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EFFECTIVENESS OF LARVICIDING AS A SUPPLEMENTARY MALARIA VECTOR CONTROL INTERVENTION IN THE LAKE ENDEMIC ZONE: A CASE STUDY OF BUSIA COUNTY, KENYA, 2022-2023
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ABSTRACT

Background: **Malaria is a leading cause of morbidity and mortality globally, mostly in tropical countries, accounting for more than one million deaths annually. Community-based larval source management using larviciding was recently introduced as a complementary tool within the context of existing Integrated Vector Management strategies. This study aimed at generating evidence on the effectiveness of microbial larvicides in reduction of mosquito larval densities in the mapped aquatic habitats to improve malaria control.**

Methods: **The biolarvicides BACTIVEC®** ***Bacillus thuringiensis*** **var.** ***israelensis*** **and GRISELESF®** ***Bacillus sphaericus*** **were used. Products applications were made aquatic habitats findable and fixed in selected areas of seven sub-counties in Busia County during the period of January to December 2022. Larval densities were determined using a standard WHO protocol at each study area prior to and after larviciding.**

***Results*: *Anophilines* and *Culicines* larval species of mosquitoes were both present in all the surveyed accumulated open water bodies. The larval type of breeding habitat predominance rates was in the order; Rice fields (24%), Dams (23%), Swamps (21%), Puddles (12%), Lagoon (9%), Fishpond (4%), Streams and Seepage pool (3%) each respectively, and Rivers (1%). Prior and post larviciding, the average reduction in relative larvae colonization rate in all habitats aggregated was 95% (P <0.001).**

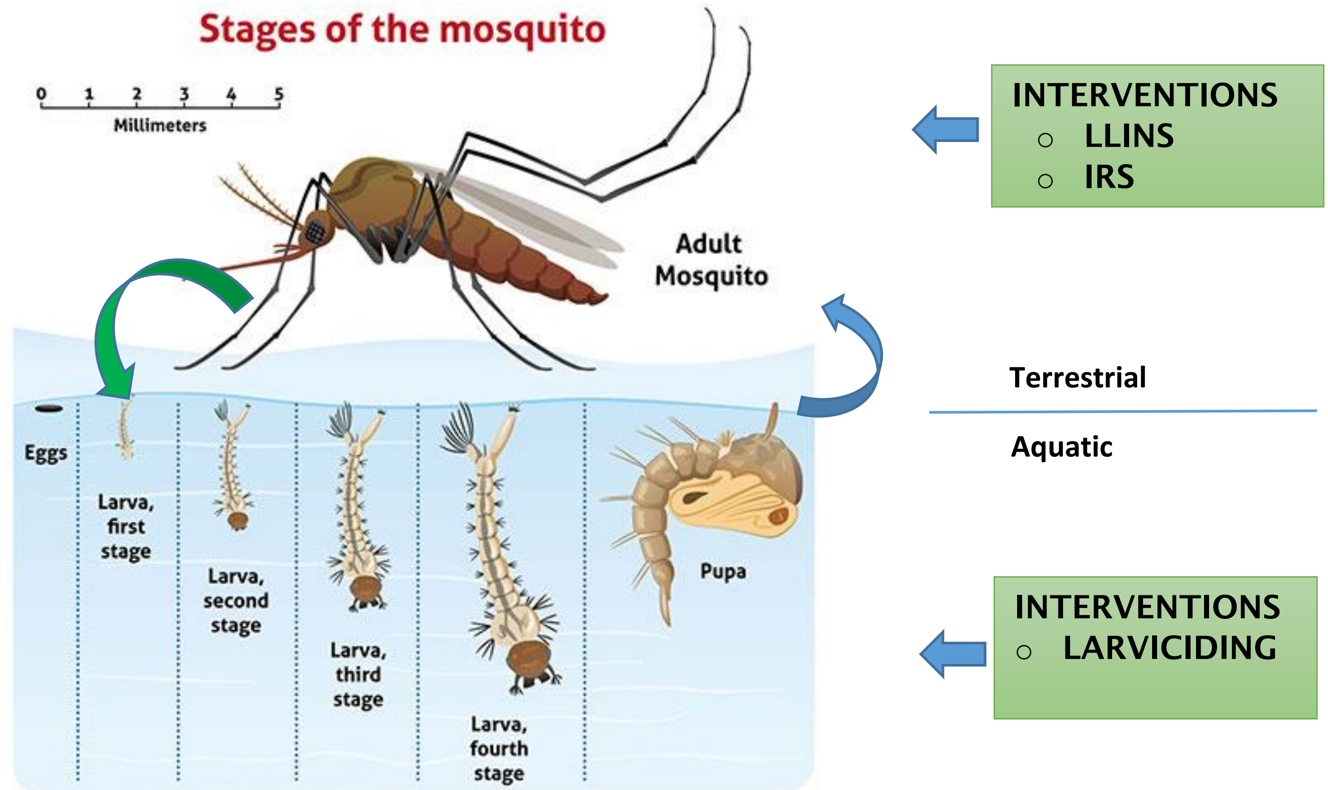
***Conclusions*: The study showed the potential effect of larviciding using biolarvicides of *bacillus* group to control vectors of mosquito borne diseases and its integration with indoor residual spraying and insecticide treated nets in malaria prevention.**

INTRODUCTION

Malaria pose an important public health concern across the world affecting both rural and urban areas (1). Global statistics indicate that an estimated 229 million malaria cases were reported in 2019 alone(2). Sub-Saharan Africa carries a disproportionately high share of the malaria burden, with 92% of cases and 93% of malaria deaths in 2017 (3) . Malaria prevalence in Kenya is at 6% while along the Lake endemic counties, previous research studies have shown the prevalence estimates to be 19% (4).

Successful control of malaria vectors requires the control of the larval and the adult stages. There is currently enough evidence on effectiveness of vector control methods through Implementation and massive scale-up of proven Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). They have been considered as the core cornerstone for malaria prevention due to their associated major changes in vector biology (5,6).

Despite the large-scale deployment of these tools, extensive use of LLINs and IRS has created intensive selection pressures for malaria vector insecticide resistance (7). Furthermore, recent scientific studies have revealed intraspecific changes in biting behavior, shift in vector species composition from previously predominant indoor biting at night to concurrently predominant species which prefer to bite and rest both indoors and outdoors, can also increase outdoor transmission (8). In addition, other potential malaria control interventions such as transmission-blocking vaccines have not been concluded or/ and genetically modified mosquitoes have not been successful. New interventions are urgently needed to augment current vector control measures such as larviciding.



Stages of mosquito life cycle with frontline interventions.

Larval source management (LSM), including habitat occupancy manipulation and larviciding, has historically proven to be highly effective for mosquito control programmes worldwide, with significant impact in control and elimination of malaria (9,10). Environmental alterations and climate change enhances the proliferation of larval habitats of malaria vectors. Monitoring larval population dynamics in different habitats over a period, has implications for vector control (11) . Larviciding constitutes several compounds including use of either synthetic organic chemicals, bio (bacterial) larvicides, or insect growth regulators.

Biolarvicides are best preferred based on the Gram-positive spore-forming bacteria, *Bacillus thuringiensis* var. *israelensis* (Bti) and *Bacillus sphaericus* (Bs) since they target both insecticide resistant and outdoor biting malaria vectors (12). It has been shown that unlike adult mosquitoes, larvae cannot change their behavior to avoid a control intervention targeted at larval habitats (13). Moreover, a larval control strategy also serves to extend the useful life of insecticides against adult mosquitoes by reducing the size of the population being selected for resistance and the strategy is equally effective in controlling both indoor and outdoor biting mosquitoes.

The WHO issued an interim position on larviciding recommending its use as a supplement to core vector interventions in areas where aquatic habitats are few, fixed and findable (5). However, this intervention is still not largely implemented for malaria control in sub-Saharan Africa, Kenya included, due to the scanty unbiased studies on its efficacy or effectiveness (14). The limited use could also be attributed to the poor knowledge on methods of implementing and monitoring the intervention, the assumed high operational costs of this intervention, the intensive labor required for its implementation and the short residual effect of previous larvicides formulations.

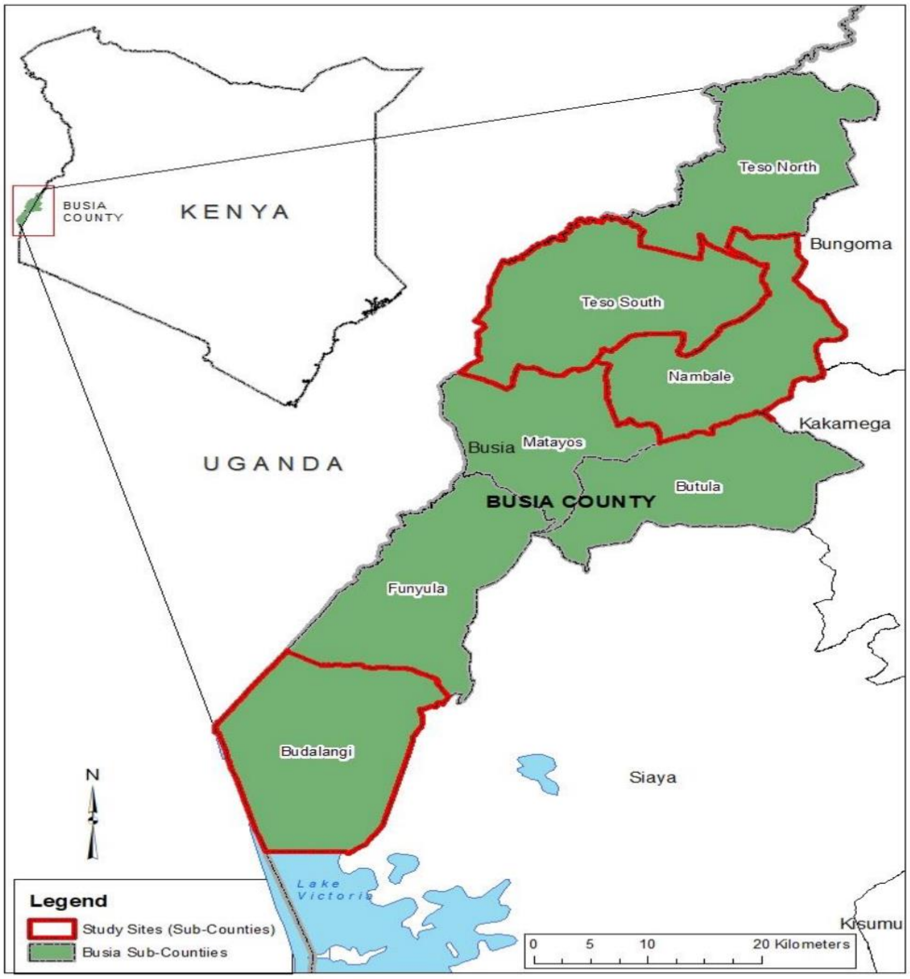
Kenya has made a good progress in piloting larviciding in the lake endemic zone, particularly with bio-larvicides, with view of need to be included in the list of viable options to intensify elimination campaigns. Thus, this study aimed on evaluating the effectiveness and feasibility of applying bio- larvicides for mosquito larvae control in the lake endemic zone, Busia County, in Kenya, 2022. Specifically, to 1) map the mosquito larvae habitat occupancy, and 2) determine the change in the larval densities pre and post bio-larvicide application.

MATERIALS AND METHODS

Study design: A descriptive retrospective cross-sectional study, was carried out on a data set available from January 2022 to December 2022, guided by previously collected routine programmatic data. The STROBE (Strengthening the Reporting of Observational studies in Epidemiology) guidelines was used to ensure the quality of reporting (15).

Study Setting: Western region, Kenya, located in Eastern Africa, has an estimated population of over 46 million in 2015 (16). Busia County has a total population of 893,681 persons, characterized by female to male ratio of 1: 1.096 respectively (17).

Specific setting: The Lake endemic region, precisely Busia, one of the eight counties administratively made up of of Teso South, Teso North, Samia, Nambale, Butula, Matayos and Bunyala Sub-Counties. Busia County borders Bungoma County to the North, Kakamega County to the East, Siaya County to the South East end and Lake Victoria one of the largest freshwater lakes in Africa Southwards respectively. Generally, the area has a coverage of approximately 1700 square kilometers, with tropical climate of average temperatures ranging from 19 to 29 °C, while altitude ranges from 1200 to 1700m above sea level.



Source, (Kenya Malaria Indicator Survey, 2020).

With two rainfall patterns annually, mosquito vector population in this region are usually high and malaria transmission is intense due to suitable climate conditions. As a result, the region experiences stable malaria transmission throughout the year.

Study population: The study villages were surveyed for the presence of mosquito larvae aquatic habitats characterized by type of breeding site, Area size (M^2), permanence, and land use types. All potential larval habitats identified were enumerated and mapped using a hand-held global positioning system (GPS) device before being sampled for mosquito larvae.

Sample size justification: Selected potential larval habitats in each of the counties were enumerated. Assuming routine sentinel entomological surveillance data as baseline density of mosquito larvae with collection conducted it was estimated the study to have over 80% power to detect reduction of the density of both *anophiline* and *culicine* species of mosquito collected using standard dipper with 10 dips at the 5% significance level.

Variables: The variables collected were the mosquito type of breeding habitat occupancy and larvae densities.

Data collection procedures: Data set from routine surveillance that has not been analyzed and presented in any scientific platform was utilized. The routine surveillance data was collected as follows:

Pre-intervention

All larval breeding habitats were surveyed in the intervention areas. All breeding sites or open water bodies were identified while walking on foot through the villages and mapped using handheld geographic positioning system (Garmin eTrex GPS) receivers and recorded in a GIS database. A unique identification number was then allocated to each site to allow quick reference during field operations.

During Intervention

From January to December 2022, all aquatic habitats which could serve as potential breeding sites for mosquitoes in the selected intervention areas were treated with the BACTIVEC® (*Bacillus thuringiensis israelensis*) and/ or GRISELESF® (*Bacillus sphaericus*) from Laboratorios Biológicos Farmacéuticos, LABIOFAM, CUBA. These two contained spores of toxic crystals 5g/L strictly aerobic gram variable bacterium *Bacillus thuringiensis israelensis* type H-14 and strictly aerobic gram variable bacterium *Bacillus sphaericus* 2362 serotype H5a5b respectively. Treatment of accumulated open water bodies in the intervention area was done fortnightly or as guided by residual effect.

Product Application: Field applicators were recruited from local communities. They were supervised during each field site visit by Cuban Technical officer and Kenyan entomologist supervisor in each selected zone and trained for one month before starting larviciding activities. Application of bio-larvicide through aerial spray, Hudson X-pert pressure spray pumps was conducted early in the morning between 7 and 11 AM to avoid the hottest time of the day. Each team performing larviciding treatments used about 07 to 610L of biolarvicide/habitat/day depending on the area size and seasonality (more during rainy days).

Endpoints

Assessment of the effect of larviciding intervention was done based on the outcomes of reduction in relative larval densities collected in breeding habitats.

Larval vector abundance assessment

The treated habitats were analyzed to assess the presence of mosquito larvae. The larval stages of mosquitoes were collected using standard dipping technique (5). Depending on habitat size, 5 to 20 dips (350 ml standard mosquito dippers) were taken from each larval habitat: 5 dips were undertaken in small larval habitats of ≤ 1 m^2; 10 dips for medium-sized habitats (2–15 m^2) and 20 dips for relatively large habitats (> 15 m^2). For too shallow habitats, larval collection was conducted using a pipette. The larval habitats were monitored immediately prior to application (day 0) and then on days 1, 3 and weekly after 7 days post-treatment for a period of 6 months. The average larval density was estimated by calculating the ratio of the number of larvae collected per dip (using a dipper with a volume of 350 ml).

Blinding.

Entomological data collection was not blinded. Field applicators were blinded to the sites selected for larval surveys. Collections were conducted each month for three consecutive days to lessen variation due to rainfall or temperature.

Data Management and Analysis

Data was abstracted from the routine surveillance database and entered in MS Excel software version 22.0 for cleaning and analyzed. Generalized Estimating Equations were used to assess the effect of larviciding treatment in various surveyed habitats. Pre- and post-larviciding periods in the study areas were compared using ANOVA in Statistix version 9.0. The percentage reduction in the larval densities were calculated using Mulla's formula (12).

Ethics consideration:

Ethical approval was granted by the Maseno University Scientific and Ethics Review Committee (MUSERC), number MUSERC/01234/23. Routinely collected program data was analyzed retrospectively. Permission to use the data for this study was granted by the Kenya Malaria Control Program. Before fieldwork, meetings had been held with the respective County leaders to inform them about the study and to seek their cooperation. Oral informed consent had been sought and obtained from land/farm owners before start of larval habitat surveys and application of biolarvicides.

RESULTS

Two species of *anopheline* and *culicine* were identified during the mapping of the open water bodies. This was done during the pre-intervention period (prior to spraying phase). Their presence was documented in all the surveyed study areas, thus qualifying them as potential breeding aquatic habitats (*Figure 1*). A total of nine (9) types of aquatic habitats were observed during the study site mapping. The predominant type of breeding site was RiceField (24%), followed by Dam (23%), swamp (21%), puddle (12%), lagoon (9%), fishpond (4%), seepage pool and stream (3%) each respectively and River (1%).

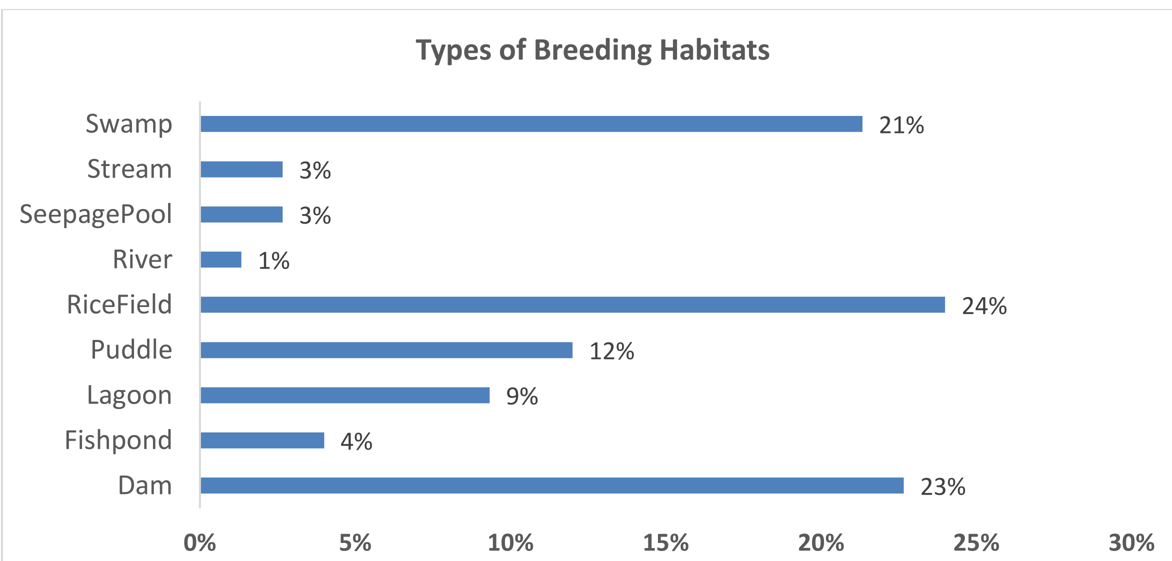


Figure 1: The proportions of types of breeding habitats mapped within Busia County

Mosquito larvae species of both *Anopheline* and *Culicine* were collected from seven (7) sub counties. A majority of the larvae concentrates were in the order; from Teso North, followed by Matayos, Samia, Butula, Bunyula, Teso South and Nambale respectively (*Table 1*). In totality, an average relative larvae density of 15, 580 were collected prior to spraying the habitats. After larviciding, the habitat colonization rate showed a significant reduction of 95% (P <0.001).

Table 1

Composition of Mosquito larvae fauna pre & post intervention in various sub-counties

Sub-county/ type breeding site	Average of Larvae before spraying (RLD L/m2)	Average of Larvae after spraying (RLD L/m2)	Average of Larvae Reduction n (%)
Bunyula Sub-County	2237	139	2098 (94)
Lagoon	433	7	426 (98)
RiceField	1024	57	967 (94)
Swamp	780	75	705 (90)
Butula Sub-County	2600	146	2454 (95)
Dam	666	27	638 (96)
Puddle	1614	119	1495 (93)
Swamp	320	0	320 (100)
Matayos Sub-County	3158	165	2993 (95)
Dam	766	0	766 (100)
Fishpond	833	37	796 (96)
SeepagePool	409	26	383 (94)
Swamp	1150	102	1048 (91)
Nambale Sub-County	202	14	188 (93)
River	202	14	188 (93)
Samia Sub-County	2869	122	2747 (96)
Dam	443	11	433 (98)
Lagoon	967	50	917 (95)
Puddle	917	38	879 (96)
Swamp	542	24	519 (96)
Teso North Sub-County	3494	175	3319 (95)
Fishpond	547	23	524 (96)
Puddle	2405	145	2261 (94)
Stream	542	8	535 (99)
Teso South Sub-County	1020	40	980 (96)
Puddle	857	40	817 (95)
Swamp	163	0	163 (100)
Grand Total	15,580	801	14,779 (95)

Among the aquatic habitats, within the seven (7) sub counties all concentrated per the type of breeding habitat, puddle was the predominant holding area with highest average relative larvae densities prior to biolarvicide spraying (*Figure 2*).

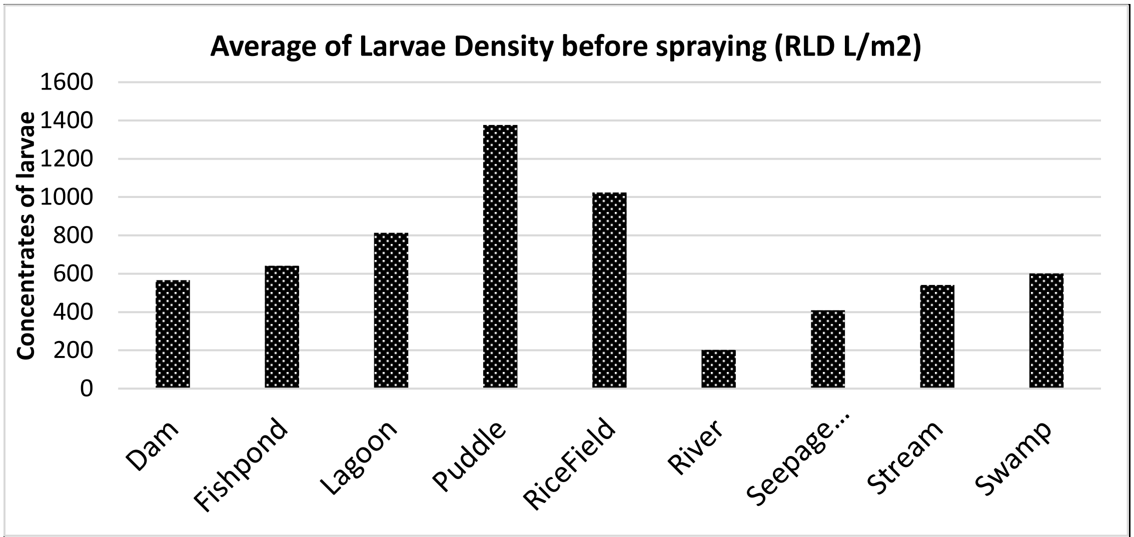


Figure 2: Relative mosquito larval densities pre intervention per aggregate of breeding sites

Among the aquatic habitats, within the seven (7) sub counties all concentrated per the type of breeding habitat, a significant reduction in the average relative larvae densities were observed in all the habitats after larviciding in all areas (*Figure 3*).

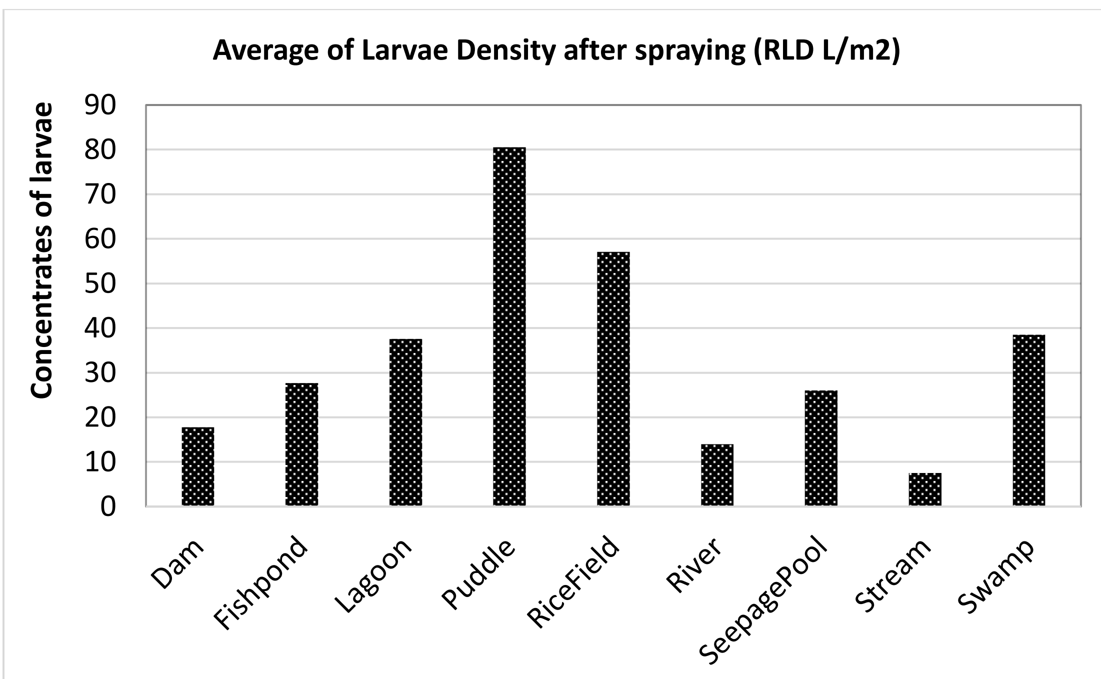


Figure 3: Relative mosquito larval densities post intervention per aggregate of breeding sites

Among the aquatic habitats, within the seven (7) sub counties all concentrated per the type of breeding habitat, the reduction rates ranged between 93 to 99%, (average 95%). This was above the WHO recommended cut-off point of >80% (*Figure 4*).

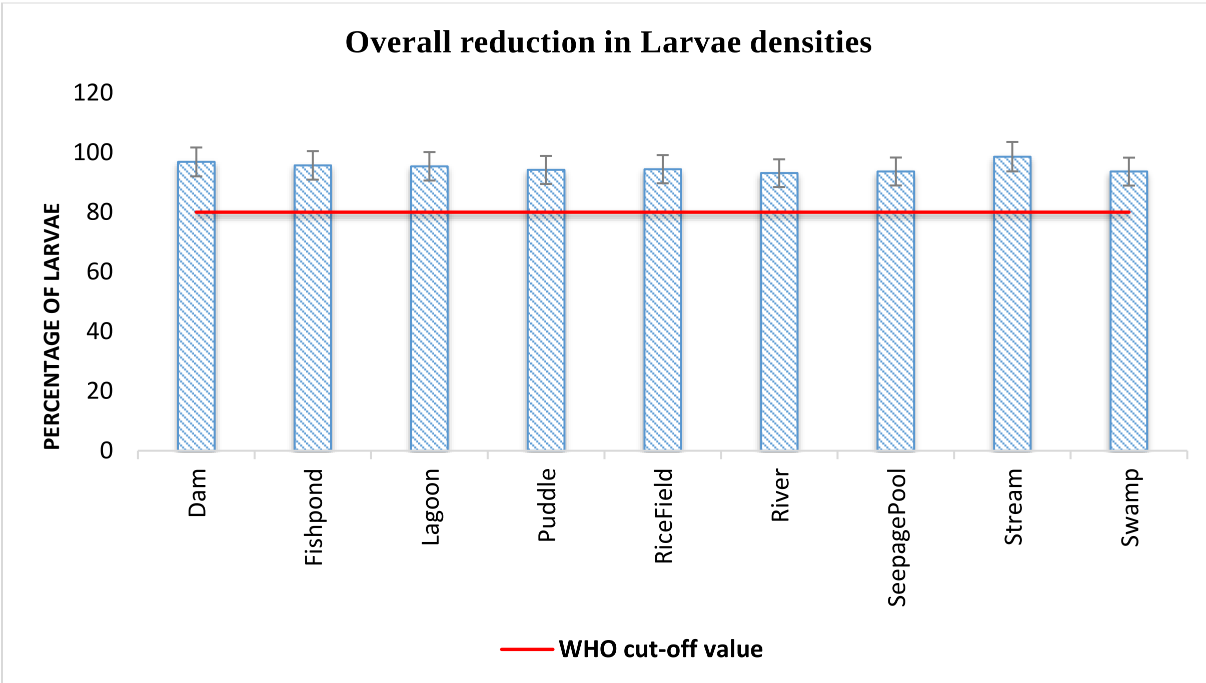


Figure 4: Relative reduction in larval densities per total aggregate of breeding sites treated

DISCUSSION

This study's primary objective was to assess the effect of larviciding on mosquito larvae densities in seven sub counties of Busia County. In mapping out the aquatic habitats, those that were potential breeding sites fell into nine categories. These findings showed presence of both mosquito larvae of *anopheline* and *culicine* species. Such results are similar to the earlier published in studies done at Tanzania, Congo and Zimbabwe region (6), where collected larvae and pupae, were reared to adult stages and confirmed taxonomically to be primary and secondary vectors of malaria. Such vectors, more so the larvae that molts to adult have demonstrated the behavior of feeding on humans (anthropophagic) or animals (zoophagic) and resting indoors (endophilic) or outdoors (exophilic), hence potential transmitters of malaria (18).

In terms of effectiveness of larvicides, this study showed a high overall reduction in relative mosquito larval densities with an average of over 95%. These figures are consistent with previous studies conducted in Bukina faso and across the continent supporting the high impact of anti-larval measures (10). The fact that the study areas covered all the seven administrative sub counties in pre-intervention and post intervention, (while being monitored by the teams led by trained entomologist, as recommended by WHO, permitted to minimize the habitat inclusion of bias and further strengthen the quality of evidence arising from the study.

During the study, continual application of bio-larvicide in various types of aquatic habitats was conducted rather than seasonal (during the rainy season) as done in previous studies in Western Kenya (6). The modified equatorial climatic conditions in the study areas include three seasons: warm and wet season (March-June), cool and dry season (July-November), and a hot and dry season (December-February) with temperatures ranges 19- 29°C. These provide ideal conditions for mosquito breeding throughout the year. This regular application of the anti-larval led to a high reduction of breeding habitats especially permanence, thus supports feasibility of regular application of bio-larvicide all year long at least during the initial years of the intervention.

Microbial larvicides are also known to be highly efficient, specific and safe to use. Moreover, the risk that resistance could emerge is very low due to the complex mode of action of these larvicides particularly Bacillus *thuringiensis* which has up to four different endotoxins (5). Recent studies of insecticide resistance has been reported to largely spread across sub-Saharan Africa but seems to have had no impact on the effectiveness of larviciding treatments, since high reduction in mosquito densities were still recorded (19). This observations showed longer larval development time for resistant mosquitoes compare to susceptible (20).

This specific characteristic could be suggestive of increasing the exposure of resistant mosquitoes to bio-larvicide and increase mortality rate among insecticide resistant larvae. These further supports the additional benefits of larviciding which could act as a complementary tool for insecticide resistance management. Bio larvicides measures could be inducing a reversal of resistance to pyrethroids and extend the efficacy of pyrethroid embedded in treated bed nets, therefore, as Busia County prepares to roll out indoor residual spray, there is no cause of alarm (21).

CONCLUSION

This study sets out to advocate the fact that the use of larviciding as a complement to LLINs and IRS could be a viable solution for controlling malaria transmission in lake endemic region, Busia County in a context of rapid expansion of insecticide resistance across Africa and outdoor malaria transmission. It also provided strong evidence supporting the use of larviciding as a main intervention in intervention areas where both habitats have clean and polluted waters, agricultural, health and environmentally safe.

Recommendations

The overall significant reduction of mosquito larval densities recorded confirmed larviciding as a promising tool for controlling malaria transmission, with potentiality of exploring the impact of climate change on larval emergence and habitats as well as use of drones and smartphone technology.

Limitations

Lack of more detailed information about larval breeding sites such as vegetation cover and unavailability of current insecticide resistance test data for the respective study sites were also important limitations of the study. The study mainly focused on entomological outcomes as primary endpoints rather than epidemiological outcomes as generally done. This has been a subject of discussion in other previous studies (22). The study did not assess the cost-effectiveness of larviciding which is very important for policymakers.

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