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 LC₉₅ and the LC₉₅ 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 habitats 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 LC₉₅ 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 LC₉₅. The trend of LC₉₅ (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; Narney 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 (Shiliilu 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 06° 39' 20.76 " E 003° 31'. 130"), Alapere-Ketu (N 06° 35' 02.34 " E 003° 23' 31.66"), Iponri (N 06° 29' 19.46" E 003° 21' 46.61"), Lagos Island (N 06° 27' 28.18" E 003° 23' 46.59") and Orile-Iganmu (N 06° 28’44.62" E 003° 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* mosquitos 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 LC95 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 LC95 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
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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 °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](image-url)
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

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

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Larvicides</th>
<th>Discriminating dose (mg/l)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Bioassay 1</td>
</tr>
<tr>
<td>Anopheles gambiae s.l</td>
<td>Temephos 0.016</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Spinosad 0.050</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Bti 0.420</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Bti/Bs 0.214</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Culex quinquefasciatus</td>
<td>Temephos 0.022</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Spinosad 0.078</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Bti 0.502</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Bti/Bs 0.356</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Aedes aegypti</td>
<td>Temephos 0.014</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Spinosad 0.054</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Bti 0.382</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Bti/Bs 0.286</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

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 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

<table>
<thead>
<tr>
<th>Mosquito Species</th>
<th>Bioassay</th>
<th>Larvicide</th>
<th>2d</th>
<th>4d</th>
<th>7d</th>
<th>14d</th>
<th>21d</th>
<th>28d</th>
<th>Estimated days of residual effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anopheles gambiae</em> s.l</td>
<td>Bioassay 1</td>
<td>Temephos</td>
<td>100</td>
<td>95</td>
<td>89</td>
<td>85</td>
<td>72</td>
<td>60</td>
<td>5.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinosad</td>
<td>87</td>
<td>93</td>
<td>100</td>
<td>90</td>
<td>68</td>
<td>54</td>
<td>2.37&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bti</em></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>98</td>
<td>91</td>
<td>86</td>
<td>17.77&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bti/Bs</em></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>97</td>
<td>91</td>
<td>81</td>
<td>17.02&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Bioassay 2</td>
<td>Temephos</td>
<td>100</td>
<td>98</td>
<td>90</td>
<td>78</td>
<td>65</td>
<td>49</td>
<td>5.64&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinosad</td>
<td>69</td>
<td>89</td>
<td>100</td>
<td>87</td>
<td>60</td>
<td>51</td>
<td>0.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bti</em></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>94</td>
<td>92</td>
<td>82</td>
<td>15.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bti/Bs</em></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>97</td>
<td>93</td>
<td>78</td>
<td>17.57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Mosquito Species</th>
<th>Bioassay</th>
<th>Larvicide</th>
<th>Mortality (%) / Days</th>
<th>Estimated days of residual effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td>Bioassay 1</td>
<td>Temephos</td>
<td>100 95 90 74 68 55 4.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinosad</td>
<td>83 90 100 90 73 41 1.64</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Br</em></td>
<td>100 100 96 89 78 64 9.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Br</em>/<em>Bs</em></td>
<td>100 100 100 96 86 81 15.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Bioassay 2</td>
<td></td>
<td>Temephos</td>
<td>100 93 83 65 53 42 3.61&lt;sup&gt;cd&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinosad</td>
<td>63 82 98 84 63 34 0.13&lt;sup&gt;ef&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Br</em></td>
<td>100 100 93 90 83 67 8.43&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Br</em>/<em>Bs</em></td>
<td>100 100 100 93 82 78 13.11&lt;sup&gt;ef&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Mosquito Species</th>
<th>Bioassay</th>
<th>Larvicide</th>
<th>Mortality (%) / Days</th>
<th>Estimated days of residual effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aedes aegypti</em></td>
<td>Bioassay 1</td>
<td>Temephos</td>
<td>100 100 100 84 76 59 10.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinosad</td>
<td>90 95 100 94 71 56 3.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Br</em></td>
<td>100 100 100 97 94 85 18.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Br</em>/<em>Bs</em></td>
<td>100 100 100 98 95 86 20.27</td>
<td></td>
</tr>
<tr>
<td>Bioassay 2</td>
<td></td>
<td>Temephos</td>
<td>100 99 94 80 62 50 6.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinosad</td>
<td>86 93 99 90 63 52 2.28&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Br</em></td>
<td>100 100 100 95 91 82 15.87&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Br</em>/<em>Bs</em></td>
<td>100 100 100 96 94 80 17.26&lt;sup&gt;cd&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

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 LC95 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 larvicide 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
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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