegypti* and *Cx. quinquefasciatus*, but not in all districts such as Dowa and Gbodje in Porto-Novo and Igolo in Ifangni. The associations *Ae. aegypti/Cx. quinquefasciatus* and *Ae. albopictus/Cx. quinquefasciatus* are identical regardless of the locality. However, this sympatric relationship was not observed in the oviposition traps for the species triplet *Aedes albopictus/Aedes aegypti/Culex quinquefasciatus*. *Ae. albopictus* was not found alone in the oviposition traps, nor was *Cx. quinquefasciatus*, unlike *Ae. aegypti*.

Table 2: Association or non-association of Culicidae species in the oviposition traps and collection sites

Species	Association or non-association in the oviposition traps							
	Municipality of Porto-Novo				Municipality of Ifangni			
	Districts				Districts			
	Anavié	Tokpota	Dowa	Gbodje	Banigbe	Igolo	Doke	Baoudjo
Aedes albopictus/Aedes aegypti	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Aedes aegypti/Culex quinquefasciatus	Yes	Yes	No	No	Yes	No	Yes	Yes
Ae. albopictus/Culex quinquefasciatus	Yes	Yes	No	No	Yes	No	Yes	Yes
Aedes albopictus/Aedes aegypti/Culex quinquefasciatus	No	No	No	No	No	No	No	No
Aedes aegypti seule	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Aedes albopictus alone	No	No	No	No	No	No	No	No
Culex quinquefasciatus alone	No	No	No	No	No	No	No	No

3.2 Evaluation of the degree of infestation by *Aedes*

According to figure 2, a large proportion of the nests were positive (nests with eggs on the isorel supports) in both Ifangni and Porto-Novo. Positive rates of 95.83%, 87.5%, 70.83% and 66.67% were recorded in the districts of Baoudjo, Banigbe, Igolo and Doke respectively. In Porto-Novo, the rates were 62.5%, 66.67%, 62.5% and 54.17% in Anavie, Tokpota, Dowa and Gbodje respectively.

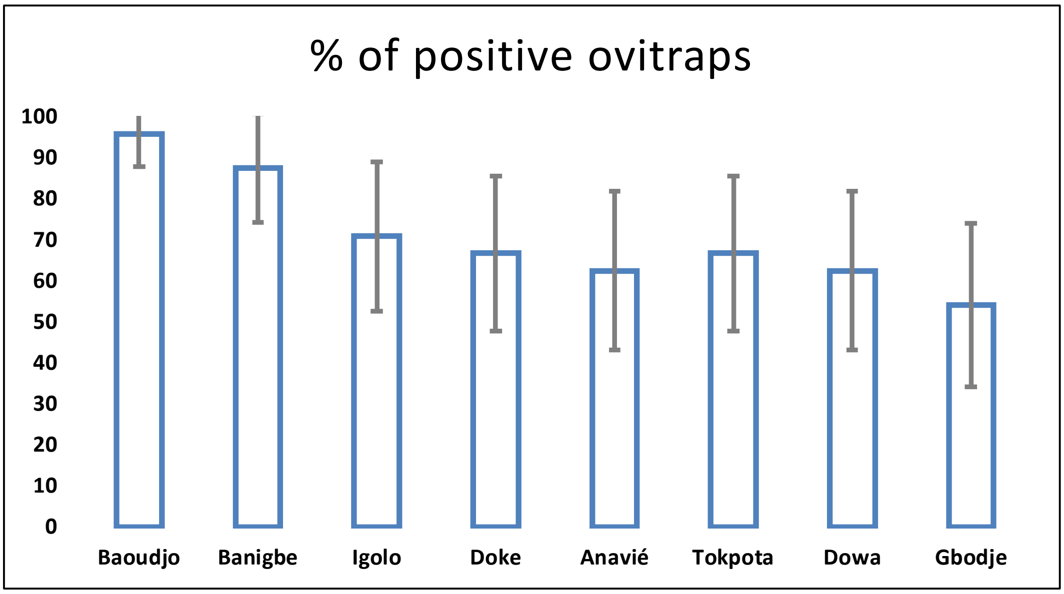

Fig 2: Proportions of positive oviposition traps in Ifangni and Porto-Novo.

3.3. Estimation of the risk of infestation of oviposition traps by *Aedes* eggs

The proportions of eggs counted from the plates of the pondoir traps vary depending on each locality and indirectly reflect the degree of infestation of the traps. In the Porto-Novo municipality, a significant difference was observed between the treated and untreated pondoir traps in the Tokpota (CTRL: 60.86%; Test: 39.12%), Dowa (CTRL: 69.40%; Test: 30.60%), and Gbodjè (CTRL: 75.89%; Test: 24.11%) districts, where the proportion of eggs in the treated traps is higher than in the control traps. At Anavie no difference was observed between the proportion of eggs collected by treated and control traps (CTRL: 46.76%; Test: 53.24%). A difference was observed between the proportions of eggs collected from the treated and untreated traps in the Igolo and Doke districts of the Ifangni municipality. The proportions of eggs collected from the treated traps (64.44%) were significantly higher than those collected from the untreated traps (35.56%) in Igolo, while the opposite was observed in Doke (CTRL: 57.55%; Test: 42.45%). In the Banigbe and Baoudjo districts, no difference was observed between the proportions of eggs in the treated and untreated traps. Overall, considering all localities, no significant difference was observed for the proportions of eggs between the two categories of traps.

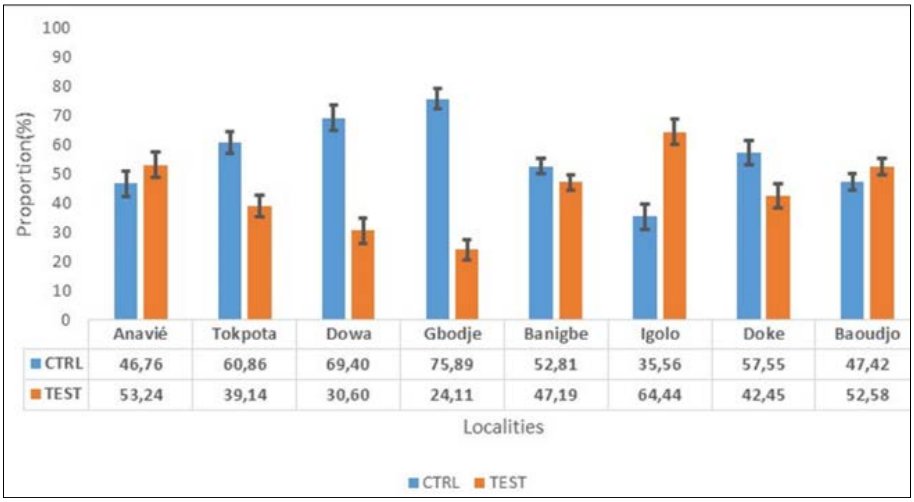

Fig 3: Degree of infestation of ovitraps by *Aedes* spp. eggs.

3.4. Impact of Mosquilarv on the inhibition of *Aedes* spp. larval development

A total of 1341 eggs were collected from the control oviposition site (untreated) and 785 from the Mosquilarv-treated oviposition site in the Porto-Novo municipality. Upon complete emergence, 1038 adults were collected from the control traps and 146 from the test traps. The emergence rates ranged from 61.46% to 92.01% with an average of 77.40% in the control group. In the test group, the emergence rates ranged from 9.96% to 26.09% with an average of 18.60%. In the Ifangni municipality, 1714 eggs were collected from the control traps compared to 1752 from the treated traps. The emergence data showed a total of 1412 adults emerged from the control group compared to 161 adults from the pondoirs treated with Mosquilarv. The emergence rates ranged from 73.33% to 88.59% with an average of 82.38% in the control group. However, in the test group, it ranged from 5.29% to 13.98% with an average of 9.19%. Overall, the emergence rates in the untreated traps were significantly higher than those in the treated traps regardless of the considered locality (P=0.001). The results revealed a considerable reduction in emergence in the treated pondoirs ranging from 64.42% to 93.53% (Table 3).

Table 3: Impact of Mosquilarv on the inhibition of larval development of *Aedes albopictus* and *Aedes aegypti*

Districts	Number of collected eggs		Number of hatched eggs		Number of emerged mosquitoes				Emergence rate (%)		P-value	Reduction (%)
					Aedes aegypti	*Aedes albopictus*	*Aedes aegypti*	*Aedes albopictus*				
	CTRL	**TEST**	**CTRL**	**TEST**	**CTRL**		**TEST**		**CTRL**	**TEST**		
Anavié	238	271	238	271	101	55	22	5	74.37	9.96	< 0.001	-86.608
Tokpota	384	247	384	247	217	15	52	2	61.46	21.86	< 0.001	-64.42
Dowa	313	138	313	138	281	7	36	0	92.01	26.09	< 0.001	-71.64
Gbodje	406	129	406	129	328	9	27	2	83.00	22.48	< 0.001	-72.91
Total	1341	785	1341	785	927	86	137	9	77.40	18.60	< 0.001	-75.97
Banigbe	677	605	677	605	557	80	32	0	81.83	5.29	< 0.001	-93.53
Igolo	165	299	165	299	112	9	21	0	73.33	7.02	< 0.001	-90.42
Doke	320	236	320	236	171	65	30	3	77.50	13.98	< 0.001	-81.957
Baoudjo	552	612	552	612	331	133	57	18	88.59	12.25	< 0.001	-86.16
Total	1714	1752	1714	1752	1171	287	140	21	82.38	9.19	< 0.001	-88.84

CTRL: Control

4. Discussion

The two main vectors responsible for arboviroses, *Aedes aegypti* and *Aedes albopictus*, are present in Benin [15, 16]. The most abundant, *Aedes aegypti*, is distributed throughout the national territory, while the second (*Aedes aegypti*) is found and widespread in a few municipalities in the south of Benin [15]. Furthermore, a recent study has shown that *Aedes aegypti* has developed resistance to pyrethroid insecticides commonly used for bed net impregnation [17]. Moreover, recent research has mentioned that not only are the arbovirus vectors abundant, but they are also carriers of the dengue virus in Benin [18]. In this worrisome situation, it is necessary to take the epidemic threat seriously and prepare for a response. The response or prevention of arbovirus epidemics involves vector control through the reduction of vector populations. This reduction can be achieved through chemical control using insecticides, including growth regulators. Mosquito oviposition traps treated with 0.4% S-methoprene (Mosquilarv) are an alternative. Our results have shown a high rate of positive oviposition traps for *Aedes* eggs regardless of the location. This result can be explained by the presence of a significant number of gravid mosquitoes in the area searching for a suitable oviposition site. These gravid mosquitoes may have bitten residents in the locations where the oviposition traps were placed. Indeed, female *Aedes aegypti* and *Aedes albopictus* require blood to bring their eggs to maturity. Being more anthropophilic, these vectors prefer to take blood from humans. Therefore, these gravid mosquitoes may have certainly taken their blood meal from unprotected individuals. Furthermore, our results report no significant difference in the proportions of eggs collected in the oviposition traps treated with Mosquilarv compared to untreated control traps. This result could be explained by the fact that Mosquilarv does not have a repellent effect but acts as a larvicide. It does not repel gravid mosquitoes but prevents larvae from the eggs laid by these mosquitoes from completing their larval development to emerge as a threat to the population.

At the emergence of larvae from the oviposition trap eggs, three species were morphologically identified. In order of abundance, they are *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus*. Similar results were obtained by Padonou *et al.* [15], who also found additional species such as *Aedes vittatus* and *Aedes anthocephalus* involved in sylvatic transmission of arboviruses. In the neighbourhoods of Porto-Novo and the Ifangni municipality, *Ae. aegypti* and *Ae. albopictus* shared the same oviposition traps and were classified as sympatric. These results are consistent with previous studies that reported in India that *Aedes* species reproduced in all available artificial containers filled with stagnant water [19]. In Central Africa, *Ae. aegypti* and *Ae. albopictus* often shared the same larval habitats, with *Ae. albopictus* preferring discarded tires while *Ae. aegypti* preferred domestic containers [20]. The associations of *Ae. aegypti*/*Cx. quinquefasciatus* and *Ae. albopictus*/*Cx. quinquefasciatus* confirm the anthropophilic nature of the three species and the coexistence of *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* in domestic environments as reported by Lopez-Solis *et al.* [21].

Our results revealed a considerable reduction in the emergence of *Aedes* larvae by Mosquilarv. This could be explained by the chemical composition of this product, with the bioactive molecule being methoprene, a growth regulator that has already proven to be a powerful larvicide against *Aedes aegypti* and *Aedes albopictus* larvae, like pyriproxyfen [22]. Growth regulators have always shown interesting larvicidal effects and significantly reduce the population of *Aedes* vectors [23]. Although our study confirmed the effectiveness of Mosquilarv as a potential future tool for combating the main arbovirus vectors, it was not conducted with positive controls such as pyriproxyfen, a powerful growth regulator whose efficacy has been confirmed by several studies [22]. Furthermore, it should be noted that community-based evaluation under natural conditions is still needed to truly confirm its effectiveness in practical vector control, considering other species of mosquitoes.

5. Conclusion

The results of this study have confirmed the anthropophilic and sympatric nature of the main dengue vector species, *Ae. aegypti* and *Ae. albopictus*. This situation poses a threat to the health of the populations in this region of Benin. Vector-borne disease control programs should strengthen entomological surveillance in this locality. The significant reduction in the emergence rate of *Aedes* spp. by Mosquilarv observed in the results of this study is valuable information for the response and control of dengue vectors during an epidemic. It is necessary to conduct a Phase 3 study with a larger spatial and temporal coverage to confirm, in a community setting, the effectiveness of 0.4% S-methoprene Mosquilarv in reducing the density of *Aedes* and other Culicidae mosquitoes in Benin.

6. Declaration of interest

The authors declare that they have no conflicts of interes

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