High efficacy of microbial larvicides for malaria vectors control in the city of Yaounde Cameroon: a cluster randomised study

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Abstract

The rapid expansion of insecticide resistance and outdoor malaria transmission are affecting the efficacy of current malaria control measures. In urban settings, where malaria transmission is focal and breeding habitats are few, fix and findable, the addition of anti-larval control measures could be efficient for malaria vector control. But field evidences for this approach remains scarce. Here we provide findings of a randomized-control larviciding trial conducted in the city of Yaoundé that support the efficacy of this approach.

A two arms random control trial design including 26 clusters of 2 to 4 km^2 each (13 clusters in the intervention area and 13 in the non-intervention area) was used to assess larviciding efficacy. The microbial larvicide VectoMax®G combining *Bacillus thuringiensis* var *israelensis* (Bti) and *Bacillus sphaericus* in a single granule was applied twice per month in all standing water collection points. The biting anopheline density collected using CDC light traps was used as the primary outcome, secondary outcomes included the entomological inoculation rate, breeding habitats with anopheline larvae, and larval density. Baseline entomological data collection was conducted for 17 months from March 2017 to July 2018 and the intervention lasted 26 months from September 2018 to November 2020.

The intervention was associated with a reduction of over 85% of habitats with anopheline larvae. The application of the larvicide also resulted in a reduction of 68% of adult anopheline biting density and of 79% of the entomological inoculation rate (OR 0.21; 95% CI 0.14–0.30, P < 0.0001). A reduction of 68.27% was recorded for indoor biting anophelines and 57.74% for outdoor biting anophelines. No impact on the composition of anopheline species was recorded. A reduction of over 35% of adult *Culex* biting densities was recorded. The study also assessed the impact of the microbial larvicide on non-target organisms and registered no significant impact of the larvicide VectoMax on the aquatic microfauna diversity.

The study indicated high efficacy of larviciding for reducing malaria transmission intensity in the city of Yaoundé. Larviciding could be part of an integrated control approach for controlling malaria vectors and other mosquito species in the urban environment.

Introduction

Africa's population almost doubled during the last two decades, from about 665 million in 2000 to 1.1 billion in 2019 [1]. This rapid demographic growth has resulted in a massive migration of the population from rural to urban areas. The rapid demographic changes in major sub-Saharan Africa cities which are also associated to large-scale unplanned urbanization including poor housing, poor drainage, inadequate waste management, multiplication of slums, have significantly influenced the epidemiology of vector-borne diseases such as malaria and arboviruses [2-4]. Malaria remains an important public health problem across the world affecting both rural and urban areas [5-8]. According to the latest world malaria report, 229 million malaria cases were reported in 2019 [9]. Twenty-nine countries account for 95% of malaria cases in the world and almost all are from sub-Saharan Africa [9]. Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are considered as the cornerstone for malaria prevention [10]. The large-scale deployment of these tools permitted to avoid about 1.5 billion malaria cases and 7.6 million malaria deaths between 2000 and 2019 [11]. Roughly 1.9 billion LLINs have been distributed in sub-Saharan Africa between 2004 and 2019 [9]. It is estimated that about 68% of

households in sub-Saharan Africa had at least one LLIN in 2019 this suggesting a terrific increase compared to 5% in 2000 [9]. However, control efforts are still affected by the rapid expansion of insecticide resistance. Almost all sub-Saharan countries have reported resistance to all four of the most commonly used insecticide classes [12,13]. Resistance to pyrethroids the compound used for impregnating bed nets is widespread [12,14,15]. Based on insecticide resistance monitoring data, many countries are now adopting new strategies to manage insecticide resistance and improve malaria control. These include switching to new control tools such as the deployment of pyrethroid-piperonyl butoxide (PBO) nets [16] or the combination of different control tools or interventions [17,18]. Larval source management (LSM) has proven in the past to be highly effective for lowering malaria transmission and even eliminating malaria vectors and disease transmission [19-21]. Historical literature reveals that the use of anti-larval mosquito control measures contributed to all successful eradication efforts [22,23]. In Egypt, the use of larviciding in the 1940s resulted in the elimination of malaria and the vector *An. arabiensis* from the region of Assouan [23]. In the Zambia, the implementation of an integrated malaria control program relying primarily on anti-larval control measures, contributed to the reduction by 97% of the annual malaria incidence from 514/1000 to 16/1000 between 1929 and 1950 [24]. Several studies reporting significant impact of larval control on malaria transmission or malaria morbidity have been registered across the continent [19,25,26]. However, despite these historical facts and new evidences on larviciding efficacy this intervention is still not largely implemented for malaria control in sub-Saharan Africa due to the limited number of unbiased studies on its efficacy or effectiveness [26,27]. The World Health Organization (WHO) issued an interim position on larviciding recommending its use in moderate to low transmission settings as a supplement to core interventions (LLINs and IRS) in areas where breeding habitats are few fixed and findable [10]. The intervention could be particularly indicated in urban settings or in highland areas where breeding habitats are less important and malaria transmission moderate. According to the WHO guidelines [28], LSM could be integrated into malaria control or general mosquito abatement programmes once transmission has been reduced to low or moderate levels after the use of LLINs or IRS, or once these interventions have reached their maximum practical impact.

In Cameroon malaria remains an important public health problem. Between 2015 and 2018, the incidence of malaria cases increased across the country highlighting the need to intensify malaria control efforts[29,30]. Treated nets are the main measure implemented by the government to prevent malaria attacks. So far there have been four distribution campaigns of treated nets to the population. It is estimated that about 80% of households own a bed net and that close to 60% use treated nets regularly [30]. Apart from LLINs which were introduced in the country in the 1990s, there have been two pilot larval control trials initiated in the country to control *Culex quinquefasciatus* populations. The first one conducted in the 1990s in the city of Maroua which consisted of two treatments per year of all breeding habitats with *Bacillus sphaericus* as larvicide had a limited impact on the biting densities of *Culex quinquefasciatus* mosquito populations [31]. The second pilot study conducted in Yaounde registered a 64% reduction of *Culex quinquefasciatus* biting densities. However, because the authors did not included any control cluster the interpretation of their findings was limited [32].

The city of Yaounde has a landscape with an alternation of both highland and lowland areas with over 90% of breeding sites located in lowland settings and could be an excellent environment to practice larviciding [33,34]. The population is approximately 3 million inhabitants and is characterised by a low malaria transmission pattern [35,36]. There have been so far not enough attempts to control malaria vectors using interventions suited

to the landscape and ecological situation of the environment. Generating evidences on the efficacy of larviciding in different epidemiological context could improve malaria control across Africa. In the course of the present study, a cluster randomised trial including 26 clusters of 2 to 4 km^2 each divided into 2 groups 13 in the intervention area and 13 in the non-intervention area was conducted to assess the impact of larviciding on malaria transmission in the city of Yaoundé. The study showed a reduction of 68% of adult anopheline densities and of 79% of the entomological inoculation rate.

Results

Household characteristics

Baseline community and entomological surveys were conducted from February 2017 to July 2018. Some data deriving from these studies have been published previously [37-40] [41-44]. Household characteristics were almost similar across the two study groups. Modern houses built up with cement (50% and 62.77%) and traditional houses constructed with mud, plank, and mix material (50% and 37.23%) were recorded. Most households (> 84%) owned at least a LLIN, 47% and 48% of households in the control and intervention area respectively had one LLIN for two people (Table 1). The majority of households had an average of 6 to 10 persons per household. Close to 20% of houses had screens on windows. The number of houses with ceilings was also similar between the two groups.

Table 1: House characteristics in the non-intervention and intervention areas

Characteristics	Factors	Non-intervention areas		Intervention areas	
		N houses	%	N houses	%
Type of house	Modern	94	50.00	118	62.77
	Traditional	94	50.00	70	37.23
Occupants	[1-5]	72	39.13	78	43.33
	[6-10]	89	48.37	88	48.89
	≥11	23	12.50	14	7.78
Holes on walls	No	116	63.74	134	72.04
	Yes	66	36.26	52	27.96
Eaves	No	65	36.52	78	43.58
	Yes	113	63.48	101	56.42
Ceiling	No	124	67.03	102	55.74
	Yes	61	32.97	81	44.26
Screened windows	No	158	85.87	144	80.00
	Yes	26	14.13	36	20.00
At least one LLIN	No	28	15.05	15	8.47
Per household	Yes	158	84.95	162	91.53
Use of LLINs	No	24	13.04	15	8.47
	Yes	160	86.96	162	91.53
owning one LLIN for 2 people	No	99	52.38	98	51.65
	Yes	90	47.62	91	48.35
Vegetation close to the house	No	41	21.69	37	19.58
	Yes	148	78.31	152	80.42
Breeding sites close to the house	No	32	16.93	35	18.52
	Yes	157	83.07	154	81.48

N houses=Number of houses selected for interview and house characterisation; % percentage of houses
LLINs=Long Lasting Insecticidal Nets

Monthly distribution of anopheline larvae

Anopheline larval abundance was seasonal with high density during the short and long rainy seasons. The annual rainfall estimates was 761.4 mm in 2017, 845.4 mm in 2018, 3011.3 mm in 2019 and 2726.2 mm in 2020. This pattern influenced breeding habitats availability and distribution in the city (Table 2).

The proportion of habitats found with early or late instar anopheles larvae at baseline was 13.32% (1150/8633) in intervention area and 18.66% (1551/8313) in non-intervention area. During the intervention period, only 0.80% of sites (1102/137120) were found with anopheline larvae after larviciding treatments whereas, in non-intervention areas, 7.52% of sites (1934/25729) were found with anopheline larvae. Taking into account the clustering by treatment group and by period, it appeared that larviciding treatment significantly reduced the chances of water bodies being colonised by anopheline larvae (OR= 0.15 95% CI = 0.07 – 0.32; P < 0.0001). The number of breeding habitats with late instar anopheline larvae was also reduced by over 73%. When was

considered the effect of larviciding treatments on culicine larvae, a significant reduction of breeding habitats with culicine larvae could also be noticed (OR = 0.37 95% CI = 0.32 – 0.42; P < 0.0001). High fluctuation in the monthly distribution of breeding habitats with anopheline larvae closely associated with the rainfall pattern was recorded (Figure 1).

Table 2: Distribution of anopheline and culicine larvae in breeding habitats at baseline and during the larviciding intervention

	Baseline		Intervention		Percent reduction*
	Non-intervention area	Intervention area	Non-intervention area	Intervention area	
Total of breeding sites Checked	8,313	8,633	25,729	137,120	
Total number of water bodies with anopheline larvae (%) (95%CI)	1551 (18.66%) (17.74 - 19.61)	1150 (13.32%) (12.56 - 14.11)	1934 (7.52%) (7.18 - 7.85)	1102 (0.80%) (0.76 - 0.85)	85.46%
Total number with late instar anopheline larvae (%) (95%CI)	1096 (70.66%) (66.54 - 74.97)	772 (67.13%) (62.48 - 72.04)	1155 (59.72%) (56.33 - 63.27)	168 (15.24%) (13.03 - 17.73)	73.13%
Total number of water bodies with culicine larvae (%) (95%CI)	1528 (18.38%) (17.47 - 19.33)	1773 (20.54%) (19.59 - 21.52)	2523 (9.80%) (9.42 - 10.19)	5538 (4.03%) (3.93 - 4.14)	69.24%

* Percent reduction = 100 – (Non LCI at baseline/LCI at baseline x LCI during intervention/non-LCI during intervention) x 100
(Non-larviciding intervention area (Non LCI), Larvicidng Intervention area (LCI))

Evolution of the frequency of breeding habitats with *Anopheles* larvae

In addition to crude data analysis, a mixed linear modelling approach was used to better assess the impact of larviciding treatments. A total of 1131 measurements in both the intervention and non-intervention areas were taken into consideration for the modelling analysis. Results confirmed a significant reduction (P < 0.001) of breeding habitats with anopheline larvae in the intervention area compared to the non-intervention area, though a significant decline in the proportion of breeding habitats with *Anopheles* larvae for both non-intervention and intervention areas was generally observed with time (Table 3). Different factors including season, flooding, agricultural activities were found associated with a significant impact on the OR of the model (P < .005).

Table 3: Mixed effects logistic regression models of the impact of larviciding on the distribution of Anopheline larvae, and influence of other parameters during baseline and intervention periods.

	Baseline		Intervention	
Parameter	OR (95% C.I.)	p-value	OR (95% C.I.)	p-value
Date (months)	0.9452 (0.9339, 0.9567)	<0.0001	0.9647 (0.9585, 0.9709)	<0.0001
Group (ref=Non-intervention)				
Intervention	1.1680 (0.6923, 1.9710)	0.5606	0.0211 (0.0129, 0.0345)	<0.0001
Season (ref=Dry)				
Rainy	Excluded		1.655 (1.444, 1.895)	<0.0001
Public works (ref=no)				
Yes	Excluded		0.8973 (0.5758, 1.398)	0.63225
Flooding (ref=no)				
Yes	0.3676 (0.2029, 0.6660)	0.0010	0.7372 (0.6125, 0.8873)	0.0013
Agricultural activities (ref=no)				
Yes	0.7125 (0.4050, 1.2530)	0.2395	0.4675 (0.2777, 0.7870)	0.0042

Ref: reference

Influence of physicochemical factors on anopheline larvae presence in breeding sites before and during intervention

The possible influence of some physicochemical factors on anopheline larvae distribution was checked at both baselines and during intervention to assess any effect of these factors on the density of anopheline in breeding sites before and during intervention. At the baseline, few parameters including sulphate (R^2 = +0.07 vs R^2 = -0.27), H_2O_2 (R^2 = -0.09 vs R^2 = +0.28) and nitrate (R^2 = -0.48 vs R^2 = +0.07) showed different correlation pattern with anopheline larvae density in breeding sites in non-intervention vs intervention areas (Table 4). During the intervention several compounds including TDS (R^2 = -0.33 vs R^2 = +0.22), organophosphates (R^2 = -0.33 vs R^2 = +0.18) and sulphate (R^2 = -0.51 vs R^2 = +0.02) were found to express different and high correlation pattern with anopheline larvae density in breeding sites in non-intervention vs intervention areas. Although differences recorded were not significant, these factors could be confounding factors and need further assessment.

Table 4: Influence of physico-chemical parameters on anopheline larvae density in breeding sites at baseline and during larviciding treatment in non-intervention and intervention areas

Baseline Parameters	Non-intervention areas				Intervention areas			
	N	Means ± SE	R^2	p-values	N	Means ± SE	R^2	p-values
pH	210	7.88±0.09	+0.15	0.03	230	7.95±0.1	+0.16	0.02
TDS (mg/l)	96	249.11±34.33	-0.02	0.87	99	185.94±17.44	-0.08	0.45
Conductivity (µs/cm)	235	427.43±19.9	+0.08	0.23	232	418.93±29.14	+0.02	0.7
Turbidity (FTU)	235	209.37±26.17	+0.21	0.001	220	285.7±109.28	+0.34	<0.001
Ammonia	71	0.53 ± 0.25	+0.05	0.54	68	0.37 ± 0.11	+0.022	0.8
Phosphate	92	0.41 ± 0.08	-0.12	0.15	98	0.6 ± 0.13	-0.056	0.54
Nitrate	101	1.71 ± 0.35	-0.48	<0.001	97	3.05 ± 0.61	+0.07	0.41
Calcium (mg/l)	70	513.58±364.37	-0.15	0.22	103	139.27±51.41	-0.07	0.5
Iron (mg/l)	114	0.67±0.09	-0.09	0.35	84	0.83±0.17	-0.19	0.09
Organophosphates (mg/l)	134	7.94±0.46	-0.06	0.46	126	15.04±4.67	-0.27	0.002
Aluminium (mg/l)	90	0.76±0.26	+0.05	0.61	110	0.56±0.13	+0.21	0.03
Sulphate (mg/l)	124	92.71±5.49	+0.07	0.44	91	81.82±4.58	-0.27	0.009
H_2O_2 (mg/l)	104	3.78±1.04	-0.09	0.36	84	9.48±2.39	+0.28	0.01
Temperature (°C)	235	28.03±0.18	+0.19	0.003	232	27.3±0.17	+0.13	0.05

Intervention Parameters	Non-intervention areas				Intervention areas			
	N	Means ± SE	R^2	p-values	N	Means ± SE	R^2	p-values
pH	51	7.45 ± 0.14	+0.38	0.04	39	7.84 ± 0.2	+0.09	0.56
TDS (mg/l)	51	627.28 ± 52.23	-0.33	0.08	39	561.18 ± 72.59	+0.22	0.18
Conductivity (µs/cm)	51	-50.47 ± 24.38	-0.1	0.62	39	-56.13 ± 15.21	+0.08	0.62
Turbidity (FTU)	64	255.42 ± 70.31	+0.16	0.34	50	302.4 ± 156.21	+0.28	0.06
Ammonia (mg/l)	64	3.54 ± 2.77	-0.04	0.8	52	0.59 ± 0.15	-0.035	0.81
Nitrate (mg/l)	64	1.92 ± 0.63	+0.11	0.54	52	9.8 ± 6.16	-0.21	0.14
Phosphates (mg/l)	64	0.4 ± 0.1	-0.06	0.72	52	0.36 ± 0.06	+0.02	0.87
Organophosphates (mg/l)	64	2.51 ± 1.16	-0.33	0.05	52	2.47 ± 2.16	+0.18	0.21
Sulphate (mg/l)	64	33.84 ± 5.65	-0.51	0.001	52	30.73 ± 5.07	+0.02	0.88
H_2O_2 (mg/l)	63	9.94 ± 1.66	+0.45	0.008	52	8.71 ± 1.1	+0.26	0.07
Temperature (°C)	51	28.49 ± 0.64	-0.26	0.17	39	26.47 ± 0.54	-0.006	0.97

N= number of breeding sites sampled and containing anopheline larvae; Mean = average concentration of the parameter in breeding sites with anopheline larvae ; SE: Standard error; R^2= correlation coefficient between anopheline larval density and physico-chemical factor concentration, TDS: Total Dissolved Solids
H_2O_2: hydrogen peroxide

Adult mosquito abundance

A total of 6,664 anophelines were collected in the course of the study. Species collected included *An. gambiae s.l., An. funestus* and *An. ziemanni* (Table 5). A subsample of 2762 *An. gambiae* s.l., was processed by PCR and both *An. coluzzii* (88.42%) and *An. gambiae* (11.58%) were recorded. Within the *An. funestus* group, out of 299 mosquitoes processed, 280 (93.65%) were *An. funestus* s.s., and 19 (6.35%) were *An. leesoni*. In almost all districts *An. coluzzii* was the predominant species; followed by *An. gambiae*. No significant variation in the composition of *An. gambiae* and *An. coluzzii* before and during the intervention was recorded in both the intervention and non-intervention areas (P>0.20) (Figure 2). *An. funestus* was recorded in few sites and was particularly abundant in the site of Mendong located close to the periphery with large swamps.

Table 5: Composition of anopheline mosquito fauna in Yaounde

	Pre-intervention		Intervention		
	Non LCI N(%)	LCI N(%)	Non LCI N(%)	LCI N(%)	Total (%)
An. funestus s.l.	152 (7.10)	462 (18.65)	72 (4.75)	131 (24.72)	817 (12.26)
An. gambiae s.l.	1976 (92.29)	1998 (80.66)	1422 (93.80)	392 (73.96)	5788 (86.8)
An. ziemanni	13 (0.61)	17 (0.69)	22 (1.45)	7 (1.32)	59 (0.89)
Total	2141 (34.98)	2477 (32.35)	1516 (24.21)	530 (8.46)	6664

Non-larviciding intervention area (Non LCI), Larviciding Intervention area (LCI)

Adult vector density was higher at baseline than for subsequent years throughout intervention in both the intervention and non-intervention areas (Figure 3). After launching the intervention, a steady decrease in vector density was recorded in the intervention area. The average density of anopheline collected in the non-intervention clusters varied from 0.42 anopheline/trap/night at baseline to 0.23 anopheline/trap/night during the intervention. In the intervention clusters the average density of anopheline collected by CDC light traps varied from 0.47 anopheline/trap/night at baseline to 0.082 anopheline/trap/night during intervention. Larviciding was associated with 68% reduction of adult anopheline biting density. The density of mosquitoes collected indoor and outdoor in control and intervention area also varied significantly. The highest reduction was recorded with mosquitoes biting indoor 68.27% vs 57.74% outdoor (Table 6). When was compared the impact of the intervention on *An. gambiae* s.l., and *An. funestus* the two main vectors species in Yaounde, it appeared that *An. gambiae* s.l., biting density was reduced by over 71% whereas *An. funestus* density was reduced by 40% (Table 7).

Table 6: Crude entomological estimates of mosquito density and malaria transmission in Yaounde during the larviciding control trial

Parameters	Baseline		Intervention		Percent reduction*
	Non-intervention area (95% CI)	Intervention area (95% CI)	Non-intervention area (95% CI)	Intervention area (95% CI)	
Mean number of Anopheles per trap per night 95%CI	0.42 (0.40-0.44)	0.47 (0.45-0.49)	0.23 (0.22-0.25)	0.082(0.075-0.09)	68.14
• Indoor	0.62 (0.59-0.65)	0.67 (0.64-0.70)	0.35 (0.33-0.37)	0.12 (0.11-0.13)	68.27
• Outdoor	0.12 (0.11-0.134)	0.12 (0.10-0.13)	0.071(0.06-0.08)	0.03 (0.02-0.03)	57.74
Mean number of Culicine per trap per night 95% CI	15.67 (15.56-15.77)	17.20 (17.09-17.31)	10.89 (10.82-10.98)	7.58 (7.51-7.64)	36.59
• Indoor	20.82 (20.66-20.98)	22.04 (21.89-22.20)	15.7 (15.57-15.83)	10.76 (10.66-10.9)	35.26
• Outdoor	7.91 (7.79-8.03)	8.40 (8.27-8.54)	4.33 (4.25-4.41)	2.65 (2.59-2.71)	42.37
Average Infection rate (%) Mean number of infected anopheles 95% CI	2.24 (1.60-3.00)	2.49 (1.86-3.25)	1.05 (0.57-1.76)	0.75 (0.20-1.93)	35.74
• Indoor	2.45 (1.7-3.4)	2.52 (1.9-3.3)	1.10 (0.58-1.9)	0.65 (0.13-1.9)	42.55
• Outdoor	0.88 (0.17-3.1)	2.11 (0.57-5.39)	0.67 (0.017-3.8)	1.47 (0.04-8.2)	8.49
[a]**Annual entomological inoculation rate** Mean number of infectious bites per person per year 95% CI	5.50 (3.75-7.86)	6.83 (4.89-9.30)	1.41 (0.74-2.57)	0.36 (0.09-1.02)	79.53
• Indoor	8.87 (5.97-12.76)	9.86 (6.97-13.63)	2.25 (1.13-4.05)	0.45 (0.08-1.44)	81.77
• Outdoor	0.62 (0.068-2.48)	1.48 (0.34-4.09)	0.28 (0.01-1.78)	0.26 (0.01-1.63)	61.34

* Percent reduction = 100 – (Non LCI at baseline/LCI at baseline x LCI during intervention/non-LCI during intervention) x 100
Non-larviciding intervention area (Non LCI), Larvicidng Intervention area (LCI)

[a] estimated biting rate*365 days per year*mean sporozoite prevalence*1.6

Table 7: Impact of larviciding treatments on biting *An. gambiae* s.l. and *An. funestus* densities in Yaounde

Species	Baseline		Intervention		Percentage reduction*
	Non-intervention area (95% CI)	Intervention area (95% CI)	Non-intervention area (95% CI)	Intervention area (95% CI)	
An. gambiae s.l.	0.39 (0.37-0.40)	0.38 (0.36-0.40)	0.22 (0.21-0.23)	0.061 (0.05-0.068)	71.54
An. funestus s.l.	0.029 (0.025-0.034)	0.088 (0.08-0.096)	0.011(0.009-0.014)	0.020 (0.017-0.024)	40.08

* Percent reduction = 100 – (Non LCI at baseline/LCI at baseline x LCI during intervention/non-LCI during intervention) x 100 ; Non-larviciding intervention area (Non LCI), Larvicidng Intervention area (LCI)

Crude analysis of infection rate estimate showed a significant reduction of the infection rate when binary logistic regression adjusting for years and group was applied (OR = 0.29; 95% CI = 0.10 – 0.80, P = 0.017). The entomological inoculation rate varied in the non-intervention area from 5.50 infected bites/person/year at the baseline to 1.41 infected bites/person/year during intervention. In the intervention area, the entomological inoculation rate dropped from 6.83 infected bites/person/year at the baseline to 0.36 infected bites/person/year during intervention. This accounted for 79% (OR 0.21; 95% CI 0.14 – 0.30, P<0.0001) reduction of EIR.

Modeling the effect of larviciding intervention using general estimating equations after adjusting for clusters, baseline data and months it appeared that at baseline, vector density and EIR in intervention and non-intervention area were readily comparable while the implementation of larviciding treatment significantly reduced the risk of being bitten by anopheline (P<0.05), all this during the entire intervention period (beginning (2018), midway (2019) and end of the study (2020)) (Table 8).

Table 8: Impact of larviciding on biting anopheline densities and malaria transmission intensity estimated using generalized estimating equations

	Parameters	Estimated means (95% CI)	P-value
Baseline	Mar 2017 - July 2018	0.1576 (-0.08759, 0.4027)	0.1898
Intervention	Sep-Dec 2018	0.09859 (0.05815, 0.1388)	0.0203
	2019	0.08299 (0.05436, 0.1116)	0.0006
	2020	0.06197 (0.009455, 0.1145)	0.0065
	EIR (total)	0.0009444 (1.795e-05, 0.001871)	0.038

Influence of house characteristics on mosquito distribution in the intervention and non-intervention areas at baseline and during the intervention

In the course of the study, various parameters enabling or preventing mosquitoes of getting into houses were assessed to check how they were affected by larviciding intervention. At baseline apart of the type of house (RR = 1.22 ± 0.19; P = 0.006) and the presence of holes in the wall (RR = 0.77 ± 0.14; P = 0.003), and the absence of breeding sites near houses (RR = 0.78 ± 0.2 ; P=0.04) the risk of being bitten by mosquitoes was similar between houses in the intervention and the non-intervention areas. During the intervention, almost all parameters measured were associated with a significant risk of being bitten by mosquitoes in the non-intervention compared to the larviciding intervention area (Table 9). The risk of being exposed to mosquito bites was always twice higher in the non-intervention compare to the intervention (RR (range) = 1.58 – 2.98; P< .001). Parameters of the house (holes on the wall, absence of ceiling, absence of screens on windows) known to increase exposure to mosquito bites were found as contributing to a less important exposure risk in intervention area compared to non-intervention area.

Table 9: Effect of house characteristics on anophelines distribution before and during intervention in non-intervention and intervention areas

Mean = average number of mosquitoes collected per trap per night
Non larviciding intervention area (Non LCI), Larviciding Intervention area (LCI), RR = Relative Risk of being exposed to anopheline bites between non-intervention and intervention areas for each parameter before and during the intervention
95% CI = 95% Confidence Interval
LLINs=Long Lasting Insecticidal Nets

Evolution of *An. gambiae* s.l. susceptibility to pyrethroids and DDT

Studies conducted indicated a slight increase in the susceptibility status of *An. gambiae* females to both permethrin (mortality rates 34.16% at beginning to 39.26% at the end of the intervention) and deltamethrin (mortality rates 35.19% at beginning to 44.20% at the end of the intervention) during the trial (Table 10).

Table 10: Evolution of *An. gambiae* sl susceptibility to permethrin, deltamethrin and DDT during preintervention and intervention period in the city of Yaounde.

	Mortality rates/1014F allele frequency			
	Pre-intervention		Intervention	
	2017	2018	2019	2020
Permethrin 0.75%	34.16% (166/486)	7.71% (34/441)	2.07% (10/484)	39.26% (106/270)
Deltamethrin 0.05%	35.19% (411/1168)	12.30% (199/1618)	22.40% (97/433)	44.20% (80/181)
DDT 4%	1.33% (2/150)	3.05% (35/1146)	1.46% (5/342)	2% (4/200)
1014F Kdr allele frequency	60%	75%	67%	73%

	Baseline				Intervention			
	Mean ± 95% CI				Mean ± 95% CI			
Characteristics	Non LCI	LCI	RR ± 95%CI	P value	Non LCI	LCI	RR ± 95%CI	P value
Type of house								
Modern	0.15± 0.01	0.22 ± 0.01	0.75 ± 0.12	0.001	0.09 ± 0.008	0.04 ± 0.005	1.58 ± 0.38	< 0.001
Traditional	0.27 ± 0.02	0.23 ± 0.02	1.22 ± 0.19	0.006	0.11 ± 0.009	0.03 ± 0.005	2.98 ± 0.73	< 0.001
Occupants per house								
> 5	0.20 ± 0.01	0.22 ± 0.01	0.97 ± 0.13	0.62	0.09 ± 0.008	0.04 ± 0.005	1.88 ± 0.38	< 0.001
≤ 5	0.23 ± 0.02	0.24 ± 0.03	1.01 ± 0.16	0.95	0.12 ± 0.01	0.03 ± 0.005	2.66 ± 0.67	< 0.001
Holes on walls								
No	0.23 ± 0.01	0.20 ± 0.01	1.07 ± 013	0.24	0.10 ± 0.008	0.029 ± 0.004	2.33 ± 0.48	< 0.001
Yes	0.18 ± 0.01	0.28 ± 0.02	0.77 ± 0.14	0.003	0.11 ± 0.01	0.058 ± 0.008	1.86 ± 0.47	< 0.001
Eaves status								
Closed	0.20 ± 0.02	0.21 ± 0.01	0.96 ± 0.17	0.59	0.09 ± 0.009	0.04 ± 0.005	1.74 ± 0.41	< 0.001
Opened	0.22 ± 0.01	0.25 ± 0.01	0.94 ± 0.13	0.36	0.11 ± 0.008	0.01 ± 0.006	2.40 0,52	< 0.001
Ceiling								
No	0.23 ± 0.01	0.29 ± 0.02	0.90 ± 0.12	0.08	0.12 ± 0.008	0.04 ± 0.006	2.13 ± 0.41	< 0.001
Yes	0.17 ± 0.01	0.16 ± 0.01	0.98 ± 0.19	0.79	0.07 ± 0.009	0.03 ± 0.006	1.85 ± 0.52	< 0.001
Screens on windows								
No	0.21 ± 0.01	0.23 ± 0.01	0.96 ± 0.11	0.48	0.11 ± 0.007	0.04 ± 0.004	2.15 ± 0.37	< 0.001
Yes	0.20 ± 0.03	0.18 ± 0.02	1.06 ± 0.32	0.67	0.10 ± 0.015	0.03 ± 0.007	2.17 ± 0.82	< 0.001
Use of LLINs								
No	0.15 ± 0.02	0.084 ± 0.01	1.17 ± 0.44	0.35	0.05 ± 0.01	0.05 ± 0.015	1.22 ± 0.7	0.40
Yes	0.14 ± 0.01	0.08 ± 0.006	0.96 ± 0.11	0.49	0.11 ± 0.006	0.04 ± 0.003	1.98 ± 0.33	< 0.001
Vegetation close to the house								
No	0.19 ± 0.02	0.26 ± 0.02	0.91 ± 0.24	0.43	0.09 ± 0.01	0.04 ± 0.007	2.38 ± 0.82	< 0.001
Yes	0.21 ± 0.01	0.22 ± 0.01	1.01 ± 0.11	0.90	0.11 ± 0.007	0.04 ± 0.004	2.17 ± 0.39	< 0.001
Breeding sites less than 10m from the house								
No	0.19 ± 0.02	0.22 ± 0.02	0.78 ± 0.2	0.04	0.08 ± 0.01	0.03 ± 0.008	2.09 ± 0.84	< 0.001
Yes	0.22 ± 0.01	0.20 ± 0.01	1.03 ± 0.12	0.59	0.11 ± 0.002	0.04 ± 0.004	2.27 ± 0.39	< 0.001
Number of Bedrooms								
5	0.22 ±	0.22 ±	0.99 ±	0.97	0.10 ±	0.04 ±	1.86 ± 0.36	<

	0.01	0.01	0.12		0.008	0.005		0.001
≥ 5	0.16 ± 0.03	0.14 ± 0.031	1.02 ± 0.45	0.92	0.07 ± 0.01	0.003 ± 0.04	2.33 ± 1.76	0.003
Number of Windows								
5	0.22 ± 0.01	0.21 ± 0.01	1.09 ± 0.14	0.19	0.11 ± 0.008	0.04 ± 0.004	2.22 ± 0.42	0.001
≥ 5	0.18 ± 0.02	0.25 ± 0.02	0.84 ± 0.16	0.05	0.11 ± 0.01	0.04 ± 0.007	2.08 ± 0.7	0.001
Number of Doors								
5	0.22 ± 0.01	0.21 ± 0.01	1.03 ± 0.11	0.57	0.11 ± 0.007	0.04 ± 0.004	2.25 ± 0.35	0.001
≥ 5	0.12 ± 0.02	0.13 ± 0.14	0.62 ± 0.86	0.28	0.06 ± 0.02	0.05 ± 0.02	1.52 ± 1.45	0.22

Effect of larviciding with VectoMax G on non-target organisms

The distribution and diversity of copepods, rotifers, ostracods and cladocerans in 290 sentinel breeding habitats were followed in both intervention and non-intervention areas to assess the influence of regular treatment with VectoMax on these non-target communities. A total of 44 species were recorded. The intervention area recorded the highest number of species (38/44 species), whereas only 17 species (17/44) were recorded in the non-intervention area. Cladocerans and Rotifers appeared as the most important groups. Species such as *Rotaria rotatoria*, *Brachionus Patulus*, *Moina micrura* and *Moina macrocopa* were abundant in non-intervention areas whereas, *Moina micrura*, *Moina macrocopa* and *Copepodes* spp. were predominant in the intervention area (Table 11). The study indicated no effect of larviciding treatments on non-target species diversity.

Table 11: Influence of larviciding treatments on non-target organisms

Groups	Species	Non-intervention areas (n=145)	intervention areas (n=145)
Copepods	*Cyclopidae* spp.	+++	++
	Copepodes spp.	+++	+++
	Calanoide spp.	+++	+
Rotifers	*Rotaria rotatoria*	++++	++
	Brachionus patulus patulus	++++	++
	Notholca salina	+	-
	Notholca striava	-	-
	Kurzia media	+	+
	Lepadella quadricarinata	+	++
	Lophocharis salpina	-	-
	Lecane physalis	-	+/-
	Platyias quadricormis	-	+
	Keratela spp.	-	+
	Lecane clara	-	+/-
	Brachionus bidentata		+/-
	Brachionus ferficala	-	+
	Frola zaralli	-	+
	Brachionus budapestinensis	+	-
	Trichocerca diurella	-	++
	Colurella geophila geophila	+/-	++
Cladocerans	*Alona protzi*	-	+/-
	Alona weltneri	+/-	+/-
	Alona guttata	-	+/-
	Alona quadrangularis	-	+/-
	Alonella exugua	-	+
	Daphnia similis	-	+
	Pleuroxus inermis	-	+/-
	Acroperus harpae	-	+
	Simocephalus exspinosus	-	+/-
	Oxyurella terulcaudia	-	+
	Sida crystallina	-	+/-
	Chydorus ovalis	-	+/-
	Disparalona rostrata	-	+/-
	Chydorus piger	-	+
	Oxyurella terulcaudia	-	+
	Alona rectangula	-	+
	Diaphranosoma brachyunum	++	+/-
	Blapertura affinis	+++	-
	Camphocercus rectirostris	-	+
	Ceriodaphnia rotunda	-	+
	Moina micrura	++++	++++
	Moina macrocopa	++++	++++
	Cyprus spp.	++	+
Ostracods	*Ostracod* spp.	+/-	-

Absent = - ; x < 1% = +/- ; 1% < x < 5% = + ; 5% < x < 25% = ++ ; 25% < x < 50% = +++ ; x> 50% ++++ ; x = density ; n= number of prospected breeding sites

Discussion

This study's main objective was to assess the impact of larviciding on biting anopheline densities and malaria transmission intensity in the city of Yaounde. The present study used entomological outcomes as primary endpoint rather than epidemiological outcomes because of limited financial means. In Yaounde, over 90% of households own at least a net and over 70% of the population report using net regularly [38]. A high reduction of vector density and malaria transmission intensity was recorded with over 68% reduction of Anopheline biting densities and 79% reduction of entomological inoculation rate. These figures are consistent with previous studies conducted across the continent supporting the high impact of antilarval measures on both entomological and epidemiological indicators [21,25,26,45]. The fact that a high number of clusters (including intervention and non-intervention areas) were used and monitored before and during the intervention, mosquito collection was undertaken using the Center for Disease Control Light trap (CDC LT) and the use of different teams involved in the treatment and the monitoring of field sites as recommended by WHO [20,46], permitted to minimise the inclusion of bias (performance bias, selection bias, low sample size …) and further strengthen the quality of evidences deriving from the study. Well-conducted vector control field trials are essential to inform policy making and for evidence-based decision-making [46]. Important reduction of both indoor and outdoor biting anopheline densities was recorded confirming larviciding as a promising tool for controlling outdoor malaria transmission in urban settings.

During the study, continual application of larvicide was conducted rather than seasonal (during the rainy season) as done elsewhere [25]. This regular application of the larvicide led to a high reduction of breeding habitats with anopheline larvae, the density of anopheline larvae and late instar stages. These figures are consistent with previous findings [47,48]. Although studies conducted so far in Yaoundé suggested seasonal malaria transmission pattern [35,37,39], it is possible that transmission could be occurring at an undetectable rate in some period of the year due to the permanent presence of *An. gambiae* sl in the city and gametocyte carriers. This observation supports regular application of larvicide all year long at least during the first years of the intervention. Analysis of the landscape of the city of Yaounde and transmission risk pattern also indicated a heterogeneous malaria risk with some districts more affected than others [37,40] and is in favor of emphasizing larviciding interventions. *An. funestus* was less intensely affected by the intervention compared to *An. gambiae* sl and could derive from the fact that *An. funestus* breed in water bodies covered by emerging vegetation which could reduce the quantity of larvicide granules getting to water surface and available for larvae whereas, *An. gambiae* s.l. is mainly found in water bodies without vegetation [49]. Limited impact of larviciding due to vegetation cover was reported in previous studies [50].

Several physico-chemical parameters were monitored in the course of the study to assess their influence on mosquito distribution or larviciding treatments efficacy. Some of them including organophosphate, sulphate, conductivity and TDS were found to display different correlation patterns with larval density in intervention compared to non-intervention areas and could translate possible interaction with the larvicide. The possible influence of physico-chemical parameters on microbial larvicide efficacy deserves further assessment.

The composition of the anopheline fauna (particularly *An. gambiae* and *An. coluzzii*) did not change significantly in the intervention and non-intervention areas before and during the intervention, which could suggest similar susceptibility status to larvicide of the two species as earlier suggested for insecticides [51]. Yet, studies conducted so far also indicated different insecticide resistance mechanisms in both *An. gambiae* and *An. coluzzii* in the city of Yaounde [42]. Insecticide resistance is largely spread across Yaounde [52,53] [54] but this seems to have had no impact on the effectiveness of larviciding treatments, since high reduction in anopheline density was recorded. A recent study in the city of Yaoundé indicated longer larval development time for resistant mosquitoes compare to susceptible [44]. This specific characteristic could increase the exposure of resistant mosquitoes to larvicide and increase mortality rate among insecticide resistant larvae. The following further supports the additional benefit of larviciding which could act as a complementary tool for insecticide resistance management [55]. Anti-larval measures could induce a reversal of resistance to pyrethroids and extend the efficacy of pyrethroid LLINs [56]. Microbial larvicides are also known to be highly efficient, specific and safe to use [20]. Moreover, the risk that resistance could emerge is very low due to the complex mode of action of these larvicides particularly *Bacillus thuringiensis* [20]. Following up the susceptibility profile of anopheline mosquitoes suggested no significant evolution of pyrethroid resistance and kdr alleles. However at this stage, it is not clear whether this pattern could be associated to the implementation of larviciding activities or reflect seasonal or temporal variations in Yaounde.

A moderate reduction of adult *Culex* species biting density was recorded. The limited impact of larviciding treatments on this species could be due to the fact that these mosquitoes breed in different types of habitats such as pit latrines, which were not targeted during larviciding treatments. It may also be possible that the impact of larviciding treatments in drains which are also preferential breeding habitats for *Culex* could have been limited due to the presence of solid wastes and many hiding places which could have limited the distribution of larvicide in the water [40]. *Culex* mosquitoes in Yaounde have also been reported to display a high resistance profile [41,57].

As for houses, various factors allow mosquitoes to easily get in, including holes in walls, presence of opened eaves or absence of ceiling, which were proven to have a limited influence on indoor biting mosquito's density during intervention, compared to the baseline period in intervention areas. Also, factors preventing mosquitoes from entering houses, such as presence of screens on windows or use of LLINs were found to induce better protection in areas where larviciding intervention was implemented compared to non-intervention areas. Better housing has always been regarded as a factor that could improve protection against mosquito bites in urban settings [43,58-60].

The impact of the use of the microbial larvicide VectoMax on non-target organisms was also monitored and no significant impact on the non-target microfauna (*Cladocerans, Rotifers, Ostracods* and *Copepods*) was recorded. A high diversity of the microfauna was instead recorded in intervention areas. The larvicide may be integrated in the food chain of some of these microorganisms. Since the study was limited to its effect on microfauna further studies are needed to assess the effect of this larvicide on aquatic macrofauna.

This study had some limitations. (i) Due to limited financial resources, the study mainly focused on entomological outcomes as primary endpoints rather than epidemiological outcomes as generally done. However, it provided a proof of concept that larviciding could be a suitable measure for reducing malaria

transmission intensity in Yaounde. (ii) The study did not assess the impact of the intervention on epidemiological outcomes. As such, further studies should urgently assess the impact of larviciding on epidemiological outcomes such as malaria incidence and parasite prevalence for evidence-based decision making. (iii) The study relied on self-report assessment to measure LLIN coverage and use. This could have biased the interpretation of the added effect of larviciding on LLINs. (iv) The study did not assess the cost-effectiveness of larviciding which is very important for policymakers.

Conclusion

This study sets out to advocate the fact that the use of larviciding as a complement to LLINs could be a viable solution for controlling malaria transmission in Yaounde, in a context of rapid expansion of insecticide resistance and outdoor malaria transmission. The study provided strong evidence supporting the use of larviciding as a main intervention in urban settings. Results obtained should be considered by national control programmes and local Government to implement tailored control approach to improve the fight against vector-borne diseases in urban settings. Further studies should be carried out to assess the impact of larviciding on epidemiological outcomes in Yaounde, the cost-effectiveness of larviciding with microbial larvicide and ways to involve community in vector control activities to ensure the sustainability of such interventions.

Materials And Methods

Study area

The study was conducted in Yaoundé the capital of Cameroon (3° 52' 12 N; 11° 31' 12 E). Yaounde is located 726 meters above sea level and receives up to 1700 mm of rainfall annually. It displays an equatorial climate with two rainy seasons extending from March to June and from September to November lasting 7 to 8 months. Despite its geographical location in the equatorial forest domain, the extension of settlements has significantly reduced the forest cover mainly found in nearby rural areas. The city extends 20 km wide and about 25 km long. Yaounde landscape comprises highlands and lowlands areas crossed by several rivers. Lowland areas are exploited during the dry season for agriculture. Houses are built on both hill slopes and in lowlands. Main rivers crossing the city include rivers Mfoundi, Ekozoa, Biyeme and Mefou.

Study design and larviciding activities

The primary objective of the trial was to assess the effect of larviciding on anopheline mosquito densities and malaria transmission rate in Yaounde. A cluster randomised trial was conducted in twenty-six districts referred to as clusters. Thirteen clusters served as control whereas the thirteen remaining were the intervention areas. Each cluster was an area of 2 to 4 km^2 crossed by a river system encompassing both lowland and highland areas. The lowland part for the majority of clusters was sparse and exploited for agriculture or with human constructions. The evaluation zone was situated at the center of each cluster always in the lowland area. Clusters were separated from one another by a distance of 500m to 1 km to minimize mosquito spillover from non-intervention to interventionsites. Baseline entomological data were collected from all clusters for 17 months, from March 2017 to July 2018. After this period microbial larvicide was applied in 13 clusters for 27 months (September 2018 to November 2020) (Figure 4). Adult biting densities collected using CDC light traps were used as the primary outcome. At the baseline, it was noticed that >90% of households owned at least one

LLIN, but only 58.5% had one LLIN for two people as requested by the WHO [30]. At the end of the baseline sampling period, all clusters were ranked according to adult anopheline biting density. Clusters with similar biting density were grouped into pairs and from each pair, one cluster was randomly selected as the intervention site and the other as control using a computer-assisted programme.

In intervention clusters, all water collection points were treated. It was assumed that when larvicide was applied to the entire cluster, the buffer zone and the fact that the evaluation was conducted at the centre of the cluster, could reduce mosquito spillover from non-intervention sites to intervention areas. Treatments were conducted using the larvicide VectoMax®G (Valent Biosciences Corporation, USA) a granule formulation (CG) containing as active ingredients both *Bacillus thuringiensis israelensis* (Bti), strain AM65-52 (45g/kg) and *Bacillus sphaericus* (Bsph) strain ABTS-1743 (27g/kg). VectoMax contains 50Bs international toxic units per mg of the product. According to WHO recommendations [61] this larvicide should be used at the dosage of 500 to 1500mg/m^2 in open water bodies (pools, temporary puddles and artificial containers) with an effect lasting for 2 to 3 weeks. During the trial breeding sites were treated once every two weeks by hand application of the larvicide. Field applicators were recruited from local communities. They were supervised during each field trip by one field supervisor in each zone and trained for one month before starting larviciding activities. Application of larvicide was conducted early in the morning between 7 and 11 AM to avoid the hottest time of the day. Teams of three to four male adult applicators conducted the application of larvicide across each cluster.

Endpoints

To assess the impact of the LSM intervention we used as primary outcome adult anopheline biting density collected using CDC light traps. Secondary outcomes included the entomological inoculation rate, the infection rate, the presence of anopheline larvae in breeding habitats and larval density.

Larval vector abundance

During the study, all breeding habitats were identified and characterised. Their size, physico-chemical characteristics and the presence or absence of anopheline and culicine larvae were recorded every month.

During the intervention, water collection points were checked every week in the intervention area to find out the number of habitats containing early and late instar larvae, to determine the effectiveness of larvicide application. Surveillance of treated breeding sites was conducted 48 hours after the treatment by a team of two people (different from those who undertook the treatment) who visited at least 50% of the treated area and all breeding habitats found with larvae were retreated. Checking larvae in breeding sites was also conducted in non-intervention sites once every month to capture the progression of mosquitoes in these sites. All water bodies encountered were geo-located using a Garmin eTrex® GPS and recorded in a GIS database for analysis.

Water bodies were analysed to check the presence of mosquito larvae. The immature stages of mosquitoes were collected using standard dipping technique [62]. Using a 350 ml deeper, three to five dips were performed for small breeding sites of less than 1 m^2; and five to ten dips for breeding sites of more than 1 m^2. For some habitats such as tyres or footprints which could be too shallow during certain periods, larval collection was conducted using a pipette. The average larval density (N) was estimated by calculating the ratio of the number of larvae collected per dip (using a dipper with a volume of 350 ml). Once collected larvae were classified

according to their stages: early instars larvae (L1 and L2), late instars (L3, L4) and pupae. Anopheline larvae were separated from the culicines using morphologically identification keys [63,64]. Each anopheline larvae specimen was stored individually at −20◦C.

Physico-chemical characterization of breeding sites

Parameters recorded in each breeding site included habitats type, size, depth, exposure to sunlight, presence/absence of vegetation, distance between each water point and the nearest human dwellings, the presence/absence of predators, organic pollution status, proportion of water surface covered by vegetation or algae, breeding sites type (stagnant water pools, gutters, well, tire print, footprint, pit latrine….). In addition to these, the following parameters were also recorded: Total Dissolved Solids (TDS), pH, temperature, conductivity using a Jenway multiparametric probe. The concentrations of sulphates, organophosphates, hydrogen peroxide (H_2O_2), turbidity, iron and calcium were analyzed using a Wagtech spectrophotometer [65].

Adult mosquitoes sampling

Adult mosquitoes were sampled using the Centre for Disease Control and Prevention Light Traps (CDC-LTs) both indoor and outdoor. Collections were performed once every two months from March 2017 to November 2020. CDC- LTs were placed indoor and outdoor in 10 homes per district. Houses were located 50 to 100 m away from each other. Collections were undertaken from 7 pm to 6 am during 3 consecutive days per district per month in each district.

Mosquito processing

Once collected, anophelines were separated from culicines using morphological identification keys of Edwards et al. [66]. Anopheline species were identified using morphological identification keys of Gillies and De Meillon [67] and Gillies and Coetzee [68]. Mosquitoes belonging to the *Anopheles gambiae* complex were further processed by PCR [69] to identify between *An. coluzzii* and *An. gambiae* the two members of the complex found in Yaoundé. Molecular identification of members of *Anopheles funestus* group was conducted according to Koekemoer et al. [70]. DNA extracted from wings and legs or the whole larvae according to Livak method [71] was used for analysis. Each anopheline specimen was stored individually in a numbered Eppendorf tube containing desiccant, archived and kept in the freezer at −20 °C. Heads and thoraxes of female anophelines were tested to check the presence of circumsporozoite protein (CSP) of *Plasmodium falciparum* by ELISA, as described by Wirtz et al. [72] or using TaqMan method [73].

Insecticide bioassay

Adult females *An. gambiae* s.l. reared from larval collections in different collection sites were tested using three insecticides (deltamethrin 0.05%, permethrin 0.75% and DDT 4%) following WHO guidelines [74]. *An. gambiae* s.l. females aged 3-4 days reared from larvae collected on site were placed in batches of 20 to 25 mosquitoes per tube. The mosquitoes were then transferred into tubes with insecticide-impregnated papers and exposed for 1 hour. The insecticide susceptible strains *An. gambiae* s.l. Kisumu and Ngousso strains were used as control to assess the quality of impregnated papers. The number of mosquitoes knocked down by the insecticide was recorded after 1h exposure; then, mosquitoes were fed with a 10% sugar solution and the number of dead

mosquitoes recorded 24 hours post-exposure. Mosquitoes subjected to untreated papers were systematically included as controls. To detect the presence of the *kdr* alleles (L1014F and L1014S) conferring resistance to DDT and pyrethroids, DNA extracted from a sub-sample of *An. gambiae* s.l. females were screened using the TaqMan assay [75].

Household surveys

Household surveys were conducted using a questionnaire. The following information was recorded: house characteristics (building material), geographical coordinates of the house, features to prevent mosquitoes from entering (screen, ceiling, close eaves) or those allowing mosquito to enter (holes on the wall, absence of ceiling, open eaves), presence and usage of LLINs, or other antimalarial measures, socio-demographic information of each household (occupation, education level, number of inhabitants per house).

Blinding

Entomological data collection was not blinded to the assignment of mosquito larval control interventions in the different clusters. Field applicators were blinded to the sites selected for larval surveys. Residents were aware of the implementation of the intervention. Adult mosquito collection was conducted using CDC light traps to avoid performance bias. Collections were conducted each month for three consecutive days to lessen variation due to rainfall or temperature. Laboratory technicians processing samples or conducting laboratory analysis were blinded to the identity of the cluster.

Ethical clearance and authorizations

The study was conducted under the ethical clearance N° 2016/11/832/CE/CNERSH/SP delivered by Cameroon National Ethics Committee on Human Health. Further informed consent was obtained from the senior division administrator of the city of Yaoundé and each local District Medical Officer. Verbal and formal informed consents were obtained from all respondents and the study purpose was explained to them. The trial was also approved by the Ministry of Public Health of Cameroon (Reference: 631-06-17). All experiments were performed in accordance with relevant guidelines and regulations.

Research and import permit for the use of VectoMax®G in Cameroon was granted by the Minister of Trade (Reference IF014167; IF021096; IF031126).

Data analysis

Data were collected on forms, checked first to ensure they were filled comprehensively, then recorded in excel databases. Linear mixed models with random intercepts and Generalized Estimating Equations were used to assess the effect of larviciding treatment on the presence of anopheline larvae (early and late instars) in water collection points as well as adult anopheline density, infection rate and Entomological Inoculation Rate (EIR) respectively, adjusting for baseline data. In a preliminary analysis, follow-up curves for the non-intervention and intervention areas were constructed to visualize differences in the responses between the two sites. Average trends and local polynomial regressions of the presence of anopheline larvae (early and late instars) in water collections, *anopheline* density, infection rate and EIR with date of evaluation were also constructed separately for the different groups to further visualize these differences. We also estimated a null model with random

intercept and calculated the intraclass correlation coefficient (ICC) associated with the presence of anopheline larvae, *anopheline* density, infection rate and EIR respectively. Generalized Estimating Equations were further used to describe the variation in *Anopheles* density, infection rate and EIR in the population under study, while controlling for baseline survey and groups. In all these cases, the identity link function with a Gaussian distribution was used, and we resorted to model with independent correlation structures since models in which within-cluster associations or correlations among the repeated measures were taken into account by defining more complex “working” correlation structures (like the autoregressive or unstructured correlation) did not converge. All analyses were carried out with the R 4.0.2 software using the R packages nlme, ggplot2, plyr, lattice, car, effects, emmeans and data.table. Odds ratios and risk ratio were calculated and adjusted for the year of intervention, cluster and season. Binary logistic regression was used to assess the distribution between species and physicochemical parameters in intervention and non-intervention areas. The Entomological inoculation rate was calculated by multiplying the mean density of mosquitoes collected in light traps in each cluster by the proportion of infected mosquitoes, by the number of days in the year and by 1.6 (the coefficient of underestimation of light trap compare to human landing catches). The percentage reduction of mosquito densities and EIR following larviciding intervention were estimated using Mulla formula [76].

Declarations

Consent for Publication

Not applicable.

Availability of data and material

The datasets supporting the findings of this paper are included in this paper.

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Authors’ contributions

Conceived and designed the study protocol: CW, CAN, Conducted field and laboratory analysis: DBP, NCS, TA, KE, BR, DDL, NL, SCN, TR, MPA, PAA critically revised the manuscript: AFD, TR, BDJ, PAA, CW; Interpreted, analysed data and wrote the paper: CAN with contribution of other authors. All the authors read and approved the final version.

Competing interests

The authors declare that they have no competing interests.

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Figures

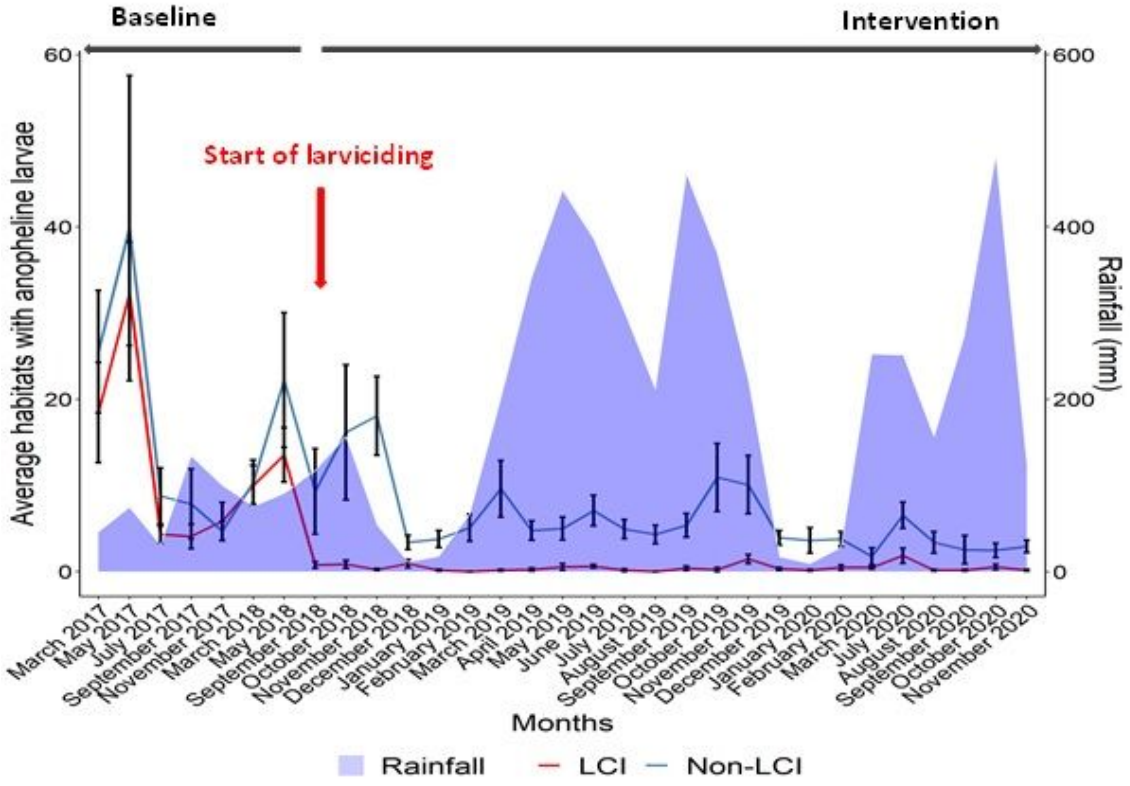

Figure 1

Evolution of breeding sites with anopheline larvae before and during the larviciding intervention ((Non-larviciding intervention area (Non LCI), Larvicidng Intervention area (LCI))

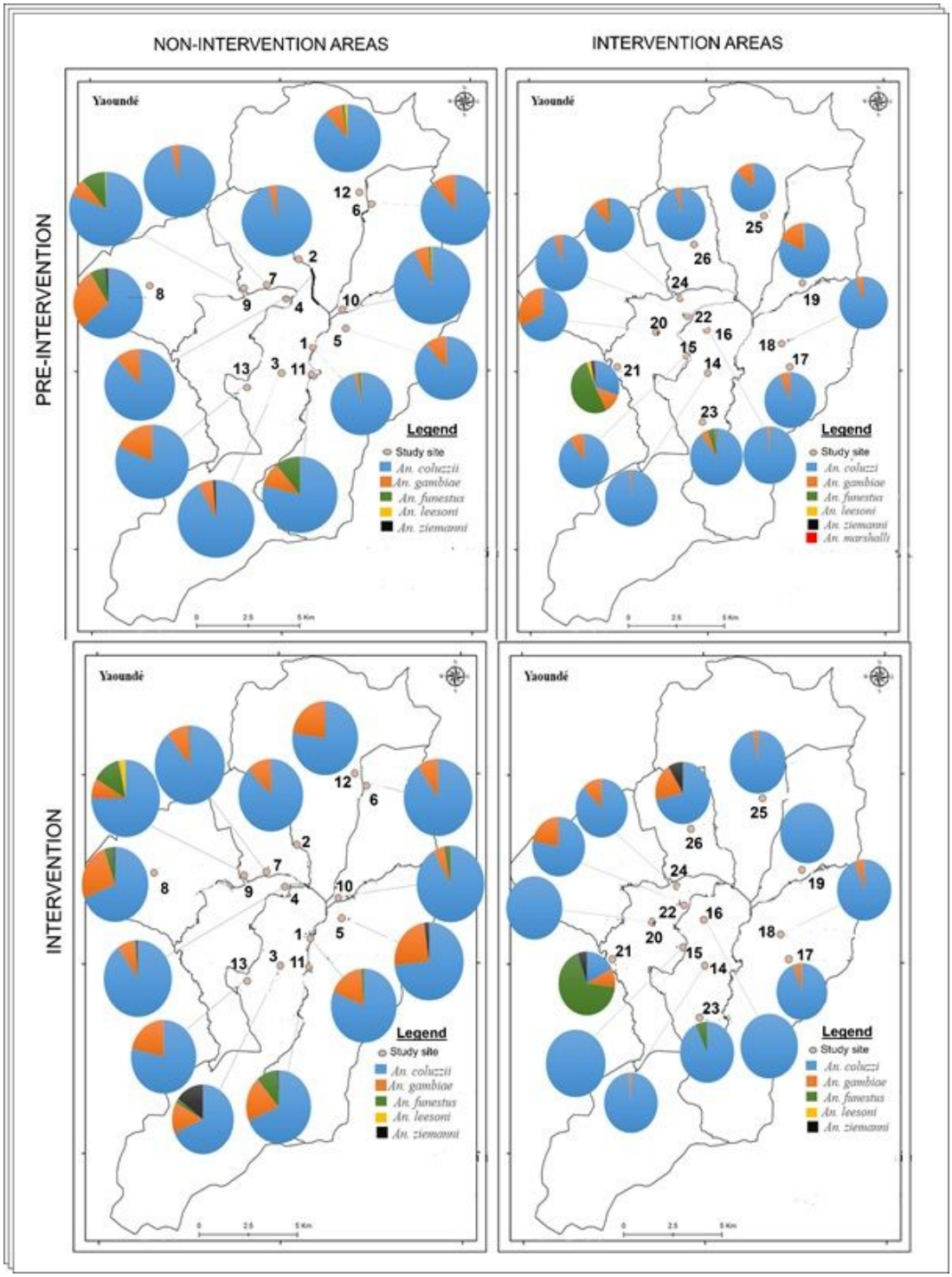

Figure 2

Distribution of Anopheline species in Yaounde before and during the intervention Legend : 1 : Mvolyé ; 2 : Bastos nouvelle route ; 3 : Efoulan ; 4 : Emia ; 5 : Etam-bafia ; 6 : Ngousso ; 7 : Nkolbikok ; 8 : Nkolbisson ; 9 : Nkolbisson nouvelle route ; 10 : Nkolndongo ; 11 : Nsam ; 12 : Santa-Barbara ; 13 : Tam-Tam; 14 : Biyemassi lac ; 15 : Biyemassi Somatel ; 16 : Cité des nations ; 17 : Ekounou-Ekié ; 18 : Ekounou-Palais ; 19 : Essos ; 20 :Etoug-Ebe ; 21 : Mendong ; 22 : Obili ; 23 : Obobogo ; 24 : Parc Labogenie ; 25 : Tongolo ; 26 : Tsinga. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or

area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

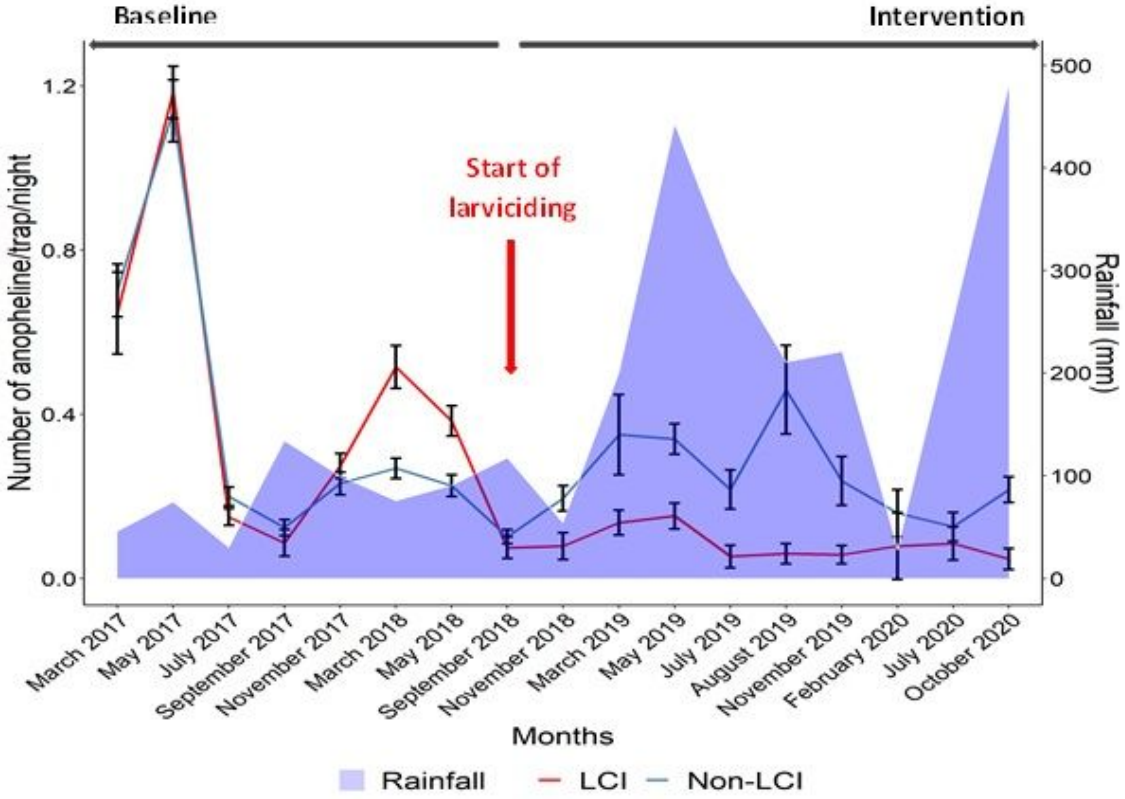

Figure 3

Evolution of anopheline biting density before and during larviciding intervention (Non-larviciding intervention area (Non LCI), Larvicidng Intervention area (LCI))

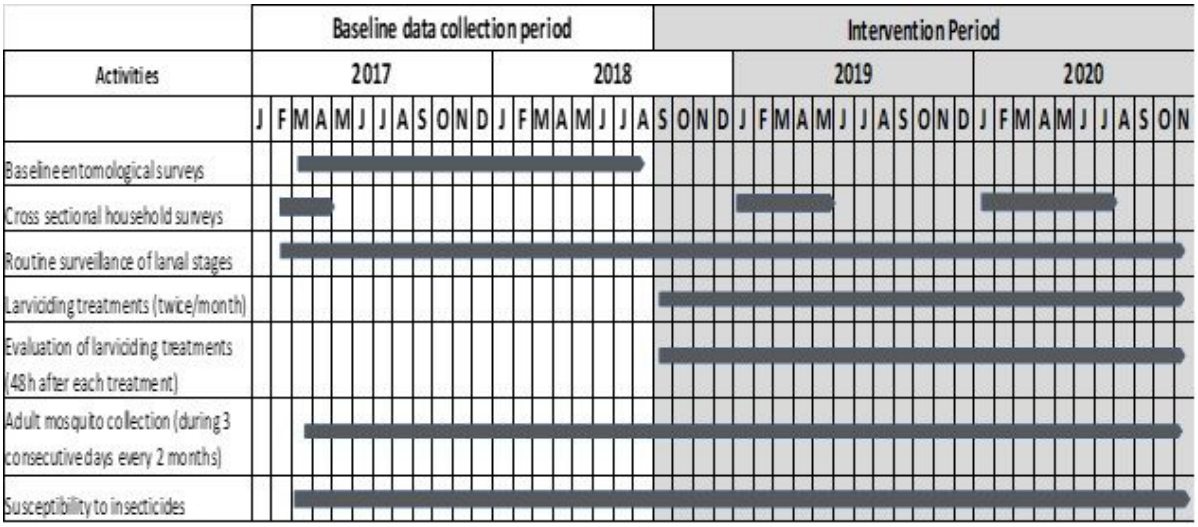

Figure 4

Activity schedule graph for larviciding trial in Yaounde