Title
Susceptibility of Anopheles gambiae complex mosquitoes to microbial larvicides in diverse ecological settings in western Kenya.

Journal
Medical and Veterinary Entomology, 33(2)

Authors
Derua, Y
Kahindi, S
Mosha, F
et al.

Publication Date
2019-06-01

DOI
10.1111/mve.12353

Peer reviewed

HHS Public Access

Author manuscript

Med Vet Entomol. Author manuscript; available in PMC 2020 February 01.

Published in final edited form as:

Med Vet Entomol. 2019 June ; 33(2): 220–227. doi:10.1111/mve.12353.

Susceptibility of *Anopheles gambiae* complex mosquitoes to microbial larvicides in diverse ecological settings in western Kenya

Y. A. DERUA[1,2], **S. C. KAHINDI**[3], **F. W. MOSHA**[1], **E. J. KWEKA**[4,5], **H. E. ATIELI**[6], **G. ZHOU**[7], **M.-C. LEE**[7], **A. K. GITHEKO**[8], **G. YAN**[7]

[1]Department of Parasitology and Entomology, Kilimanjaro Christian Medical University College, Tumaini University Makumira, Moshi, Tanzania,

[2]Department of Research Programmes, National Institute for Medical Research, Amani Research Centre, Tanga, Tanzania,

[3]Department of Zoology, School of Pure and Applied Sciences, Pwani University, Kilifi, Kenya,

[4]Division of Livestock and Human Diseases Vector Control, Tropical Pesticides Research Institute, Arusha, Tanzania,

[5]Department of Medical Parasitology and Entomology, Catholic University of Health and Allied Sciences, Mwanza, Tanzania,

[6]Department of Public Health, Maseno University, Kisumu, Kenya,

[7]Programme in Public Health, College of Health Sciences, University of California Irvine, Irvine, CA, U.S.A.

[8]Climate and Human Health Research Unit, Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya

Abstract

The microbial larvicides *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) (Bacillales: Bacillaceae) are well known for their efficacy and safety in mosquito control. In order to assess their potential value in future mosquito control strategies in western Kenya, the current study tested the susceptibility of five populations of *Anopheles gambiae* complex mosquitoes (Diptera: Culicidae), collected from five diverse ecological sites in this area, to *Bti* and *Bs* under laboratory conditions. In each population, bioassays were conducted with eight concentrations of larvicide (*Bti*/*Bs*) in four replicates and were repeated on three separate days. Larval mortality was recorded at 24 h or 48 h after the application of larvicide and subjected to probit analysis. A total of 2400 *An. gambiae* complex larvae from each population were tested for their susceptibility to *Bti* and *Bs*. The mean (± standard error of the mean, SEM) lethal concentration values of *Bti* required to achieve 50% and 95% larval mortality (LC_{50} and LC_{95}) across the five populations were 0.062 (± 0.005) mg/L and 0.797 (± 0.087) mg/L, respectively. Corresponding mean (± SEM)

Correspondence: Guiyun Yan, Programme in Public Health, College of Health Sciences, University of California Irvine, Irvine, CA 92697, U.S.A. Tel.: + 1 9498240164; Fax: + 1 9498240249; guiyuny@uci.edu.

The authors declare no conflicts of interest.

values for *Bs* were 0.058 (± 0.005) mg/L and 0.451 (± 0.053) mg/L, respectively. Statistical analysis indicated that the five populations of *An. gambiae* complex mosquitoes tested were fully susceptible to *Bti* and *Bs*, and there was no significant variation in susceptibility among the tested populations.

Keywords

Anopheles arabiensis; *Anopheles gambiae sensu stricto*; *Bacillus sphaericus*; *Bacillus thuringiensis* var. *israelensis*; larval bioassays

Introduction

The microbial larvicides *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) have gained a positive reputation in recent decades as a result of their effectiveness in controlling mosquito vectors and their limited impact on non-target organisms. The unique mode of action of *Bti* and *Bs* allows a high level of selective toxicity to target only a variety of insects, mainly mosquitoes and black flies (Lacey, 2007). Both *Bti* and *Bs* are considered important tools for integrated vector management and have the potential to control both indoor and outdoor biting mosquito vectors (Walker & Lynch, 2007). Further, their use may represent an important strategy that extends the useful life of chemical insecticides by reducing the selection pressure that results in the development of resistance. Both *Bti* and *Bs* are manufactured in various formulations such as wettable powders, a variety of granules (water-dispersible granules, corn cob grits and sand granules), flowable concentrates, slow-release tablets and briquettes (Lacey, 2007). The slow-release formulations are designed to offer flexibility in application and relatively high levels of persistence to overcome the previous operational constraints of conventional products (Afrane *et al.*, 2016; Zhou *et al.*, 2016).

Because of combination of complement toxins and unique mode of action, the development of resistance to *Bti* in target vectors was previously suggested to be unlikely (Lacey, 2007; Wirth, 2010). Field studies with repeated applications of a full complement of *Bti* toxins over several years did not observe the development of resistance in the target vectors (Becker & Ludwig, 1993; Wirth *et al.*, 2001), but other studies using fewer than four complement toxins of *Bti* indicated that resistance may develop in target vectors (Georghiou & Wirth, 1997). Of particular concern, a high level of *Bti* resistance in a field population of *Culex pipiens* (Diptera: Culicidae) was reported from New York (Paul *et al.*, 2005). The development of resistance in mosquito larvae after the use of *Bs* has also been reported (Rao *et al.*, 1995; Nielsen-Leroux *et al.*, 2002; Mulla *et al.*, 2003). Although no resistance to *Bti* and *Bs* in malaria vectors has been reported, it has been suggested that the persistence of *Bti* and *Bs* toxins in the environment may impose continuous selection pressure on mosquito populations and hence increase the risk for the evolution of resistance (Tilquin *et al.*, 2008; Paris *et al.*, 2011).

Several environmental factors and the prolonged use of chemical insecticides have been associated with the development of insecticide resistance in mosquito vectors. Thus, exposure to agrochemicals and urban pollutants has been linked to the evolution of

insecticide resistance in malaria mosquito vectors (Djouaka *et al.*, 2007; Nkya *et al.*, 2012; Tetreau *et al.*, 2014). The spread of pyrethroid resistance has reached unprecedented levels, with the rapid development of cross-resistance affecting other classes of insecticide [World Health Organization (WHO), 2017]. Cross-resistance between chemical and microbial larvicides has not been established in the field to date. However, as for most agents applied to control mosquito vectors, prolonged use of *Bti* and *Bs* may increase the risk for the development and spread of resistance. This may be further amplified by the advent and use of longlasting formulations which potentially offer the sustained release of *Bti* and *Bs* toxins over prolonged periods of time compared with short-lived conventional products.

To design effective resistance management options, it is crucial to establish the natural susceptibility of mosquito vectors to candidate insecticides before their widespread use. This information is important for detecting changes in the sensitivity of the target population so that alternative control measures can be implemented well before resistant traits spread (Wirth *et al.*, 2001). Although *Bti*/*Bs* resistance in malaria vectors has not been established in field interventions, there is no guarantee that resistance will not develop under a strategy of continuous use for target vector control in the future. Hence, there is a need to maintain regular monitoring of the susceptibility of malaria vectors in interventions deploying either chemical or microbial larvicides. The current study monitored the level of susceptibility of larvae of *Anopheles gambiae* complex mosquitoes to *Bti* and *Bs* in diverse ecological settings in western Kenya to inform a programme proposed for the control of malaria transmission using longlasting microbial larvicides based on *Bti* and *Bs*.

Materials and methods

Study area

The study was conducted in five different locations in western Kenya, namely Ahero (0.13862°S, 34.94173°E), Kisian (0.08709°S, 34.68099°E), Chulaimbo (0.03525°S, 34.61929°E), Iguhu (0.16176°N, 34.76160°E) and Bungoma (0.63255°N, 34.39565°E). Ahero, Kisian and Chulaimbo are in Kisumu County, and Iguhu and Bungoma are in Kakamega and Bungoma Counties, respectively (Fig. 1, Table 1). *Anopheles* vectors at these sites have developed various levels of resistance to several major insecticides that are commonly used for malaria vector control or for agricultural pest control (Wanjala *et al.*, 2015a). Details on the topography, weather conditions, human settlements and agricultural activities of the study sites have been reported elsewhere (Petrarca *et al.*, 1991; Githeko *et al.*, 2006; Bukhari *et al.*, 2011; Kweka *et al.*, 2012; Ochomo *et al.*, 2013; Zhou *et al.*, 2013; Degefa *et al.*, 2017; Ngugi *et al.*, 2017). In brief, Ahero is located about 22 km east of Kisumu City and has a well-developed irrigation scheme mainly used for rice cultivation. Its annual rainfall ranges from 1000 mm to 1800 mm. Malaria transmission occurs through-out the year. *Anopheles gambiae sensu stricto*, *Anopheles arabiensis* and *Anopheles funestus* are the main vectors.

Kisian is a lowland village located on the shores of Lake Victoria, about 10 km west of Kisumu City. It is characterized by a semi-urban to rural setting and the local population is engaged in formal and informal economic activities including subsistence agriculture and livestock keeping. Annual rainfall averages 1000–1500 mm; there is a long rainy season

from March to May and a relatively shorter one from September to November. Malaria is holoendemic and transmitted by *An. gambiae s.s.*, *An. arabiensis* and *An. funestus*. Chulaimbo is a small town located about 19 km west of Kisumu City along the Kisumu–Busia road. Its average annual rainfall is 1352 mm. The area is densely populated and most inhabitants are small-scale subsistence farmers. Brick making is an important economic activity and the pits left after soil has been excavated for bricks create important breeding habitats for malaria mosquitoes.

By contrast with the lowland sites, Bungoma and Iguhu are located in the western Kenyan highlands. Bungoma has both urban and rural areas. The main economic activities in the rural areas (where most malaria transmission occurs) are small-and large-scale farming and livestock keeping. The area has two rainy seasons (annual average: 2488 mm), with the long rains occurring from March to May and the short rains from October to December. Iguhu is a densely populated, malaria epidemic-prone village in the western Kenyan highlands. Its topography is characterized by hills and valleys in which human habitations are sited and farming is conducted. The inhabitants practise subsistence farming and livestock keeping. Small-scale gold mining is also practised in the valley bottom and pits left by miners provide favourable breeding habitats for malaria mosquitoes. Iguhu has been the site of intensive entomological studies and interventions. Mosquito larvae control interventions implemented in this village between 2010 and 2012 included the application of two rounds of non-residual *Bti* (CG formulation, VectoMax; Valent BioSciences Corp., Libertyville, IL, U.S.A.) and one round of a longlasting microbial larvicide formulation that combined both *Bti* and *Bs* (FourStar™; Adapco, Inc., Sanford, FL, U.S.A.) (Zhou *et al.*, 2013; Afrane *et al.*, 2016). During the current study, Iguhu village was under preparation for the implementation of a malaria transmission control strategy using longlasting microbial larvicides (Zhou *et al.*, 2016).

Larvae collections

Anopheles gambiae complex larvae were collected from different habitats in the five study sites using 350-mL mosquito dippers. Searches were conducted in typical *An. gambiae* complex larval habitats (small, clear, temporary water bodies exposed to direct sunlight). A sub-sample of fourth instars from each collection were re-examined subsequently to confirm their species identity in the laboratory based on morphological criteria. Efforts were made to collect larvae from a variety of anopheline larval habitats at different points in each of the collection sites in order to expand the gene pool of the samples collected. Upon collection, larvae were transferred to the insectary, sorted by larval instars and maintained by following recommended standard mosquito rearing techniques (Benedict, 2007). Larvae were fed on TetraMin® fish food (Tetra Holding, Inc., Blacksburg, VA, U.S.A.) before and during bioassays. Standard insectary-reared larvae of *An. gambiae s.s.* (Kisumu strain), a colony that had been maintained for several generations at the insectary of the Kenya Medical Research Institute, Kisumu, were used for the purposes of comparison. The Kisumu strain of *An. gambiae s.s.* is a reference strain susceptible to all insecticides and has been used extensively in bioassay experiments across Africa (Chandre *et al.*, 1999). Efforts were made to include only third instars in subsequent microbial larvicide bioassays.

Preparation of test larvicides

Microbial larvicides *Bti* [potency: 7000 international toxic units (ITU)/mg] and *Bs* (potency: 1000 ITU/mg), both sourced from Becker Microbial Products, Inc. (Parkland, FL, U.S.A.), were used in larval bioassays. Stock solutions of *Bti* and *Bs* were prepared by dissolving 200 mg of powder of the respective microbial larvicide in 20 mL of distilled water. The resultant 10-mg/mL stock solution was kept frozen in 2-mL aliquots until use. On the day of the experiment, one aliquot of stock solution of either *Bti* or *Bs* was thawed and serially diluted in distilled water as recommended (WHO, 2005). In brief, a 10-fold dilution series was prepared by first transferring 2 mL of stock solution to 18 mL of distilled water to make a 1.0-mg/mL concentration. This procedure was subsequently repeated by transferring 2 mL of the most recent solution to 18 mL of distilled water to make 0.1-mg/mL, 0.01-mg/mL and 0.001-mg/mL concentrations of the respective *Bti* or *Bs* larvicide. The last three dilutions (0.1 mg/mL, 0.01 mg/mL and 0.001 mg/mL) were used in subsequent larvicide bioassays. Distilled water was used in control test cups.

Larval Bioassays

For each of the five selected study sites, two larval bioassays that tested the susceptibility of wild populations of *An. gambiae* complex mosquito larvae to *Bti* and *Bs*, respectively, were conducted. Two additional bioassay experiments testing *Bti* and *Bs* against a susceptible reference laboratory strain (*An. gambiae s.s.*, Kisumu strain) were conducted for the purposes of comparison. Each larval bioassay (with either *Bti* or *Bs*) involved the testing of eight larvicide concentrations (including a negative control) in four replicates and was repeated on three different days. At the beginning of the experiments, 25 third instars were transferred from the larvae rearing pans to labelled disposable styrofoam test cups (Hotpack Packaging Ind. LLC, Dubai, UAE) containing 100 mL of rainwater using disposable Pasteur pipettes. Using pipettes with disposable tips, and starting with the lowest concentration, appropriate volumes (0.2–1.0 mL) of each of the three last dilutions of *Bti*/*Bs* (0.1 mg/mL, 0.01 mg/mL and 0.001 mg/mL) were added to the experimental cups (with mosquito larvae) to give final concentrations of 0.0 (control), 0.005, 0.01, 0.03, 0.05, 0.1, 0.2/0.3 and 1.0 parts per million (p.p.m.; equivalent to mg/L). The last three dilutions were selected after a series of bioassays conducted to establish mortality in test larvae ranged from 0% to 100%. The test cups were maintained at 28 °C and under an LD 12 : 12 h photoperiod. In *Bs* experiments, which were run for 48 h, test larvae were provided with larval food at 24 h after the onset of each experiment. Larval mortality was recorded at 24 h and 48 h after the addition of *Bti* and *Bs*, respectively.

Data analysis

Data were entered into Excel and subsequently transferred to text file (Notepad) for analysis. The concentrations of the larvicides *Bti* and *Bs* that caused 50% and 95% mortality (LC_{50} and LC_{95}, respectively) in test larvae, and lethal concentration ratios including 95% confidence limits, were calculated using the probit/logit analysis program PoloPlus (Robertson & Preisler, 2003).

Results

To examine the degree of variation in the susceptibility of *An. gambiae* complex larvae to *Bti* and *Bs*, larval collections from five sites with varying ecological characteristics were bioassayed with the two larvicides in 2016 and 2017 (Table 1). From each collection site, a total of 2400 (2100 subjects and 300 control larvae) third instars of *An. gambiae* complex larvae were tested for their susceptibility to each larvicide.

For *Bti*, overall LC_{50} and LC_{95} values for all five tested sites ranged from 0.052 mg/L to 0.081 mg/L and from 0.544 mg/L to 1.014 mg/L, respectively. Based on LC_{95} values, Chulaimbo had the least and Kisian the most susceptible larvae (Table 2). The mean (± standard error of the mean, SEM) LC_{50} and LC_{95} values for *Bti* across the five tested sites were 0.062 (± 0.005) mg/L and 0.797 (± 0.087) mg/L, respectively. The LC_{50} and LC_{95} values for the susceptible laboratory strain (*An. gambiae s.s.*,Kisumu strain) bioassayed alongside the wild-collected larvae were 0.054 mg/L and 0.584 mg/L, respectively (Table 2).

For *Bs*, the overall LC_{50} and LC_{95} values for all five tested sites ranged from 0.046 mg/L to 0.070 mg/L and from 0.353 mg/L to 0.628 mg/L, respectively. Based on LC_{95} values, Chulaimbo had the least and Bungoma the most susceptible larvae (Table 2). The mean (± SEM) LC_{50} and LC_{95} values for *Bs* across the five tested sites were 0.058 (± 0.005) mg/L and 0.451 (± 0.053) mg/L, respectively. The LC_{50} and LC_{95} values for the susceptible laboratory strain (*An. gambiae s.s.*, Kisumu strain) bioassayed alongside the wild-collected larvae were 0.056 mg/L and 0.545 mg/L, respectively (Table 2). Examination of the 95% confidence limits for LC_{50} and LC_{95} values in the five populations of *An. gambiae* complex mosquitoes showed extensive overlaps, indicating a lack of significant variation in susceptibility to both *Bti* and *Bs*.

To further examine whether there was any variation in the susceptibility of *An. gambiae* complex larvae from the five populations to *Bti* and *Bs*, lethal concentration ratios were calculated by comparing the wild-collected larvae with the reference laboratory strain (Table 3). For *Bti*, lethal concentration ratio values at LC_{50} and LC_{95} for all five *An. gambiae* complex populations ranged from 0.667 to 1.035 and from 0.576 to 1.074, respectively. For *Bs*, these values ranged from 0.818 to 1.213 and from 0.792 to 1.546, respectively (Table 3). For both *Bti* and *Bs*, lethal concentration ratios at LC_{50} and LC_{95} were < 2.0 at all tested sites, thus confirming a lack of significant variation in the susceptibility of larvae to the microbial larvicides tested (Table 3).

Discussion

Despite widespread insecticide resistance, the control of malaria vectors still relies heavily on the use of chemical insecticides applied indoors in the form of longlasting insecticidal nets (LLINs) and indoor residual spraying (IRS). However, the application of larvicide, preferably using microbial agents, has been shown to complement indoor-based interventions. It also has the potential to delay the development of resistance and to control both indoor and outdoor biting vectors. The current study monitored the level of susceptibility of *An. gambiae* complex larvae collected from five sites with diverse

ecological characteristics relevant to the evolution and spread of insecticide resistance. The sites included an area known for pyrethroid resistance, a rice-growing agricultural ecosystem, an area characterized by the use of indoor insecticide and microbial larvicide, an area without history of microbial larvicide use, and a semi-urban area predisposed to urban pollutants (Table 1).

In the current study, wild *An. gambiae* complex larvae collected from five study sites were tested for their level of susceptibility to *Bti* and *Bs* under laboratory conditions. The lethal concentration values (especially LC_{90} or LC_{95}) obtained in laboratory bioassays represent the minimum effective dosages from which field application concentrations are derived, usually as multiple concentrations of LC_{90} or LC_{95} (WHO, 2005). The LC_{95} values recorded in this study compare fairly well with concentrations (amount of active ingredient per surface area in kg/ha) achieved in field interventions to control malaria vectors in African settings (Fillinger & Lindsay, 2006; Fillinger *et al.*, 2008). The larvae tested were found to be fully susceptible to *Bti* and *Bs* when compared with a reference susceptible laboratory strain. The findings also showed that *An. gambiae* complex larvae from the five tested sites had limited and insignificant variation in their levels of susceptibility to *Bti* and *Bs* at both LC_{50} and LC_{95}. The slight variation in lethal concentration values (LC_{50} and LC_{95}) observed at the five different test sites most likely represented a natural biological variability in susceptibility, which has also been reported in other bioassay studies (Becker & Ludwig, 1993; Wirth *et al.*, 2001; Vasquez *et al.*, 2009).

The susceptibility of larvae of the *An. gambiae* complex to *Bti* and *Bs* at the site with a history of repeated applications of microbial larvicides (Iguhu) did not differ significantly from that at the site at which no microbial larvicides had been used (Chulaimbo). Interestingly, the lethal concentration values (LC_{50} and LC_{95}) for both *Bti* and *Bs* were slightly higher in Chulaimbo than in Iguhu, but the difference was not statistically significant. This may reflect the fact that no *Bti* or *Bs* resistance has been reported in *An. gambiae* complex mosquitoes following repeated applications, although resistance has been reported in *Cx. pipiens* and *Culex quinquefasciatus* (Rao *et al.*, 1995; Nielsen-Leroux *et al.*, 2002; Mulla *et al.*, 2003; Paul *et al.*, 2005). This particular finding corroborates that of Becker & Ludwig (1993), who reported a lack of variation in susceptibility between *Bti*-treated and untreated field populations of *Aedes vexans* (Diptera: Culicidae). In the same study, the population from treated areas was found to be even more susceptible to *Bti* than untreated populations (Becker & Ludwig, 1993).

Of particular relevance to the control of malaria vectors, larvae collected in a site with a high level of pyrethroid insecticide resistance (Bungoma) were fully susceptible to *Bti* and *Bs*. The lethal concentration values recorded in samples from this site were comparable with those in samples from the other four tested sites, which had been reported to have low to moderate resistance (Ochomo *et al.*, 2013; Wanjala *et al.*, 2015a, 2015b). Although pyrethroid insecticides are known to select and confer cross-resistance to multiple classes of insecticide used in mosquito control (Kulkarni *et al.*, 2006; Protopopoff *et al.*, 2013), this phenomenon has not been reported to occur with the microbial larvicides *Bti* and *Bs*. Studies conducted elsewhere have shown a lack of cross-resistance between chemical insecticides and microbial larvicides (Vasquez *et al.*, 2009; Araújo *et al.*, 2013). On the other hand,

larvae collected from the rice irrigation ecosystem (Ahero) and the semi-urban area (Kisian) were fully susceptible to *Bti* and *Bs*. The susceptibility of larvae populations from these two sites did not differ significantly from that at the remaining three tested sites. These findings suggest that, at least for now, the sensitivity of *An. gambiae* complex larvae to *Bti* and *Bs* has not been affected by agricultural activities and urban pollutants present in the study areas. Agricultural activities and urban pollutants are known to contribute to the evolution of resistance to chemicals used in mosquito control or agricultural activities (Djouaka *et al.*, 2007; Nkya *et al.*, 2012; Tetreau *et al.*, 2014).

The susceptibility of mosquito larvae to microbial larvicides has been found to be influenced by a range of biotic and abiotic factors (Becker *et al.*, 1992, 1993; Lacey, 2007). Of relevance to the current study, the species of the target mosquito is among the factors reported to affect the activity of *Bti* and *Bs*. In this study, the susceptibility of larvae from sites at which *An. gambiae s.s.* is the predominant species (Iguhu, Bungoma and Chulaimbo) did not differ significantly from that of larvae from sites at which *An. arabiensis* predominates (Kisian and Ahero). A previous study reported a lack of variation in susceptibility to *Bti* between *An. gambiae s.s.* and *An. arabiensis* at LC_{50}, but the former was found to be significantly more susceptible at LC_{90} (Ketseoglou *et al.*, 2011). However, comparisons of susceptibility between *An. gambiae s.s.* and *An. arabiensis* in this study should be interpreted with caution as some degree of the co-occurrence of the two sibling species in any one of the test sites is possible (Table 1). As comparisons between bioassay-based studies have been discouraged in view of variations in test conditions and formulations (Skovmand *et al.*, 1998; Otieno-Ayayo *et al.*, 2008), the present findings suggest a lack of significant variation in susceptibility to *Bti* and *Bs* in the populations of *An. gambiae s.s.* and *An. arabiensis* tested.

Conclusions

Mosquito larvae bioassays are crucial for detecting resistance in the early stages of a control strategy, before the widespread use of larvicides, and for monitoring future changes in susceptibility. The five populations of *An. gambiae* complex mosquitoes tested were fully susceptible to *Bti* and *Bs* and no significant variation in susceptibility between the tested sites was recorded. The present findings suggest that the microbial larvicides *Bti* and *Bs* can still be used in areas in which insecticide resistance has been recorded, in areas with a previous history of microbial larvicide use, in areas predisposed to urban pollutants and in areas with intensive agricultural activities.

Acknowledgements

The authors are grateful to the inhabitants of the study villages for allowing investigators to collect larvae in habitats located in their villages and farms. The project technicians: Jesse Agalomba, Maxwell Machani, Charles Otieno, Bernard Alele and Enock Onyango (Climate and Human Health Research Unit, Kenya Medical Research Institute, Kisumu, Kenya), are thanked for their technical support during the field larvae collection, mosquito rearing and laboratory bioassays. This study received financial support from the National Institutes of Health (grant nos. R01 AI050243, U19 AI129326 and D43 TW001505). Drawings of the mosquito larvae and adult included in the graphical abstract were generously provided by Cheryl White-horn, London School of Hygiene and Tropical Medicine, London, U.K. This paper is published with the permission of the director of the Kenya Medical Research Institute.

References

Afrane YA, Mweresa NG, Wanjala CL et al. (2016) Evaluation of long-lasting microbial larvicide for malaria vector control in Kenya. Malaria Journal, 15, 577. [PubMed: 27903292]

Araújo AP, Diniz DFA, Helvecio E et al. (2013) The susceptibility of *Aedes aegypti* populations displaying temephos resistance to *Bacillus thuringiensis israelensis*: a basis for management. Parasites & Vectors, 6, 297. [PubMed: 24499507]

Becker N & Ludwig M (1993) Investigations on possible resistance in *Aedes vexans* field populations after a 10-year application *of Bacillus thuringiensis israelensis*. Journal of the American Mosquito Control Association, 9, 221–224. [PubMed: 8350079]

Becker N, Zgomba M, Ludwig M, Petric D & Rettich F (1992) Factors influencing the activity of *Bacillus thuringiensis* var. *israelensis* treatments. Journal of the American Mosquito Control Association, 8, 285–289. [PubMed: 1357088]

Becker N, Ludwig M, Beck M & Zgomba M (1993) The impact of environmental factors on efficacy of *Bacillus sphaericus* against *Culex pipiens*. Bulletin of the Society of Vector Ecologists, 18, 61–66.

Benedict MQ (2007) Methods in Anopheles Research. Centers for Disease Control and Prevention, Atlanta, GA.

Bukhari T, Takken W, Githeko AK & Koenraadt CJM (2011) Efficacy of aquatain, a monomolecular film, for the control of malaria vectors in rice paddies. PLoS One, 6, e21713. [PubMed: 21738774]

Chandre F, Darrier F, Manga L et al. (1999) Status of pyrethroid resistance in *Anopheles gambiae sensu lato*. Bulletin of the World Health Organization, 77, 230–234. [PubMed: 10212513]

Degefa T, Yewhalaw D, Zhou G et al. (2017) Indoor and outdoor malaria vector surveillance in western Kenya: implications for better understanding of residual transmission. Malaria Journal, 16, 443. [PubMed: 29110670]

Djouaka RF, Bakare AA, Bankole HS et al. (2007) Does the spillage of petroleum products in *Anopheles* breeding sites have an impact on the pyrethroid resistance? Malaria Journal, 6, 159. [PubMed: 18053173]

Fillinger U & Lindsay SW (2006) Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. Tropical Medicine and International Health, 11, 1629–1642. [PubMed: 17054742]

Fillinger U, Kannady K, William G et al. (2008) A tool box for operational mosquito larval control: preliminary results and early lessons from the Urban Malaria Control Programme in Dar es Salaam, Tanzania. Malaria Journal, 7, 20. [PubMed: 18218148]

Georghiou GP & Wirth MC (1997) Influence of exposure to single versus multiple toxins of *Bacillus thuringiensis* subsp. *israelensis* on development of resistance in the mosquito *Culex quinquefasciatus* (Diptera: Culicidae). Applied and Environmental Microbiology, 63, 1095–1101. [PubMed: 16535542]

Githeko AK, Ayisi JM, Odada PK et al. (2006) Topography and malaria transmission heterogeneity in western Kenya highlands: prospects for focal vector control. Malaria Journal, 5, 107. [PubMed: 17096835]

Ketseoglou I, Koekemoer LL, Coetzee M & Bouwer G (2011) The larvicidal efficacy of *Bacillus thuringiensis* subsp. *israelensis* against five African *Anopheles* (Diptera: Culicidae) species. African Entomology, 19, 146–150.

Kulkarni MA, Rowland M, Alifrangis M et al. (2006) Occurrence of the leucine-to-phenylalanine knockdown resistance (kdr) mutation in *Anopheles arabiensis* populations in Tanzania, detected by a simplified high-throughput SSOP-ELISA method. Malaria Journal, 5, 56. [PubMed: 16820067]

Kweka EJ, Zhou G, Munga S et al. (2012) Anopheline larval habitats seasonality and species distribution: a prerequisite for effective targeted larval habitats control programmes. PLoS One, 7, e52084. [PubMed: 23272215]

Lacey LA (2007) *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. Journal of the American Mosquito Control Association, 23, 133–163. [PubMed: 17853604]

Mulla MS, Thavara U, Tawatsin A, Chomposri J & Su T (2003) Emergence of resistance and resistance management in field populations of tropical *Culex quinquefasciatus* to the microbial control agent *Bacillus sphaericus*. Journal of the American Mosquito Control Association, 19, 39–46. [PubMed: 12674533]

Ngugi HN, Mutuku FM, Ndenga BA et al. (2017) Characterization and productivity profiles of *Aedes aegypti* (L.) breeding habitats across rural and urban landscapes in western and coastal Kenya. Parasites & Vectors, 10, 331. [PubMed: 28701194]

Nielsen-Leroux C, Pasteur N, Prètre J, Charles J-F, Sheikh HB & Chevillon C (2002) High resistance to *Bacillus sphaericus* binary toxin in *Culex pipiens* (Diptera: Culicidae): the complex situation of west Mediterranean countries. Journal of Medical Entomology, 39, 729–735. [PubMed: 12349855]

Nkya TE, Akhouayri I, Kisinza W & David JP (2012) Impact of environment on mosquito response to pyrethroid insecticides: facts, evidences and prospects. Insect Biochemistry and Molecular Biology, 43, 407–416. [PubMed: 23123179]

Ochomo E, Bayoh MN, Brogdon WG et al. (2013) Pyrethroid resistance in *Anopheles gambiae s.s.* and *Anopheles arabiensis* in western Kenya: phenotypic, metabolic and target site characterizations of three populations. Medical and Veterinary Entomology, 27, 156–164. [PubMed: 22861380]

Otieno-Ayayo ZN, Zaritsky A, Wirth MC et al. (2008) Variations in the mosquito larvicidal activities of toxins from *Bacillus thuringiensis* ssp. *israelensis*. Environmental Microbiology, 10, 2191–2199. [PubMed: 18637949]

Paris M, Tetreau G, Laurent F, Lelu M, Despres L & David JP (2011) Persistence of *Bacillus thuringiensis israelensis* (*Bti*) in the environment induces resistance to multiple *Bti* toxins in mosquitoes. Pest Management Science, 67, 122–128. [PubMed: 21162152]

Paul A, Harrington LC, Zhang L & Scott JG (2005) Insecticide resistance in *Culex pipiens* from New York. Journal of the American Mosquito Control Association, 21, 305–309. [PubMed: 16252522]

Petrarca V, Beier JC, Onyango F et al. (1991) Species composition of the *Anopheles gambiae* complex (Diptera: Culicidae) at two sites in western Kenya. Journal of Medical Entomology, 28, 307–313. [PubMed: 1875359]

Protopopoff N, Matowo J, Malima R et al. (2013) High level of resistance in the mosquito *Anopheles gambiae* to pyrethroid insecticides and reduced susceptibility to bendiocarb in north-western Tanzania. Malaria Journal, 12, 149. [PubMed: 23638757]

Rao DR, Mani TR, Rajendran R, Joseph AS, Gajanana A & Reuben R (1995) Development of a high level of resistance to *Bacillus sphaericus* in a field population of *Culex quinquefasciatus* from Kochi, India. Journal of the American Mosquito Control Association, 11, 1–5. [PubMed: 7616173]

Robertson JL & Preisler HRR (2003) PoloPlus: Probit and Logit Analysis User's Guide. LeOra Software, Petaluma, CA.

Service MW (1977) Mortalities of the immature stages of species B of the *Anopheles gambiae* complex in Kenya: comparison between rice fields and temporary pools, identification of predators, and effects of insecticidal spraying. Journal of Medical Entomology, 13, 535–545. [PubMed: 845895]

Skovmand O, Thiery I, Benzon GL, Sinègre G, Monteny N & Becker N (1998) Potency of products based on *Bacillus thuringiensis* var. *israelensis*: interlaboratory variations. Journal of the American Mosquito Control Association, 14, 298–304. [PubMed: 9813828]

Tetreau G, Chandor-Proust A, Faucon F et al. (2014) UV light and urban pollution: bad cocktail for mosquitoes? Aquatic Toxicology, 146, 52–60. [PubMed: 24275062]

Tilquin M, Paris M, Reynaud S et al. (2008) Long lasting persistence of *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) in mosquito natural habitats. PLoS One, 3, e3432. [PubMed: 18941501]

Vasquez MI, Violaris M, Hadjivassilis A & Wirth MC (2009) Susceptibility of *Culex pipiens* (Diptera: Culicidae) field populations in Cyprus to conventional organic insecticides, *Bacillus thuringiensis* subsp. *israelensis*, and methoprene. Journal of Medical Entomology, 46, 881–887. [PubMed: 19645293]

Walker K & Lynch M (2007) Contribution of *Anopheles* larvae control to malaria suppression in tropical Africa: review of achievements and potential. Medical and Veterinary Entomology, 21, 2–21. [PubMed: 17373942]

Wanjala CL, Mbugi JP, Ototo E et al. (2015a) Pyrethroid and DDT resistance and organophosphate susceptibility among *Anopheles* spp. mosquitoes, western Kenya. Emerging Infectious Diseases, 21, 2178–2181. [PubMed: 26583525]

Wanjala CL, Zhou G, Mbugi J et al. (2015b) Insecticidal decay effects of long-lasting insecticide nets and indoor residual spraying on *Anopheles gambiae* and *Anopheles arabiensis* in Western Kenya. Parasites & Vectors, 8, 588. [PubMed: 26567915]

World Health Organization (2005) Guidelines for Laboratory and Field Testing of Mosquito Larvicides WHO Pesticides Evaluation Scheme: WHO/CDS/WHOPES/GCDPP/2005.13, WHO, Geneva.

World Health Organization (2017) World Malaria Report 2017. WHO, Geneva.

Wirth M (2010) Mosquito resistance to bacterial larvicidal toxins. Open Toxicology Journal, 3, 126–140.

Wirth MC, Ferrari JA & Georghiou GP (2001) Baseline susceptibility to bacterial insecticides in populations of *Culex pipiens* complex (Diptera: Culicidae) from California and from the Mediterranean island of Cyprus. Journal of Economic Entomology, 94, 920–928. [PubMed: 11561853]

Zhou G, Githeko AK, Minakawa N & Yan G (2010) Community-wide benefits of targeted indoor residual spray for malaria control in the western Kenya highland. Malaria Journal, 9, 67. [PubMed: 20199674]

Zhou G, Afrane YA, Vardo-Zalik AM et al. (2011) Changing patterns of malaria epidemiology between 2002 and 2010 in western Kenya: the fall and rise of malaria. PLoS One, 6, e20318. [PubMed: 21629783]

Zhou G, Afrane YA, Dixit A et al. (2013) Modest additive effects of integrated vector control measures on malaria prevalence and transmission in western Kenya. Malaria Journal, 12, 256. [PubMed: 23870708]

Zhou G, Wiseman V, Atieli HE, Lee M-C, Githeko AK & Yan G (2016) The impact of long-lasting microbial larvicides in reducing malaria transmission and clinical malaria incidence: study protocol for a cluster randomized controlled trial. Trials, 17, 423. [PubMed: 27558161]

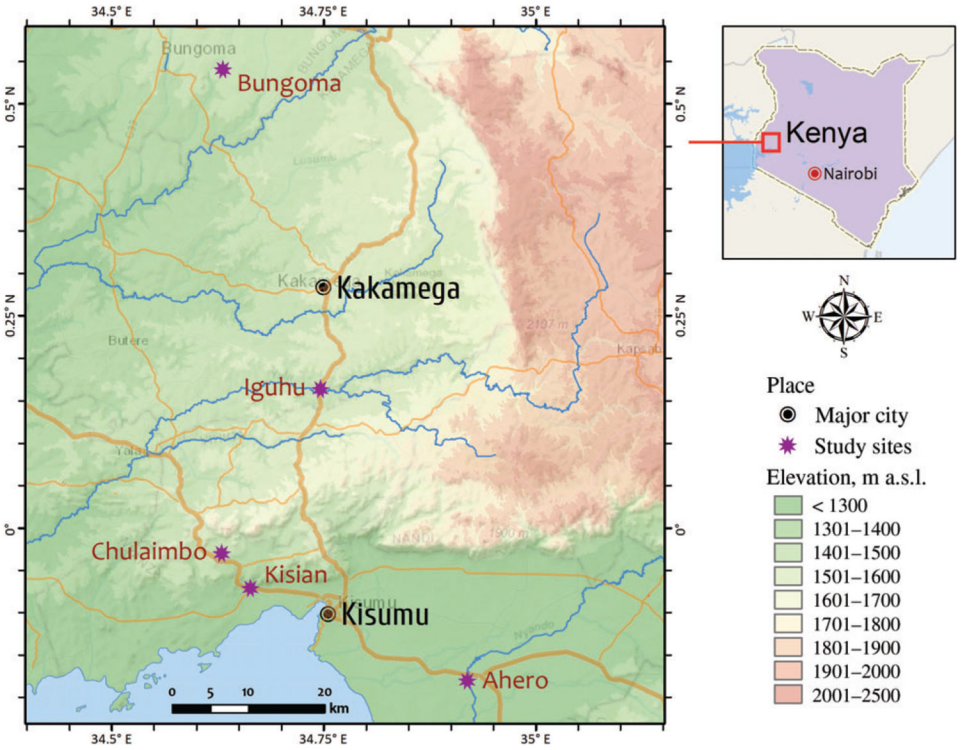

Fig. 1.
Location of study sites in western Kenya.

Table 1.

Characteristics of the five study sites in western Kenya at which *Anopheles gambiae* complex larvae were collected and tested for susceptibility to *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*).

Study site	Altitude, m a.s.l.	Collection date	Larval habitat type	Site-specific attributes	Composition of *An. gambiae* complex	References
Iguhu	1450	February 2016	Abandoned gold mines	Malaria vector control by LLINs, IRS and microbial larvicides reported[1]	~ 88% *An. gambiae sensu stricto*[2]	[1]Zhou *et al.*, 2010, 2011, 2016; Afrane etal., 2016
						[2]Degefa *et al.*, 2017
Kisian	1149	July 2016	Roadside canals, lakeside pools	Lakeside, predisposed to urban pollutants	~ 64% *An. arabiensis*[3]	[3]Wanjala *et al.*, 2015a
Ahero	1150	July 2017	Rice fields, roadside canals	Intensive use of pesticides, herbicides and fertilizers[4]	~ 100% *An. arabiensis*[5]	[4]Service, 1977; Bukhari *et al.*, 2011
						[5]Ochomo *et al.*, 2013; Degefa *et al.*, 2017
Chulaimbo	1397	July 2017	Pools and puddles *	Malaria vector control limited to LLINs	~ 72% *An. Gambiae s.s.*[6]	[6]Wanjala *et al.*, 2015a
Bungoma	1381	March 2017	Roadside canals, drainage ditches, ponds, pools	High levels of pyrethroid, DDT and bendiocarb resistance[7]	~ 88% *An. gambiae s.s.*[7]	[7]Ochomo *et al.*, 2013; Wanjala *et al.*, 2015a

* Created by brick makers.

IRS, indoor residual spraying; LLINs, longlasting insecticidal nets.

Table 2.

Level of susceptibility of larvae of *Anopheles gambiae* complex to microbial larvicides *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) in different ecological settings in western Kenya.

Mosquito source	Biolarvicide	Larvae tested, *n* *	LC_{50} † (95% CI)	LC_{95} † (95% CI)	Slope ± SE	χ^2	Heterogeneity
Laboratory strain	*Bti*	2400	0.054 (0.034–0.087)	0.584 (0.278–2.467)	1.591 ± 0.062	62.852	12.57
	Bs	2400	0.056 (0.041–0.076)	0.545 (0.325–1.212)	1.661 ± 0.066	27.450	5.49
Iguhu	*Bti*	2400	0.053 (0.037–0.076)	0.743 (0.393–2.094)	1.434 ± 0.057	33.481	6.70
	Bs	2400	0.049 (0.039–0.062)	0.387 (0.261–0.685)	1.839 ± 0.079	17.673	3.53
Kisian	*Bti*	2400	0.052 (0.034–0.078)	0.544 (0.287–1.668)	1.616 ± 0.063	48.096	9.62
	Bs	2400	0.057 (0.043–0.074)	0.370 (0.243–0.706)	2.021 ± 0.081	28.355	5.67
Ahero	*Bti*	2400	0.058 (0.036–0.092)	0.713 (0.342–2.795)	1.510 ± 0.062	50.950	10.19
	Bs	2400	0.066 (0.053–0.080)	0.515 (0.363–0.833)	1.840 ± 0.083	12.107	2.42
Chulaimbo	*Bti*	2400	0.067 (0.048–0.095)	1.014 (0.548–2.642)	1.393 ± 0.057	26.140	5.23
	Bs	2400	0.070 (0.054–0.091)	0.628 (0.400–1.226)	1.726 ± 0.076	18.711	3.74
Bungoma	*Bti*	2400	0.081 (0.066–0.101)	0.973 (0.642–1.693)	1.523 ± 0.059	12.835	2.57
	Bs	2400	0.046 (0.036–0.058)	0.353 (0.236–0.631)	1.859 ± 0.078	19.129	3.83

*2100 subject and 300 control larvae (control mortality did not exceed 3% in any experiment) for each experiment.

†mg/L (p.p.m.) at 24 h and 48 h for *Bti* and *Bs*, respectively.

CI, confidence interval; SE, standard error.

Table 3.

Lethal concentration ratios for microbial larvicides *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) in the five study sites in western Kenya.

	Bti*: lethal concentration ratios		***Bs*: lethal concentration ratios***	
Collection site	**LC_{50} (95% CI)**	**LC_{95} (95% CI)**	**LC_{50} (95% CI)**	**LC_{95} (95% CI)**
Iguhu	1.019 (0.883–1.175)	0.786 (0.572–1.079)	1.130 (0.985–1.296)	1.408 (1.062–1.868)
Kisian	1.035 (0.901–1.189)	1.074 (0.801–1.439)	0.982 (0.861–1.120)	1.473 (1.130–1.920)
Ahero	0.930 (0.805–1.075)	0.819 (0.599–1.119)	0.849 (0.737–0.978)	1.059 (0.793–1.415)
Chulaimbo	0.806 (0.695–0.936)	0.576 (0.414–0.801)	0.818 (0.706–0.949)	0.792 (0.577–1.088)
Bungoma	0.667 (0.579–0.768)	0.600 (0.439–0.821)	1.213 (1.060–1.389)	1.546 (1.169–2.045)

*Compared with a susceptible reference laboratory strain (*Anopheles gambiae s.s.*, Kisumu strain).

CI, confidence interval.