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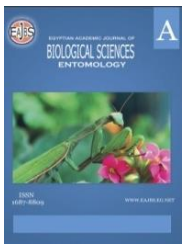

Comparative Evaluation of Larvicides for Larval Source Management of Mosquitoes in Lagos, Nigeria.

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ABSTRACT

The use of larval source management (LSM) in sub-Saharan Africa is limited, however, widespread report of insecticide resistance in adult mosquitoes has engendered renewed interest in LSM; especially larviciding. Hence, this study evaluated bioefficacy and residual efficacy of commercially available larvicides; Temephos, Spinosad, *Bacillus thuringiensis var. israelensis (Bti)* and *Bacillus thuringiensis var. israelensis*/*Bacillus sphaericus (Bti/Bs)* on larvae of *Anopheles gambiae s.l*, *Culex quinquefasciatus* and *Aedes aegypti* in Lagos, Nigeria. Acute toxicity assay was used to determine LC_{95} and the LC_{95} was doubled to determine discriminating doses. Residual efficacy was assessed by exposing larvae to discriminating doses of larvicides in deionised water and in water collected from larval habitat for 28 days and 24 hr mortality post-introduction of larvae was checked for 2nd, 4th, 7th, 14th, 21st and 28th day. Probit analysis was used to estimate LC_{95} and residual efficacy at mortality ≥95%. T-Test was used to determine the level of significance ($P<0.05$) of the residual effect. Temephos (0.007mg/l) was the most toxic considering 24h LC_{95}. The trend of LC_{95} (Temephos < Spinosad < Bti/Bs < Bti) in *Anopheles gambiae s.l* was the same for *Culex quinquefasciatus* and *Aedes aegypti*. Residual effect of *Bti*/*Bs* > *Bti* > Temephos > Spinosad on mosquito larvae from Lagos, Nigeria. The trend of residual efficacy is the same in both bioassays albeit reduced when larvicides were dissolved in water collected from larval habitats. Strong bioefficacy and better residual capacity of *Bti/Bs* make it a better larviciding agent against *Anopheles gambiae s.l*, *Culex quinquefasciatus* and *Aedes aegypti* in Lagos, Nigeria.

INTRODUCTION

Mosquito-borne diseases still remain one of the main public health challenges in the world. This is because mosquitoes are an efficient transmitter of a good number of diseases in different parts of the world. One major way employed in addressing the challenges of these diseases is the control of adult mosquitoes. Of all the control methods targeting the adult vector, 2 major strategies; Indoor residual spray (IRS) and long-lasting insecticide net (LLIN) are prominent, especially in Africa (Omotayo *et al*., 2021). Scale-

up of these 2 methods in past years has been attributed to a decline in the incidence of diseases such as malaria (WHO, 2015). Unfortunately, the success achieved in past years as regards reduction of the disease has been stalled since 2015 (WHO, 2019b) and this has been majorly attributed to the development of insecticide resistance (IR) by adult mosquito vectors (Ranson and Lissenden, 2016).

The development of IR has prompted an inquiry into other methods that could be used in addressing vector control and one of such methods is larval source management (LSM). Larval source management is the management of breeding sites by the use of various strategies such as habitat modification and manipulation, insect growth regulator (IGR), larviciding and biological control (Gimnig *et al*., 2020). LSM is aimed at controlling the vector at the larval stage and it has gained attention in recent times due to reports of its effectiveness in reducing the incidence and prevalence of malaria in East Africa and Asia (Tusting *et al*., 2013) as well as in eradication of *Anopheles gambiae* in Brazil (Killen *et al*., 2002). Derua *et al*., (2019) also noted that LSM could help to extend the usefulness of currently available insecticides to adult mosquitoes as it will reduce the population of mosquitoes being selected for resistance. Despite the success attributed to the use of LSM in malaria control, it is still largely forgotten and not well used, especially in Africa (Worrall and Fillinger, 2011).

Of all the LSM strategies, there is increasing interest in the concept of larviciding. Historically, larviciding is not new to mosquito control (Killen *et al*., 2002) but its use waned down when huge success was recorded during the period DDT was used for IRS (Fillinger and Lindsay, 2011). The reduction in its use is also strengthened by the adverse impact of chemical larvicides on non-target organisms (Walker and Lynch, 2007). However, it has recently been recommended to be used as a complementary intervention to ITN and/or IRS in areas where insecticide resistance is already established and spreading (Gimnig *et al*., 2020). The World Health Organisation also recommends that it can be used as a supplemental strategy in moderate or low transmission settings where larval habitats are few, fixed and findable (WHO, 2019a).

As much as there are concerns about the adverse impact of chemical larvicides, reports on the use of bacterial larvicides have been satisfactory (Derua *et al*., 2002). The safety concerns engendered by the adverse impact of chemical larvicides on a non-target organism are eliminated with the use of bacterial larvicides such as *Bacillus thuringiensis var israelensis (Bti)* and *Bacillus sphaericus (Bs)*. Bacterial larvicides are species-specificity thereby mitigating concerns about their ecological and public health impact (Walker and Lynch, 2007). The potency of different formulations of *Bti* and *Bs* against larvae of specific mosquito species coupled with the fact that there is no known adverse effect on the environment and other organisms (Gimnig *et al*., 2020) makes them an ideal tool for larviciding in an integrated vector management (IVM) plan.

Laboratory trials establishing the bioefficacy of *Bti* and *Bs* on the local population of mosquitoes have been conducted in a different part of Africa (Fillinger *et al*., 2003; Nartey *et al*., 2013; Ketseoglou *et al*., 2011). Numerous semi-field trials (Majambere *et al*., 2010; Majori *et al*., 1987; Demissew *et al*., 2016; Diedhiou *et al*., 2016) and field trials (Shililu *et al*., 2007; Msellemu *et al*., 2016; Obopile *et al*., 2018) have also reported the effectiveness of these larvicides in mosquito control. In as much that numerous studies have established the bioefficacy of *Bti* and *Bs* in Africa, there are reports of varying control efficacy influenced by several factors which include the target mosquito populations and larval habitat conditions which vary from one area to another. Considering this fact, WHO (2013) posited that larviciding strategy that is known to be effective in a particular area may be ineffective in another area. Therefore, for effective application of *Bti* or *Bs* in African countries, comprehensive knowledge of local mosquito

populations (Mittal, 2003), as well as the bioefficacy on local populations, need to be adequately assessed. This points to the country-specific sound evaluation of the impact of *Bti* and *Bs* application in different African settings and ecological climates before it can be totally absorbed into the national integrated vector management plan.

Considering this fact coupled with positive reports from other studies in some African countries, there is a need for the sound evaluation of *Bti*, *Bs* and other larvicides on the local population of mosquitoes from Nigeria, unfortunately, these studies are limited. Hence, the present study seeks to evaluate the efficacy of selected larvicides on mosquito populations in Nigeria from an area where insecticide resistance in adult mosquitoes is already documented. This will provide baseline data towards the development of effective and ecological-based Integrated Vector Management (IVM) strategies for mosquito control in Nigeria.

MATERIALS AND METHODS

Mosquitoes:

Third instar larvae of wild *Anopheles gambiae s.l, Culex quinquefasciatus and Aedes aegypti* were collected between June and October 2018 from areas within Lagos, Nigeria (Fig. 1). Larvae of mosquitoes were sampled from five locations, Ikorodu (N 06º 39' 20.76 " E 003º 31'. 130"), Alapere-Ketu (N 06º 35' 02.34 " E 003º 23' 31.66"), Iponri (N 06º 29' 19.46" E 003º 21' 46.61"), Lagos Island (N 06º 27' 28.18" E 003º 23' 46.59") and Orile-Iganmu (N 06º 28‘’44.62" E 003º 20' 57.96"). Larvae of the different species were identified using morphological keys (Gilles and Coetzee, 1987) and kept in the Zoology laboratory, University of Lagos. Colony of susceptible Kisumu *Anopheles gambiae s.s* that has been maintained in the insectarium of Nigerian Institute of Medical Research (NIMR), Yaba, Lagos for more than ten years were used to validate the larvicides.

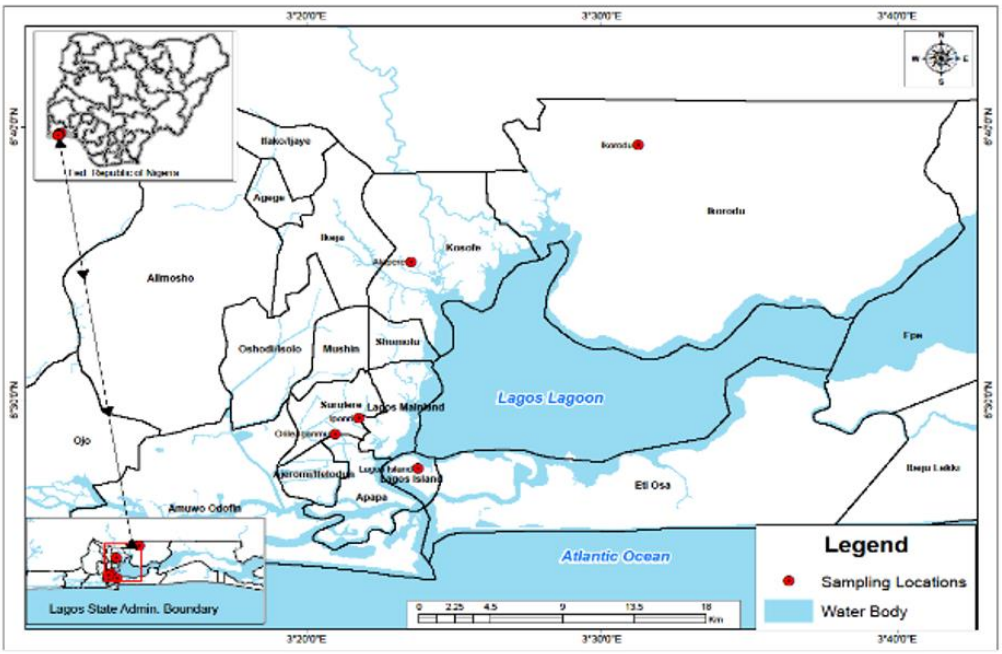

Fig. 1: Map of Lagos, Nigeria showing the sampling locations

Identification of Mosquitoes:

Mosquito larvae from the different locations were mixed up to get a representative of mosquitoes obtainable in Lagos and cohort from the larvae were reared

till adult at the Zoology Laboratory, Faculty of Sciences, University of Lagos, Lagos, Nigeria. Adult *Culex quinquefasciatus* and *Aedes aegypti* were identified morphologically using keys by Gilles and Coetzee (1987) while Adult *Anopheles gambiae s.l* mosquitoes were identified to species level using the procedure in Scott *et al*., (1993), however, molecular differentiation between *Anopheles gambiae s.s* and *Anopheles coluzzii* was not done.

Test Larvicides*:*

Four (4) larvicides were evaluated for their acute effect and residual efficacy against wild strains of *Anopheles gambiae s.l, Culex quinquefasciatus and Aedes aegypti* larvae. The organophosphate larvicide; Temephos (Skeeter Abate®; 5% EC) Pellets was sourced from Harvestfield Nigeria Limited, a commercial pesticide marketing company based in Lagos State, Nigeria. The other three larvicides are; Spinosad (Spintor Dust®; 1.25g/kg WP) produced by Dow Agrosciences LLC, Indianapolis, USA. *Bacillus thuringiensis var. israelensis* (*Bti*) (Vectobac®; Serotype H-14, Strain AM65-52) and *Bacillus thuringiensis var. israelensis/Bacillus sphaericus* (*Bti*/*Bs*) (Vectomax®; *Bti* Strain AM65-52/ *Bs* strain ABTS 1743) granules by Valent Biosciences LLC, Illinois, USA.

Stock Solution Preparation:

Stock solution for each larvicide was prepared by weighing 200 mg of the larvicides and adding 20 ml of distilled water in a screw-cap container with aluminium foil over the mouth of the vial. Thereafter, the solution was vigorously shaken to dissolve or disperse each of the larvicides. The stock solution was then serially diluted ten-fold by adding 2 ml solution to 18 ml of distilled water. Test concentration was then obtained by adding 0.001–1.0 ml of the stock solution diluents to 200 ml of distilled water in five replicates (WHOPES, 2005). Larvicide dilutions from respective stock solutions were introduced into experimental cups by means of micro-pipettes.

Acute Toxicity and Determination of Discriminating Dose of Selected Larvicides on Mosquito Larvae:

Direct-contact mortality bioassay was used to evaluate the acute toxicity of the larvicides. Susceptible Kisumu *Anopheles gambiae s.s* larvae were first exposed to recommended doses of Temephos, Spinosad, *Bti* and *Bti/Bs* to validate the larvicides. To determine the acute toxicity, new batches of mosquito larvae were exposed to a wide range of lower concentrations of the larvicides to determine the activity range of each of the larvicides. After recording larval mortality in these wide ranges of concentrations, a narrower range of 4 – 5 concentrations yielding between 10% and 100% mortality in 24 h were used to determine LC50 and LC95 values. The discriminating doses of larvicides were then derived by doubling the extrapolated 24h LC_{95} values for the different mosquito species.

Bioefficacy of Discriminating Doses of Selected Larvicides on Mosquito Larvae*:*

Mosquito larvae were exposed to discriminating doses of larvicides derived by doubling the extrapolated 24h LC_{95} values. Exposure of larvae was done via two different media; deionised water (Bioassay 1) and larval habitat water (Bioassay 2). The exposure using larval habitat water was done to simulate habitat conditions in a semi-field bioassay considering the fact that the larval habitat has been reported to contain heavy metals that may affect field application. Twenty (20) larvae of *Anopheles gambiae s.l, Culex quinquefasciatus,* and *Aedes aegypti* in five replicates were separately exposed to the selected larvicides dissolved in deionized water and larval habitat water separately, and mortality response was obtained after 24 hours. Control was done by exposing 20 larvae of different mosquito species in 2 replicates to the exposure media without the larvicides. Larval mortality was evaluated 24h post-larvicide exposure by calculating the number of

dead larvae. Dead mosquito larvae were identified as those that do not show the characteristic diving reaction when probed with a stirrer. All experimental procedure was conducted in the laboratory at 26 ± 3.1 ^{0}C and relative humidity at 70 ± 2.4%.

Residual Efficacy Of Selected Larvicides On Mosquitoes:

Residual efficacy of larvicides was assessed through the introduction of twenty (20) 3rd instar larvae into bioassay test cups in five (5) replicates containing discriminating larvicidal concentrations. The larvae were introduced on days 2, 4, 7, 14, 21 and 28 and mortality was checked after 24 h of introduction. The larvicidal concentration was prepared using deionized water and larval habitat water as done when assessing the bioefficacy of discriminating doses. Control was established by exposing 20 larvae of the different mosquito species in 2 replicates to the exposure media without the larvicides. Percentage mortality was evaluated 24h post-larval introduction and all larvae (both dead and alive) were removed from the bioassay after 24h treatment to allow for the introduction of new batches of larva. Larval food was added on larval introduction days to each test replicate and control in order to eliminate starvation as a confounding factor for mortality. The residual effect of the assay is considered to be efficient when larva mortality is ≥95%.

Data Analysis*:*

Concentration mortality and estimated days of the residual effect of the larvicides on mosquito larvae were analysed using Probit Analysis. Mortality was calculated in percentage. Paired sample T-Test was employed to determine the level of significance ($P<0.05$) of the residual efficacy of the larvicides. All analysis was done using IBM Statistical Package for Social Sciences (SPSS) (Version 22.0).

RESULTS

Mosquito Identification*:*

Seventy- Three (73) adult *Culex* mosquitoes were identified morphologically as *Culex quiquefasciatus* while eighty-four (84) *Aedes* species were identified morphologically to be *Aedes aegypti*. Likewise, ninety (90) *Anopheles* species were identified molecular with eighty-seven (87) of the samples *Anopheles gambiae* s.s (Fig 2) and three (3) *Anopheles arabiensis.*

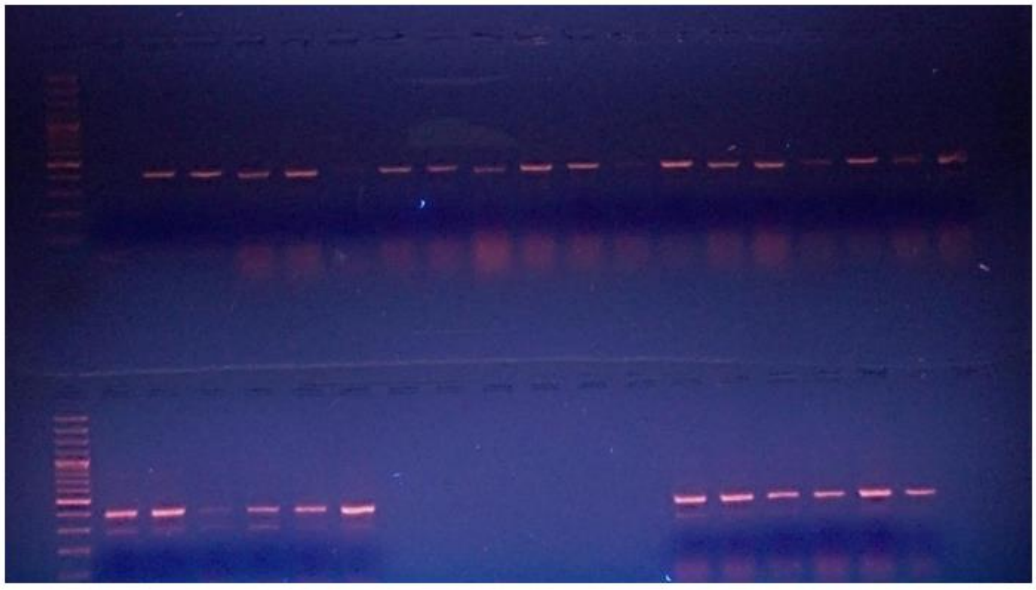

Fig. 2: Photomicrograph of PCR product of *Anopheles* samples showing band length for *Anopheles gambiae* s.l

Acute Toxicity of Selected Larvicides on Mosquito Larvae in Laboratory Bioassay:

The result of acute toxicity is presented in Table 1. Out of the 4 larvicides, Temephos is the most toxic on larvae of all the 3 species of mosquito. 24h LC_{95} of *Anopheles gambiae* s.l, *Culex quinquefasciatus* and *Aedes aegypti* larvae for Temephos were 0.008mg/l, 0.11 mg/l, and 0.007mg/l respectively. 24h LC_{95} for all the larvicides in *Culex quinquefasciatus* larvae were generally higher than in *Anopheles gambiae* s.l and *Aedes aegypti* larvae (*Culex quinquefasciatus* > *Aedes aegypti* > *Anopheles gambiae s.l*). Based on the 24h LC_{95} values, Temephos-induced mortality on *Anopheles gambiae s.l* larvae was 3x, 13x and 26x more than Spinosad, *Bti*/*Bs* admixture and *Bti* respectively. A similar trend was observed for *Culex quinquefasciatus* and *Aedes aegypti* larvae exposed to the larvicides (Table 1).

Table 1: Acute toxicity of larvicides against different mosquito species after 24hours

Mosquito Species		Temephos (mg/l)	Spinosad (mg/l)	*Bti* (mg/l)	*Bti/Bs* (mg/l)
Anopheles gambiae s.l	LC_{50} (CL)	**0.005** (0.003-0.007)	**0.014** (0.005–0.023)	**0.118** (0.097–0.150)	**0.073** (0.052–0.102)
	LC_{95} (CL)	**0.008** (0.003–0.011)	**0.025** (0.012–0.039)	**0.210** (0.142–0.301)	**0.107** (0.088–0.195)
Culex quinquefasciatus	LC_{50} (CL)	**0.005** (0.002–0.008)	**0.017** (0.009–0.031)	**0.130** (0.111–0.216)	**0.102** (0.089-0.214)
	LC_{95} (CL)	**0.011** (0.072–0.016)	**0.036** (0.021–0.058)	**0.251** (0.210–0.408)	**0.178** (0.126–0.265)
Aedes aegypti	LC_{50} (CL)	**0.004** (0.001–0.006)	**0.014** (0.009–0.027)	**0.109** (0.089–0.202)	**0.091** (0.052–0.178)
	LC_{95} (CL)	**0.007** (0.004–0.010)	**0.027** (0.017–0.041)	**0.191** (0.109–0.310)	**0.143** (0.107–0.208)

Mortality Response of Mosquito Larvae to Discriminating Doses of Larvicides:

The 24h-mortality response of all the species of mosquito larvae exposed to discriminating doses of larvicides is presented in Table 2. The discriminating doses when dissolved in deionised water (bioassay 1) achieved 100% mortality in all the mosquito species. However, when the larvicides were dissolved in water collected from the habitat of the larvae (bioassay 2), 100% mortality was achieved only for *Anopheles gambiae* s.l and *Aedes aegypti*, while mortality in *Culex quinquefasciatus* was 99% and 97% for Temephos and Spinosad respectively (Table 2). Larval mortality was below 5% in all controls.

Table 2: 24hr mortality response of mosquito larvae exposed to discriminating doses of larvicides

Species	Larvicides	Discriminating dose (mg/l)	Mortality (%)		
			Control	Bioassay 1	Bioassay 2
***Anopheles gambiae* s.l**	**Temephos**	0.016	3	100	100
	Spinosad	0.050	2	100	100
	Bti	0.420	2	100	100
	Bti/Bs	0.214	0	100	100
Culex quinquefasciatus	**Temephos**	0.022	2	100	99
	Spinosad	0.078	1	100	97
	Bti	0.502	0	100	100
	Bti/Bs	0.356	0	100	100
Aedes aegypti	**Temephos**	0.014	0	100	100
	Spinosad	0.054	2	100	100
	Bti	0.382	0	100	100
	Bti/Bs	0.286	0	100	100

Bioassay 1 – Larvicides in deionised water - Bioassay 2 – Larvicides in larval habitat water

Residual Efficacy of Larvicides on Mosquito Larvae*:*

Results of residual efficacy of larvicides on *Anopheles gambiae* s.l larvae showed that *Bti/Bs* possess more residual capacity than the three other larvicides. In bioassay 1, the residual impact of Temephos, Spinosad, *Bti* and *Bti/Bs* on *Anopheles gambiae* s.l larvae lasted for 5.01, 2.37, 17.77 and 17.02 days respectively, while the effect on *C. quinquefasciatus* larvae was very minimal at 4.6, 1.64, 9.34 and 15.31 respectively (Table 3 and Fig. 3). The differences in the residual impact of the larvicides on *Anopheles gambiae* s.l larvae were only significant between Temephos and *Bti* (P=0.028), Temephos and *Bti/Bs* (P=0.019), Spinosad and *Bti* (P=0.037) and also spinosad and *Bti/Bs* (P=0.030). Likewise, the difference in residual effect on *Culex quinquefasciatus* larvae was significant in Temephos and *Bti* (P=0.016) and Temephos and *Bti/Bs* (P=0.022). As recorded in bioassay 1, the general trend for the residual effect of the larvicides in bioassay 2 (Fig. 4) was *Bti/Bs* > *Bti* > Temephos > Spinosad. The residual efficacy of the larvicides on *Culex quinquefasciatus* (Table 4) and *Aedes aegypti* (Table 5) mosquito larvae followed the same trend as seen in *Anopheles gambiae* s.l larvae. Also, the residual efficacy of the larvicides on the 3 species was lower in bioassay 2 than in bioassay 1. Generally, the residual effect of the larvicides was higher for *Aedes aegypti* species than *Anopheles gambiae* s.l and *Culex quinquefasciatus* except for *Bti* that the effect on *Aedes aegypti* and *Anopheles gambiae* s.l were almost of the same value. In bioassay 2, the differences in the residual effect were more diverse as significant differences (P<0.05) were noticed in Spinosad-*Bti* and Spinosad-*Bti/Bs* residual impact on *Aedes aegypti* larvae unlike in bioassay 1. Significant differences were observed when the residual impact of Temephos and *Bti*, Temephos and *Bti/Bs*, Spinosad and *Bti* and also spinosad and *Bti/Bs* were compared separately for both *Anopheles gambiae* s.l and *Culex quinquefasciatus*. Mortality in all controls was below 5%.

Table 3: Residual efficacy of selected larvicides on *Anopheles gambiae* s.l larvae

Mosquito Species	Bioassay	Larvicide	Mortality (%) / Days						Estimated days of residual effect
			2d	4d	7d	14d	21d	28d	
Anopheles gambiae s.l	Bioassay 1	Temephos	100	95	89	85	72	60	5.01^{ab}
		Spinosad	87	93	100	90	68	54	2.37^{cd}
		Bti	100	100	100	98	91	86	17.77^{ac}
		Bti/Bs	100	100	100	97	91	81	17.02^{bd}
	Bioassay 2	Temephos	100	98	90	78	65	49	5.64^{ef}
		Spinosad	69	89	100	87	60	51	0.26^{gh}
		Bti	100	100	100	94	92	82	15.24^{eg}
		Bti/Bs	100	100	100	97	93	78	17.57^{fh}

Numbers with same superscripts are significantly different at P < 0.05
Bioassay 1 – Larvicides in deionised water
Bioassay 2 – Larvicides in larval habitat water

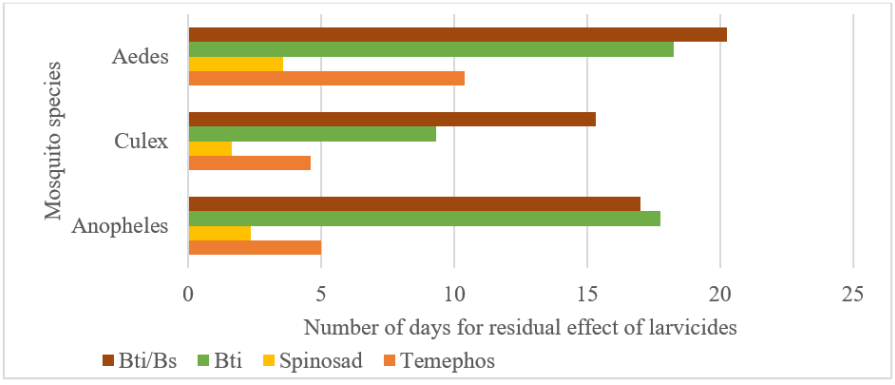

Fig. 3: Residual effects of larvicides on field mosquito larvae in deionised water.

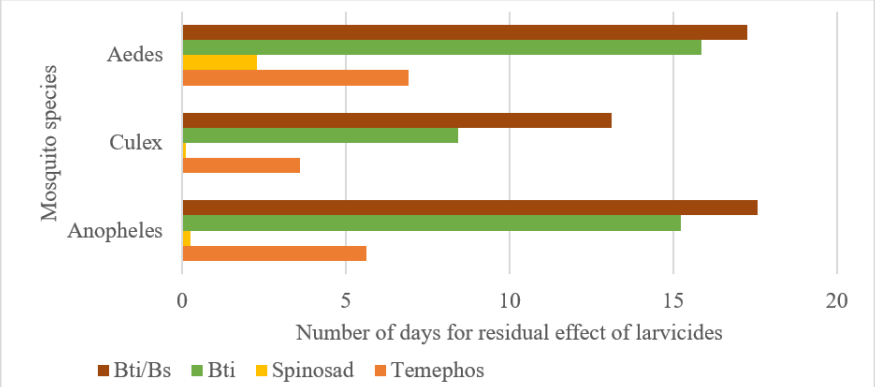

Fig. 4: Residual effects of larvicides on-field mosquito larvae in larval habitat water

Table 4: Residual efficacy of selected larvicides on *Culex quinquefasciatus* larvae

Mosquito Species	Bioassay	Larvicide	Mortality (%) / Days						Estimated days of residual effect
			2d	4d	7d	14d	21d	28d	
Culex quinquefasciatus	Bioassay 1	Temephos	100	95	90	74	68	55	4.60^{ab}
		Spinosad	83	90	100	90	73	41	1.64
		Bti	100	100	96	89	78	64	9.34^{a}
		Bti/Bs	100	100	100	96	86	81	15.31^{b}
	Bioassay 2	Temephos	100	93	83	65	53	42	3.61^{cd}
		Spinosad	63	82	98	84	63	34	0.13^{ef}
		Bti	100	100	93	90	83	67	8.43^{ce}
		Bti/Bs	100	100	100	93	82	78	13.11^{df}

Numbers with same superscripts are significantly different at $P < 0.05$
Bioassay 1 – Larvicides in deionised water
Bioassay 2 – Larvicides in larval habitat water

Table 5: Residual efficacy of selected larvicides on *Aedes aegypti* larvae

Mosquito Species	Bioassay	Larvicide	Mortality (%) / Days						Estimated days of residual effect
			2d	4d	7d	14d	21d	28d	
Aedes aegypti	Bioassay 1	Temephos	100	100	100	84	76	59	10.39
		Spinosad	90	95	100	94	71	56	3.56
		Bti	100	100	100	97	94	85	18.27
		Bti/Bs	100	100	100	98	95	86	20.27
	Bioassay 2	Temephos	100	99	94	80	62	50	6.93
		Spinosad	86	93	99	90	63	52	2.28^{ab}
		Bti	100	100	100	95	91	82	15.87^{a}
		Bti/Bs	100	100	100	96	94	80	17.26^{b}

Numbers with same superscripts are significantly different at $P < 0.05$
Bioassay 1 – Larvicides in deionised water
Bioassay 2 – Larvicides in larval habitat water

DISCUSSION

Continuous spread and increase in the intensity of insecticide resistance especially in sub-saharan Africa (WHO, 2019b) have engendered debate as regards the sufficiency of the most common methods (ITN and IRS) employed in the region in alleviating the mosquito-borne diseases. While interventions using one of the two methods have successfully reduced the transmission intensity of some diseases, it is not yet clear if the use of the two methods singly or together will achieve the critically low levels that result in the elimination of diseases such as malaria (Beier *et al*., 2008). Likewise, Fillinger and Lindsay (2011) noted that considering the fact that both IRS and ITN target the adult vector, complementary strategies such as larval source management

targeting the larval stage will be necessary for areas at the stage of malaria elimination. Historically, LSM has been found to be effective in reducing disease transmission in certain regions (Carlson, 2006; Becker, 2010), hence, evidence, as regards its effectiveness in sub-saharan Africa, is essential to its integration into the country-specific integrated vector management program. In line with this, this study was initiated to assess bioefficacy and compare the residual efficacy of some larvicides that can be employed for LSM in Lagos, Nigeria.

Acute toxicity of the selected larvicides on mosquito larvae showed that Temephos was the most toxic of the 4 larvicides on larva of *A. gambiae* s.l, *C. quinquefasciatus* and *A. aegypti* mosquito. Based on the 24h LC_{95} values, Temephos-induced mortality on *A. gambiae* s.l larvae was 3 times, 13 times and 26 times more toxic than Spinosad, *Bti*/*Bs* admixture and *Bti* respectively. A similar trend was observed for *C. quinquefasciatus* and *A. aegypti* larvae exposed to the selected larvicides. The low acute toxicity of *Bti*-based larvicide, when compared with Spinosad and Temephos in the study, conforms with previous works by Lacey (2007) and de Melo-Santos *et al*., (2009) where the larvicidal activity of *Bti* on *Culex* and *Aedes* spp was poor. Also, Marina *et al* (2014) had earlier reported significant acute toxicity of Temephos over bio-rational larvicides. Cetin *et al* (2005) and Marina *et al* (2014) have also reported significantly higher bioefficacy of Spinosad on mosquito larvae as against *Bti*. The observed high acute toxicity of mosquito larvae to Temephos and Spinosad may be due to their contact action which affects the nervous system (Gimnig *et al*., 2020) as against systemic mode of action wherein insect larvae are required to ingest considerable amounts of active ingredients for appropriate response in the case of *Bti*-based larvicides (Walker and Lynch, 2007).

The 24h-mortality response of discriminating doses of larvicides calculated from this study achieved 100% mortality on *A. gambiae* s.l and *A. aegypti* larvae when the exposure medium is deionised water and also when larvicides were dissolved in water from larvae's natural habitat. However, there was a slight change in the case of *C. quinquefasciatus* larvae when larval habitat water was the medium of exposure as mortality was a little lower than 100% for the 4 larvicides except for *Bti*. The slight differences in the activity of the discriminating doses point in the direction of species-specific activity of larvicides as documented in the works of Das and Amalraj (1997) and Hertlein *et al*., (2010).

Similarly, residual efficacy of *Bti*/*Bs* and *Bti* sustained ≥95% mosquito larval mortality between 18-21 days, which was about three times that of Temephos which sustained ≥95% larval mortality between 6-7 days. Several authors (Karch *et al,* 1991; Cetin *et al*., 2007; Anderson *et al*, 2011) have all reported on the high residual activities of *Bti*-based larvicides on mosquito larvae. Also, the low residual ability of Spinosad as recorded in the study in comparison with *Bti*-based larvicides can be explained by the ease with which Spinosyn breakdown in the presence of light. The result of the residual effect of *Bti*-based larvicides recorded in this study is similar to the results of previous research (Lacey and Lacey, 1990; Mittal, 2003), however, it was noticed that *Bti/Bs* formulation had a longer residual effect than larvicide made from *Bti* only. This has also been documented by Romi *et al*., (1993). This is attributable to the persistence of *Bs* due to the fact that *Bs* formulation uses live spores that possess a strong recycling capacity when applied (Nicolas *et al*., 1987). This recycling ability helps *Bs* to persist in the environment for a longer time than *Bti*. The higher residual efficacy of *Bti/Bs* as shown in this study makes it a better larviciding agent to be employed for an integrated vector control activity in Lagos, Nigeria. It will reduce the number of repeated applications on the field, unlike the 3 other larvicides, there reducing cost and helping to avoid several

logistic complications.

A comparison of residual efficacy of the larvicides when applied in deionised water and when dissolved in water scooped from larval habitat showed that residual efficacy of the larvicides was reduced when applied in the assay using larval water. Use of larval habitat water in this study was an attempt to simulate field operations, however, the assay was performed in the laboratory and the conditions of the laboratory are not representative of natural larval habitat conditions. This is one of the limitations of this study, however, the results from exposure to larval habitat water give an insight into possible field dynamics when larvicides are applied in Lagos, Nigeria. The reduction in residual efficacy of larvicides is probably due to differences in physico-chemical characteristics of the two water bodies. Consoli *et al*., (1995) had earlier noted that the high presence of organic matter and some other physico-chemical conditions may accelerate the breakdown or inactivation of *Bti*-based larvicides. Altogether, this result calls for proper monitoring of variables which are potential confounding factors that may reduce the efficacy of larvicides when applied on the field. These variables may include but are not limited to wide fluctuations in physico-chemical parameters of larval habitats, local eco-climatic conditions, and pollution burden, especially via man-induced activities. Another major consideration that should be looked at to make a sound choice for any larviciding activity in Lagos, Nigeria should be the formulation of the larvicides. In as much as this present study did not consider the formulations of different larvicides for comparison, a detailed review on the potentials of larvicides as a major vector control tool (Walker and Lynch, 2007) have shown that larvicide formulations are also very important. In essence, the consideration of larvicides for the control of insect larvae must take cognizance of not only the mode of action of such insecticides but the appropriate formulation must be deployed for maximum residual efficacy.

LSM through the use of larvicides has been included in Nigeria's national malaria control plan, however, its application has been very limited. Likewise, considering efforts and funds expended on IRS and ITN, appropriate use of larval source management through the application of larvicides to compliment these two other strategies is not only wise but imminent. This is particularly true in the urban part of Lagos and other urban centres in Nigeria where habitats are usually few and findable. With reference to findings from this study, the use of an appropriate discriminating dose of *Bti/Bs* for larviciding can be blended into the nation's IVM plan. While the integration of larviciding into the country IVM plan may have a significant impact on some factors like funding, larviciding requires no substantial change in human behaviour or management of key resources such as water and land. Also, its introduction as a community-driven project may help to reduce the funds that will be committed to its application and other logistics issues. The potential seen in bioefficacy of commercial formulations of different larvicides in this study calls for more serious action on the use of larval source management as a complementary strategy to IRS and ITN in Nigeria, as it has been shown to possess enough potential to complement other strategies to help eliminate malaria from the country.

Conclusion

The findings of the study revealed that commercially available Temephos, Spinosad, *Bti* and *Bti/Bs* are effective at different concentrations against *A. gambiae* s.l, *C. quinquefasciatus* and *A. aegypti* larvae in Lagos, Nigeria. Temephos is the most potent and needed in very small concentrations. Of the two *Bti*-based larvicides, an admixture of *Bti/Bs* is more potent. The residual effect of the *Bti*-based larvicides is higher than both Temephos and Spinosad. *Bti/Bs* has the highest residual impact even when used in polluted water collected from larval habitats. Pollution of larval habitat interacted with

the efficacy of the larvicides by reducing both their bioefficacy and residual effect. Considering bioefficacy and residual effect, *Bti/Bs* is a good larvicide to be employed in larval source management of mosquitoes in Lagos, Nigeria.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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