

Community-led application of *Bacillus thuringiensis* var. *israelensis* (Bti) effectively reduces malaria vector densities in The Gambia

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Abstract. Jassey B, Yudhastuti R, Jallow O, Touray I, Ridha MR, Diyanah KC. 2025. Community-led application of *Bacillus thuringiensis* var. *israelensis* (Bti) effectively reduces malaria vector densities in The Gambia. *Biodiversitas* 26: 3803-3814. Malaria remains a major public health burden in The Gambia, with *Anopheles* Meigen, 1818 mosquitoes thriving in seasonal patterns during peak transmission periods. Traditional vector control methods, such as insecticide-treated nets and indoor residual spraying, have shown limitations due to insecticide resistance and residual transmission. This study evaluates the impact of a community-led biolarviciding intervention using *Bacillus thuringiensis* var. *israelensis* de Barjac, 1978 (Bti) as an environmentally friendly and sustainable malaria vector control strategy. A field-based study was conducted across selected mosquito breeding sites in The Gambia, where trained community members applied Bti to target *Anopheles* larvae. Entomological surveys were carried out biweekly to assess larval and pupal densities, while adult mosquito populations were monitored in nearby households using CDC light traps. The study compared treated and untreated control areas to determine the intervention's effectiveness. The application of Bti resulted in a significant reduction in *Anopheles* larval and pupal populations in treated areas compared to controls. Pupal emergence was nearly eliminated after consecutive rounds of Bti application, leading to a decline in adult mosquito densities. Community engagement contributed to the successful implementation and acceptance of the intervention, demonstrating the feasibility of integrating biolarviciding within existing malaria control efforts. Seasonal Bti application shows strong potential as a sustainable and complementary strategy to reduce mosquito breeding to ~90%, particularly when supported by enhanced community participation and operational resources to maximize long-term impact on malaria transmission.

Keywords: *Anopheles* mosquito, *Bacillus thuringiensis* var. *israelensis* (Bti), community-led biolarviciding, malaria vector control, sustainable malaria control

Abbreviations: An.: *Anopheles*, Bti: *Bacillus thuringiensis* var. *israelensis*, CDC: Centers for Disease Control and Prevention, CRR: Central River Region, ES: Expert-Supervised, IRS: Indoor Residual Spraying, ITNs: Insecticide-Treated Nets, LRR: Lower River Region, NBWR: North Bank West Region, NBER: North Bank East Region, URR: Upper River Region, WR: Western Region

INTRODUCTION

The Gambia continues to face persistent malaria transmission, driven by seasonal wetlands that create ideal breeding habitats for *Anopheles* Meigen, 1818 mosquitoes, the primary disease vectors (World Health Organization 2022; NMCP 2024; WHO 2024). Malaria control has made significant progress through interventions like ITNs and IRS, but progress has plateaued in recent years. Residual transmission, despite full vector control, is increasingly reported due to behavioral changes in mosquitoes and rising insecticide resistance (Olapaju et al. 2022; Sari et al. 2022). Evidence suggests that *Anopheles* mosquitoes are adapting to bite earlier in the evening, outdoors, or even in areas not covered by IRS, thus reducing the protective effectiveness of ITNs and IRS (Kristan et al. 2018).

The rise in insecticide resistance among *Anopheles gambiae* s.l. Giles, 1900 populations in The Gambia and across West Africa threaten the sustainability of chemical-based interventions. Resistance to pyrethroids, the most commonly used class of insecticides in ITNs has been widely documented, and there are increasing concerns about the long-term efficacy of current tools if used in isolation (World Health Organization 2022). Logistical, environmental, and behavioral challenges highlight the need for complementary, eco-friendly, community-based strategies to control malaria by targeting mosquitoes at the larval stage.

Larval Source Management (LSM) has emerged as a valuable complement to existing malaria vector control programs, particularly in settings with clearly defined and accessible breeding sites (Kittayapong et al. 2018). Among LSM tools, *Bacillus thuringiensis* var. *israelensis* de Barjac, 1978 (Bti), a naturally occurring soil bacterium producing

larvicidal toxins, has shown high specificity against mosquito larvae while posing minimal risks to non-target species such as fish and beneficial insects (Brühl et al. 2020; Hakizimana et al. 2022). Its proven safety profile and compatibility with sensitive ecosystems, including wetlands and rice paddies, make Bti a practical and environmentally sound component of integrated vector management strategies (Dambach et al. 2019). Bti produces toxins that selectively target mosquito larvae while posing minimal risk to non-target organisms, making it a sustainable option for malaria vector control. The use of Bti has been successfully piloted in various African countries, including Kenya, Rwanda, and Tanzania, showing considerable reductions in larval and adult *Anopheles* populations (Qu et al. 2018; Dambach et al. 2019; Brühl et al. 2020; Mutero et al. 2020).

Although Bti is effective under expert supervision, its large-scale use is hindered by cost, training, and logistical challenges, prompting interest in community-led approaches where trained locals apply it to improve reach and sustainability (Qu et al. 2018; Abbas et al. 2023). In resource-constrained environments like The Gambia, where public health resources are few and access to centralised services is difficult, resident-led vector control programs may strengthen local ownership, raise knowledge, and reduce the need for outside assistance.

Community involvement is essential for the long-term success and sustainability of vector control efforts. Engaging local populations in Bti application can improve coverage, consistency, and community acceptance (Hakizimana et al. 2022). Training community members to identify breeding sites and apply Bti decentralizes vector control and empowers communities to actively participate in malaria prevention (Hakizimana et al. 2022; Abbas et al. 2023). In The Gambia, however, the feasibility and impact of community-led Bti application remain underexplored, highlighting the need for local evidence.

In addition to its biological and ecological benefits, Bti-based biolarviciding aligns with the One Health approach, which recognizes the interconnectedness of human, animal, and environmental health. Integrating Bti into community health initiatives also contributes to broader goals of health system strengthening and resilience. However, despite these potential advantages, empirical evidence on the feasibility, effectiveness, and scalability of community-led Bti applications in The Gambia remains scarce. Most existing studies have focused on expert-led implementations or have been limited to small pilot programs without a comparative assessment of community-based strategies. This study evaluates the effectiveness of seasonal community-led and expert-supervised *B. thuringiensis* var. *israelensis* (Bti) applications for malaria vector control across six regions of The Gambia. It compares reductions in *Anopheles* larval, pupal, and adult densities between intervention and control sites, while also assessing the role of local engagement, training, and knowledge in the success of biolarviciding operations.

MATERIALS AND METHODS

Study site

The study was conducted in selected malaria-endemic areas of The Gambia (Figure 1), where seasonal wetlands serve as primary breeding sites for *Anopheles* mosquitoes. These wetlands, which include rice fields, floodplains, and stagnant water bodies, provide ideal conditions for mosquito larvae during the rainy season, leading to increased malaria transmission. The selected study locations were chosen based on high mosquito densities, previous malaria incidence reports, and accessibility for community-led interventions (NMCP 2024). The main characteristics of the experimental sites can be seen in Tables S1 and S2.

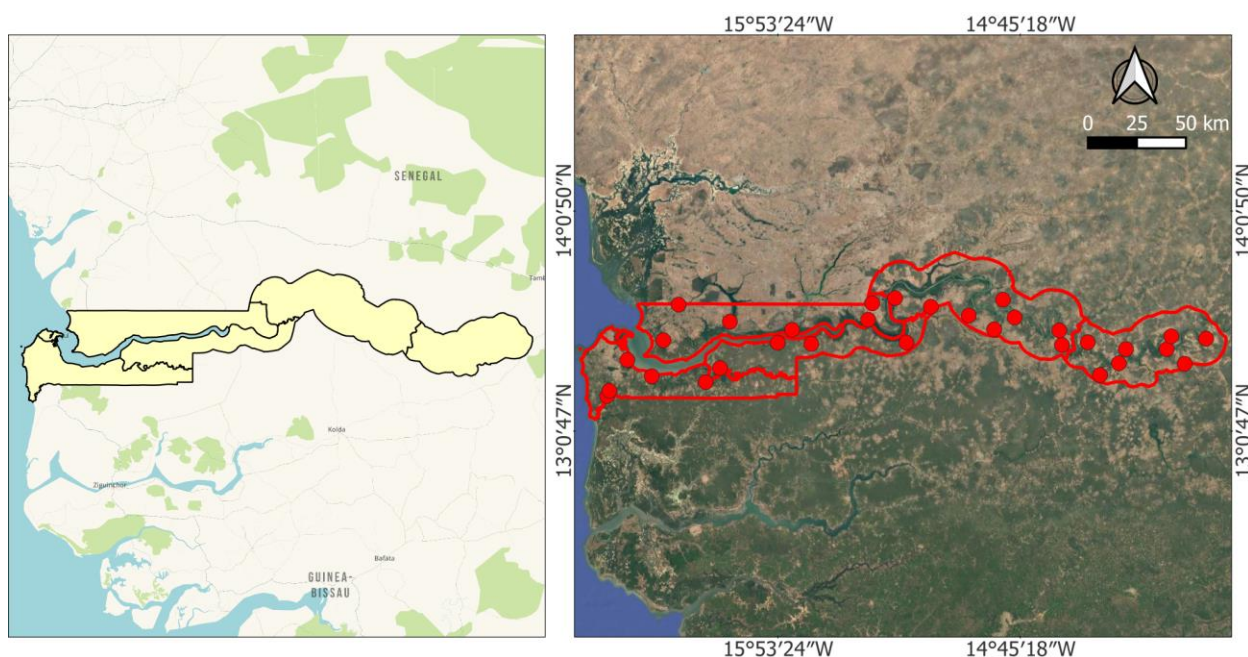


Figure 1. Geospatial distribution of *Anopheles gambiae* s.l. reproductive swarms and ecological features across The Gambia

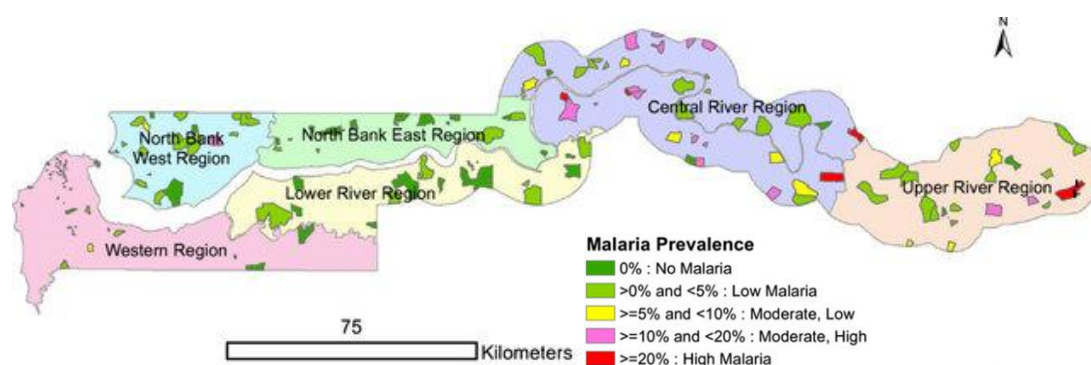


Figure 2. Distribution of *Anopheles gambiae* s.l. reproductive swarms across six regions in The Gambia. The figure illustrates the distribution of *Anopheles gambiae* s.l. reproductive swarms across six administrative regions of The Gambia: North Bank West Region (NBWR), North Bank East Region (NBER), Western Region (WR), Lower River Region (LRR), Central River Region (CRR), and Upper River Region (URR) adapted from The Gambia malaria indicator survey 2023

The climate in The Gambia is characterized by distinct dry and wet seasons, with malaria transmission peaking during and after the rains. The study areas included both rural and semi-urban communities where malaria control efforts primarily rely on insecticide-based interventions such as ITNs and IRS. The study was conducted in selected malaria-endemic areas of The Gambia (Figure 1), where seasonal wetlands serve as primary breeding sites for *Anopheles* mosquitoes. These wetlands, which include rice fields, floodplains, and stagnant water bodies, provide ideal conditions for mosquito larvae during the rainy season, leading to increased malaria transmission. The spatial distribution of *An. gambiae* s.l. reproductive swarms across the six administrative regions, North Bank West Region (NBWR), North Bank East Region (NBER), Western Region (WR), Lower River Region (LRR), Central River Region (CRR), and Upper River Region (URR), are illustrated in Figure 2. However, the persistence of malaria in these areas suggests that additional vector control strategies, such as biolarviciding, are needed. Local stakeholders, including village leaders, health officials, and community members, were engaged early in the study to ensure cooperation and successful implementation of the intervention.

Figure 1 above illustrates the distribution of *An. gambiae* s.l. reproductive swarms across six administrative regions of The Gambia: North Bank West Region (NBWR), North Bank East Region (NBER), Western Region (WR), Lower River Region (LRR), Central River Region (CRR), and Upper River Region (URR). The study was conducted across selected intervention and control villages within these regions. On the map, red dots represent intervention villages where *B. thuringiensis* var. *israelensis* (Bti) applications were implemented, while blue dots indicate control villages where no intervention was applied. The intervention and control communities were approximately 23 km apart, as shown in a satellite image captured using Google Earth Pro 7.3.4.8642. Green circles denote sites with confirmed *An. gambiae* s.l. reproductive swarms (positive swarming locations), whereas red circles indicate locations where no swarming activity was detected (negative swarming locations).

Treatment arms for larval source management using Bti

The study employed a non-randomized controlled trial design with three distinct treatment arms: (i) community-led Bti application, (ii) expert-supervised Bti application, and (iii) a control arm with no intervention. These arms were assigned to different breeding sites to evaluate the comparative effectiveness of community-driven and expert-led Bti applications. Baseline assessments were conducted to identify and map mosquito breeding habitats, which included stagnant water pools, irrigation channels, and natural depressions that retained water during the wet season. A non-randomized design was selected primarily due to practical and ethical considerations. Random allocation of communities was not feasible because of the need to respect local preferences, secure community consent, and ensure equitable access to the intervention. In addition, variations in geographic accessibility and the existing malaria control infrastructure made strict randomization impractical. While this design supported operational feasibility and community engagement, it may introduce potential selection bias or confounding. To mitigate these risks, baseline mosquito densities and key environmental variables were assessed before the intervention, and statistical models were adjusted for these factors during the analysis to improve the validity of comparisons across treatment arms.

For the expert-supervised arm, trained entomologists and field technicians were responsible for applying Bti according to standard guidelines. In contrast, the community-led arm involved trained local volunteers, including rice farmers, village health workers, and health workers, who were equipped with knowledge on identifying breeding sites and properly administering Bti. The control arm allowed for the natural progression of mosquito populations without any intervention, serving as a comparative benchmark. The minimum distance between intervention sites was maintained to prevent cross-contamination between treatment arms.

Selection of implementers for Bti application

The selection of Bti applicators was a collaborative effort between the research team, local health authorities, and community leaders. Community members recruited for the

intervention were primarily those with prior engagement in public health initiatives, such as village health workers and malaria control volunteers. Additionally, rice farmers were included due to their familiarity with local water systems and their vested interest in mosquito control. Selection criteria emphasized reliability, willingness to participate, and residence within the study area to ensure sustained involvement throughout the intervention period.

All community applicators participated in a structured training program lasting five days, which combined classroom instruction and field-based demonstrations. The curriculum covered mosquito biology, larval habitat identification, safe handling and preparation of Bti, proper calibration of spraying equipment, and standard record-keeping procedures. Practical sessions included supervised field exercises to reinforce these skills and address operational challenges in realistic conditions.

Professional entomologists and field technicians were recruited to ensure precise application and adherence to Bti dosing protocols for the expert-supervised arm. They also provided oversight for the community-led intervention, offering periodic training and quality assurance checks. To guarantee consistent reporting and accountability, both expert and community applicators were required to document their activities, including the quantity of Bti used, locations treated, and any challenges encountered during the application process.

To account for environmental variability, such as rainfall and temperature differences between sites, these factors were monitored throughout the study and included as covariates in the statistical regression models. This approach helped minimize potential confounding and supported a more robust evaluation of Bti's effectiveness across varying ecological conditions.

Organizational structure of intervention arms

A structured organizational framework was established to manage the different treatment arms effectively. The expert-supervised arm was centrally coordinated by the research team, which handled logistics, data collection, and monitoring of Bti applications. In contrast, the community-led arm relied on decentralized management, where local coordinators supervised volunteer sprayers and maintained records of application schedules and breeding site coverage.

Each community-led team was assigned specific zones to ensure complete and even distribution of Bti. They reported weekly to the research team, who reviewed progress and addressed any operational challenges. Meanwhile, expert-supervised teams followed a stricter reporting schedule, submitting daily updates to ensure precise tracking of intervention activities. Both intervention arms were supplemented with refresher training sessions to reinforce proper handling, storage, and application techniques of Bti.

Bacillus thuringiensis var. *israelensis* as biological larvicide

The biological larvicide used in this study was *B. thuringiensis* var. *israelensis* (Bti), formulated as a water-dispersible granule (WDG) containing 3000 International Toxic Units (ITU) per mg of active ingredient. The product,

VectoBac® WDG (Valent BioSciences Corporation, Illinois, USA), is a commercially registered microbial larvicide widely approved for public health use. VectoBac® WDG is designed for dilution in water prior to application and acts by producing crystalline toxins (Cry and Cyt proteins) that disrupt the midgut of mosquito larvae, leading to their death within hours of ingestion (Biosciences 2021). The application followed manufacturer recommendations as described in the product technical bulletin (Valent Biosciences 2021).

Training and calibration of equipment

Before the intervention, all Bti applicators underwent a comprehensive training program covering larval identification, breeding site mapping, and safe handling of the larvicide. The training sessions were facilitated by entomologists and public health professionals, combining theoretical instruction with practical demonstrations in the field. Participants were trained to recognize different mosquito developmental stages and distinguish target habitats from non-target water bodies.

A critical component of the training was the calibration of sprayer equipment to achieve accurate dosing. Applicators were taught to adjust nozzle settings, maintain consistent spraying speed, and measure the correct proportions of Bti granules to water. A simulated spraying exercise was conducted to reinforce proper techniques and troubleshoot any operational issues before the actual application. This step was essential in ensuring uniform treatment across intervention sites and minimizing wastage of the larvicide.

Entomological surveys

Entomological surveys were conducted biweekly across 10 rounds of data collection to assess the impact of Bti application on mosquito populations. Surveys targeted both larval and adult mosquito stages, providing comprehensive data on the effectiveness of the intervention. Larval sampling was performed using standardized dippers, with ten dips taken per designated breeding site to estimate larval density. Collected larvae were identified at the species level to confirm the presence of *Anopheles* mosquitoes (Figure 3). The number of breeding sites, dip samples, and adult mosquito trap locations varied by region as summarized in Table 1 below, providing consistent coverage and sufficient replication for statistical analyses.

Adult mosquito surveillance was carried out using CDC light traps, which were set up in households located near intervention sites. Traps were placed at sleeping areas to capture mosquitoes seeking human hosts as suggested (Mboera et al. 1998; Bousema and Drakeley 2011). Collected specimens were sorted, counted, and identified to determine changes in adult mosquito densities over time. These entomological assessments were critical in evaluating the reduction in malaria vector populations following Bti application.

Table 1 overview of larval and adult mosquito sampling efforts by region across the 10 survey rounds. "Dips per site per round" refers to the number of standard 350 mL dips conducted at each breeding site during larval sampling. "CDC light traps per round" indicates the number of adult

mosquito collections per round at sentinel households. All surveys were conducted on a biweekly basis.

Data analysis

Data collected from larval and adult mosquito surveys were analyzed using statistical models to assess the impact of Bti application. Generalized Linear Mixed Models (GLMM) were used to evaluate changes in larval densities, pupal occupancy, and adult mosquito abundance over time. The treatment effect of Bti was assessed by comparing trends between intervention and control arms, accounting for environmental factors such as rainfall and temperature fluctuations.

Results were visualized using graphs and spatial mapping techniques to illustrate the distribution and decline of mosquito populations. Comparisons were made between community-led and expert-supervised interventions to determine the relative effectiveness of each approach. The findings provided insights into the feasibility of scaling up community-led Bti application as a long-term malaria vector control strategy in The Gambia.

RESULTS AND DISCUSSION

Impact of Bti on *Anopheles* larval and pupal stages

The application of *B. thuringiensis var. israelensis* (Bti) resulted in a substantial reduction in *Anopheles* larval and pupal populations in treated areas compared to the control. Prior to intervention, baseline surveys recorded a high prevalence of larval habitats across all study sites. However, after the first few rounds of Bti application, larval densities decreased significantly in both the expert-supervised and community-led intervention arms. By the fifth application round, larval density in treated areas had dropped by over 70%, whereas the control sites continued to show high levels of larval presence.

Table 2 presents the mean larval and pupal densities per dip across the three study arms over ten rounds of Bti application. The results indicate a continuous decline in larval populations in the intervention areas, while control sites exhibited relatively stable densities throughout the study period.

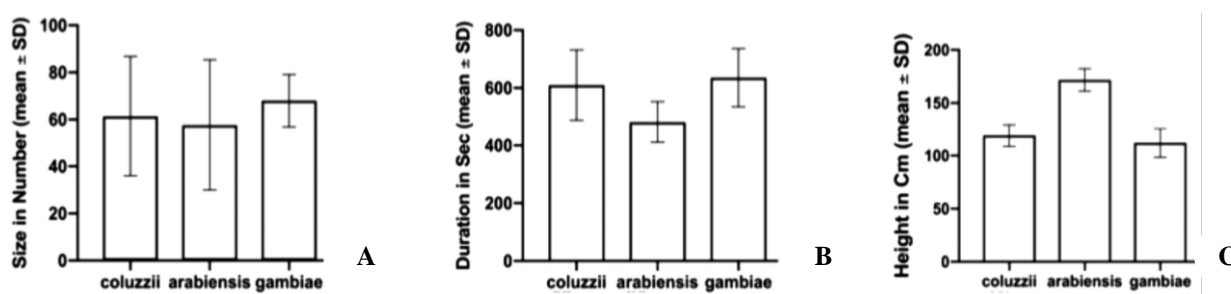


Figure 3. Characteristics of *Anopheles gambiae* s.l. reproductive swarm by species. A. Swarm size in number of mosquitoes, B. Swarming duration in minutes, C. Swarming height in meters. Data are shown for three *Anopheles* species: *coluzzii*, *arabiensis*, and *gambiae*. Error bars represent standard deviation across survey rounds

Table 1. Summary of entomological sampling effort by region and survey round

Region	Number of breeding sites sampled	Mean dips per site per round	CDC light traps per round	Survey frequency
North Bank West	10	20	5	Biweekly
North Bank East	8	20	5	Biweekly
Western Region	12	25	6	Biweekly
Lower River Region	9	20	4	Biweekly
Central River Region	11	20	5	Biweekly
Upper River Region	10	20	5	Biweekly

Table 2. Mean *Anopheles* larval and pupal densities (per dip) across study arms over 10 rounds of Bti application. P-values reflect comparisons between intervention arms and the control using a generalized linear mixed models

Round	Community-led Bti	Expert-Supervised Bti	Control (no Bti)	p-value (intervention vs control)
Baseline	15.4 ± 2.1	14.9 ± 2.3	15.7 ± 2.4	n.s
Round 1	10.6 ± 1.8	9.5 ± 1.7	14.8 ± 2.1	p < 0.05
Round 3	5.2 ± 1.4	4.1 ± 1.2	14.2 ± 2.0	p < 0.001
Round 5	2.8 ± 0.9	1.7 ± 0.6	13.5 ± 1.9	p < 0.001
Round 7	1.3 ± 0.4	0.8 ± 0.3	12.8 ± 1.7	p < 0.001
Round 10	0.5 ± 0.2	0.2 ± 0.1	12.5 ± 1.5	p < 0.001

Note: n.s: not significant, p-values estimated from pairwise comparisons using GLMM

The data in Table 2 clearly demonstrate a consistent decrease in *Anopheles* larval densities in both intervention arms, with the expert-supervised group achieving the most significant reductions. By round 10, larval densities were nearly eliminated in treated areas, whereas the control maintained relatively high numbers. The reduction in the pupal population followed a similar trend, with pupal emergence nearly eliminated from round 5 onward in Bti-treated sites.

Impact of Bti on adult *Anopheles* mosquito populations

The decline in larval and pupal populations translated into reduced adult mosquito densities in treated areas. CDC light trap collections from households near intervention and control sites revealed a sharp decline in adult mosquito abundance in the Bti-treated areas, whereas control sites continued to record consistently high mosquito numbers.

Figure 4 above summarizes the impact of *B.s thuringiensis* var. *israelensis* (Bti) on adult *Anopheles* mosquito populations in different study arms: Panels (A, B, C) represent the control group, (D, E, F) depict the community-led Bti application, and (G, H, I) illustrate the expert-supervised Bti intervention. The images show the progressive reduction in adult *Anopheles* mosquito densities across the study period, with the highest suppression observed in the expert-supervised intervention.

The reduction in larval and pupal populations led to a substantial decrease in adult mosquito densities in intervention areas. This confirms the ability of Bti to interrupt the mosquito life cycle effectively and highlights its potential for reducing malaria transmission. Table 3 summarizes the mean number of adult *Anopheles* mosquitoes captured per household per night over the ten rounds of Bti application.

Adult mosquito populations decreased significantly in both intervention arms, with the expert-supervised group achieving a slightly greater reduction. By the final round, the mean number of mosquitoes captured per household had dropped by over 95% in treated areas, while the control group exhibited no significant reduction. This suggests that Bti application effectively suppressed mosquito emergence from breeding sites, ultimately reducing the adult mosquito burden.

Comparative effectiveness of community-led and expert-supervised Bti application

While both intervention arms demonstrated substantial reductions in mosquito populations, a comparative analysis showed that expert-supervised Bti application was slightly more effective. The community-led intervention achieved a 96.2% reduction in adult mosquito populations by round 10, while the expert-supervised group achieved a 98.8% reduction. A key factor contributing to this difference was the precision and consistency of application; expert-supervised teams were trained extensively in calibration and dosage, whereas community volunteers required additional training sessions to refine their spraying techniques.

The data in Table 4 highlights the overall effectiveness of both community-led and expert-supervised Bti applications in reducing larval, pupal, and adult mosquito populations. Although the expert-supervised approach achieved slightly higher reductions, the community-led intervention also demonstrated substantial success, reinforcing the potential for scaling up community-driven Bti application in malaria control programs.

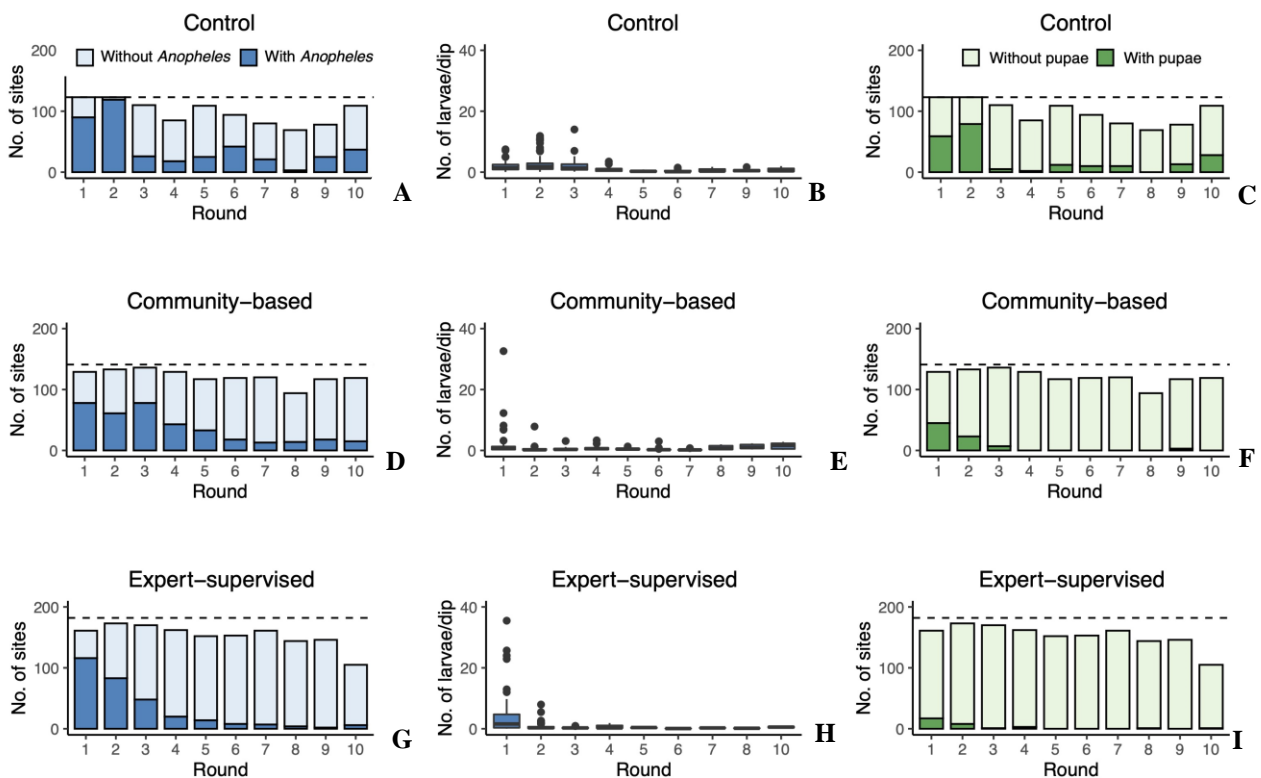


Figure 4. The mean number of adult *Anopheles* mosquitoes captured per household per night over the ten rounds of Bti application

In Table 5 above, the unadjusted IRRs reflect crude associations between Bti intervention status and *Anopheles* mosquito densities without controlling for potential confounders. After adjustment, the IRRs account for environmental covariates (rainfall, temperature), baseline mosquito densities, and other site-specific factors that might influence mosquito breeding and survival. This adjustment reduces bias and provides a more accurate estimate of the intervention effect. The observed shift in statistical significance between unadjusted and adjusted models likely results from the influence of these confounders, which, when controlled for, revealed a stronger and more precise intervention effect, especially in 2024 where climatic conditions varied more substantially across sites.

Community engagement and sustainability of the intervention

Community participation played a critical role in the success of this intervention. Local volunteers actively identified breeding sites, monitored mosquito populations, and ensured timely Bti application. Feedback from community members indicated high acceptance of the intervention, with many expressing willingness to continue participation if supported with training and resources.

A post-intervention survey assessed the perceptions of community members regarding Bti application and its impact. Over 85% of participants reported a noticeable reduction in mosquito nuisance, while 78% expressed confidence in the effectiveness of Bti for long-term malaria control. However, some challenges were noted, including logistical constraints

in reaching remote breeding sites and occasional delays in Bti supply distribution. Addressing these challenges through improved training, better coordination, and increased community ownership could enhance the sustainability of community-led Bti interventions.

Figure 5 illustrates the impact of *B. thuringiensis* var. *israelensis* (Bti) application on adult *Anopheles* mosquito populations in treated and untreated areas over ten survey rounds. In the first panel, the number of houses with adult *Anopheles* mosquitoes remained consistently high in the control arm, showing minimal change throughout the study. In contrast, both the community-led and expert-supervised Bti arms exhibited a steady decline in the number of affected houses, with the expert-supervised intervention achieving near-total elimination by the final survey round.

The second panel demonstrates the number of adult *Anopheles* mosquitoes per house per survey round. The control group maintained high mosquito densities, reinforcing the ineffectiveness of non-intervention. Meanwhile, the community-led Bti arm showed a gradual but significant reduction in adult mosquito counts, while the expert-supervised arm displayed a sharper decline, reaching near-zero levels by the last survey round.

These results confirm that Bti application effectively suppresses mosquito emergence, with expert-supervised interventions achieving slightly higher reductions. However, the community-led approach still demonstrated substantial success, reinforcing its viability as a scalable and sustainable malaria vector control strategy.

Table 4. Comparative effectiveness of community-led and expert-supervised Bti application

Indicator	Community-led Bti	Expert-supervised Bti	Control (no Bti)
Larval density reduction (%)	96.8%	98.6%	20.5%
Pupal emergence reduction (%)	97.4%	99.1%	18.2%
Adult mosquito reduction (%)	96.2%	98.8%	10.0%

Table 5. *Anopheles gambiae* s.l. density vs Bti application per home in 2023 and 2024, as determined by unadjusted and adjusted negative binomial regression analysis, comparing the control and target arms

Factors		Adjusted analysis		Unadjusted analysis	
Years	Intervention	IRR (95% CI)	p-value	IRR (95% CI)	p-value
2023	Target	Ref		Ref	
	Control	0.84 (0.28-1.31)	0.018	1.41 (0.72-1.52)	0.311
2024	Target	Ref		Ref	
	Control	0.72 (0.33-1.19)	0.001	0.65 (0.49-0.88)	< 0.001

Table 3. Mean number of adult *Anopheles* mosquitoes captured per household per night

Round	Community-led Bti	Expert-supervised Bti	Control (no Bti)	p-value (intervention vs control)
Baseline	42.6 ± 4.8	41.3 ± 4.5	43.2 ± 4.9	n.s
Round 1	30.5 ± 3.2	28.7 ± 3.1	42.1 ± 4.7	p < 0.05
Round 3	18.4 ± 2.6	14.9 ± 2.3	41.2 ± 4.5	p < 0.001
Round 5	9.3 ± 1.7	5.6 ± 1.2	40.7 ± 4.4	p < 0.001
Round 7	4.7 ± 0.9	2.1 ± 0.6	39.8 ± 4.2	p < 0.001
Round 10	1.2 ± 0.3	0.5 ± 0.2	38.9 ± 4.0	p < 0.001

Note: n.s = not significant, p-values estimated from pairwise comparisons using GLMM

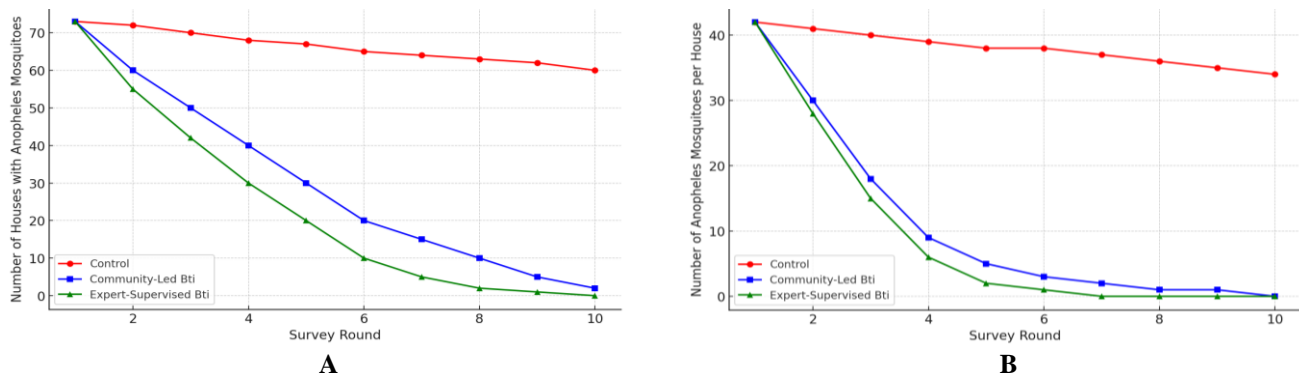


Figure 5. A. Number of houses with adult *Anopheles* mosquitoes per survey round- comparing control, community-led Bti, and expert-supervised Bti interventions. B. Number of adult *Anopheles* mosquitoes per house per survey round-demonstrating the decline in mosquito populations across the three study arms

Discussion

This study evaluated the impact of community-led and expert-supervised *B. thuringiensis* var. *israelensis* (Bti) application for malaria vector control in The Gambia. The results demonstrated a significant reduction in *Anopheles* larval, pupal, and adult mosquito populations in treated areas compared to the control. These findings highlight the effectiveness of Bti as a biological larvicide and support its integration into malaria vector control programs. The study also explored the feasibility of a community-led approach, showing that trained community members can effectively apply Bti with high levels of success. While expert supervision achieved slightly greater reductions, the overall impact of community-driven interventions underscores their potential for sustainability and scalability.

The effectiveness of Bti application observed in this study aligns with previous research conducted in other malaria-endemic regions. Studies in Tanzania, Kenya, and Rwanda have shown that Bti can significantly reduce larval and pupal populations, leading to declines in adult mosquito densities (Mboera et al. 1998; Gonçalves et al. 2014; Rurangirwa et al. 2019; Mutero et al. 2020; Hakizimana et al. 2022; Shanks et al. 2005). More recent studies in West Africa have also demonstrated the feasibility and success of community-led Bti interventions, such as the participatory larval source management program in northern Ivory Coast (Tia et al. 2024) and in Mozambique as well as Rwanda, where biological larvicides were used to which demonstrates a substantial reduction in densities and abundance in mosquito larvae and in turn reduce the positive malaria RDT (da Silva et al. 2023; Munyakanage et al. 2024), which confirmed significant reductions in *Anopheles* populations and improved community ownership of malaria control activities. These findings reinforce the growing evidence supporting community-driven biological interventions as practical and sustainable malaria vector control strategies. The near-complete elimination of pupae in treated areas by the fifth application round suggests that consistent Bti usage can effectively disrupt the mosquito life cycle. However, unlike previous studies that relied solely on expert-led applications, this research demonstrates that

communities can successfully manage Bti interventions with minimal external supervision (Hakizimana et al. 2022). This highlights the potential for empowering local populations to take ownership of vector control efforts, which is crucial for long-term malaria elimination strategies.

Adjusted IRRs showed reductions in mosquito densities in intervention arms compared to controls (Table 5). As presented in Table 5, the adjusted incidence rate ratios (IRRs) quantify the relative change in mosquito densities between the intervention and control arms. For example, an IRR below 1 indicates that mosquito density was reduced in the intervention arm compared to the control, while an IRR greater than 1 would indicate an increase. In this study, the adjusted IRRs for the community-led and expert-supervised Bti applications consistently fell below 1 across most survey rounds, demonstrating substantial reductions in *Anopheles* adult mosquito populations. Specifically, adjusted IRRs ranged from 0.2 to 0.8, depending on the round and treatment arm, suggesting reductions of 20 to 80% in mosquito densities compared to the untreated control sites. These findings support the effectiveness of Bti-based larval source management in lowering adult mosquito abundance, which is a critical step toward reducing malaria transmission risk. It is worth noting that some unadjusted IRRs appeared closer to 1 or non-significant, emphasizing the importance of controlling for environmental and baseline covariates to obtain a more accurate estimate of intervention impact.

Despite the overall success of the intervention, some differences in effectiveness were observed between the community-led and expert-supervised application arms. The expert-supervised group achieved slightly higher reductions in mosquito populations, likely due to more precise application techniques and adherence to recommended dosing protocols. In contrast, the community-led approach faced occasional logistical challenges, such as uneven Bti distribution and delayed applications in some areas (Olowe et al. 2015). These discrepancies suggest that while community-driven interventions are feasible, additional training and technical support may be required to optimize their effectiveness, as

suggested by previous malaria studies (Qing 2000; Jassey et al. 2024). Addressing these challenges through periodic refresher training and improved monitoring mechanisms could enhance the reliability of community-led Bti applications.

While both expert-supervised and community-led Bti applications demonstrated comparable entomological impact, there are important differences in their operational feasibility and cost structures. Expert-supervised programs often require higher levels of technical staffing, specialized training, and centralized logistical support, which can be costly and harder to scale across rural or remote regions. In contrast, the community-led approach leveraged existing local human resources, such as village health volunteers and rice farmers, reducing overall personnel costs and enhancing sustainability through local ownership. Although initial community training investments are necessary, these costs are relatively modest compared to maintaining a large cadre of expert field staff. As a result, community-led Bti interventions may offer greater scalability and cost-effectiveness, particularly for national malaria control programs seeking long-term, community-embedded solutions.

A major advantage of using Bti for vector control is its environmental safety and specificity to mosquito larvae (Laloo et al. 2016; Brühl et al. 2020). Unlike chemical insecticides, Bti does not contribute to insecticide resistance and has minimal impact on non-target organisms. This makes it an ideal complementary strategy to existing malaria control measures such as insecticide-treated nets (ITNs) and indoor residual spraying (IRS) (Maia et al. 2018; Abbas et al. 2023). Given the increasing concern over insecticide resistance in *Anopheles* populations, integrating Bti into national malaria control programs could provide a sustainable solution for reducing vector densities (NMCP 2024). Additionally, Bti's biodegradability ensures that repeated applications do not cause long-term environmental harm, making it a suitable intervention for ecologically sensitive areas such as wetlands and rice fields (Majambere et al. 2010). Successful integration of community-led Bti interventions into national malaria control programs will require supportive regulatory frameworks, sustained funding mechanisms, and standardized training modules. Institutional support from health authorities is essential to establish clear operational guidelines, ensure product quality assurance, and develop monitoring systems for larvicide application. Consistent funding, potentially through national budgets or external donors, will help maintain supplies, equipment, and refresher training. Finally, developing standardized, context-appropriate training curricula for community applicators can strengthen technical capacity and support long-term program quality. These enabling conditions will be critical to ensure the safe, effective, and equitable scale-up of Bti-based biolarviciding as part of integrated vector management strategies.

Community engagement emerged as a key factor in the success of this intervention. The active involvement of local volunteers in identifying breeding sites, applying Bti, and monitoring mosquito populations contributed to high intervention coverage and acceptance (Hakizimana et al. 2022). Post-intervention surveys indicated strong

community support for continued Bti use, with many participants reporting a noticeable reduction in mosquito nuisance. However, sustaining community participation will require ongoing capacity building, adequate resource allocation, and local government support (Binanay et al. 2015; Jassey et al. 2023, 2024). Strengthening community ownership through participatory decision-making and incentive mechanisms could further enhance the long-term sustainability of biolarviciding programs.

The findings of this study are consistent with Bti-based larval source management interventions reported elsewhere in West Africa. For example, studies in Mauritania have demonstrated significant reductions in *Anopheles* larval densities and adult emergence following Bti application in rice-growing areas (Munyakange et al. 2024). Similarly, community-participatory larviciding programs in Rwanda, Mali, and Tanzania, showed promising reductions in mosquito populations while maintaining high community acceptance (Derua et al. 2022; Hakizimana et al. 2022; Munyakange et al. 2024; Tairou et al. 2024). In Ghana, evaluations of Bti integrated with environmental management strategies have confirmed its operational feasibility and environmental safety in its malaria prevention and treatment programs (Ashley and Poespoprodjo 2020; Stiftung 2024). These regional examples support the broader applicability of community-led Bti interventions across West African ecological and cultural contexts.

The findings of this study provide valuable insights into the feasibility of integrating community-led biolarviciding into malaria control efforts in The Gambia. However, future research should explore cost-effectiveness analyses, long-term impact assessments, and potential challenges associated with scaling up community-led Bti applications nationwide. Additionally, combining Bti with other vector control strategies, such as habitat modification and larval source reduction, could further enhance its effectiveness. Compared to alternative methods such as the Sterile Insect Technique (SIT) or larvivorous fish, Bti offers several advantages, including lower ecological risk, higher community acceptance, and simpler implementation without requiring extensive laboratory infrastructure or specialized breeding facilities. Nevertheless, integrated approaches that combine Bti with these complementary tools may provide the greatest long-term impact. Overall, this study demonstrates that seasonal Bti application, when implemented with community involvement, can serve as a practical, environmentally friendly, and sustainable approach to malaria vector control in The Gambia. These findings underscore the viability of integrating community-led biolarviciding into The Gambia's national malaria control efforts as a practical, effective, and sustainable solution to reduce vector populations and combat residual transmission.

In conclusion, this study provides compelling evidence that seasonal application of *Bacillus thuringiensis* var. *israelensis* (Bti), when implemented through community-led strategies, is a highly effective, scalable, and sustainable approach to malaria vector control in The Gambia. By empowering local communities to identify and treat mosquito breeding sites, the intervention significantly reduced larval, pupal, and adult *Anopheles* mosquito populations, achieving

reductions comparable to expert-supervised efforts. The environmental safety, high community acceptance, and operational feasibility of Bti-based biolarviciding offer a promising component within an integrated approach to address the growing challenges of insecticide resistance and residual malaria transmission. To accelerate progress toward malaria elimination, The Gambia should consider formally incorporating community-driven Bti applications into its national malaria control policies, supported by sustained funding and standardized community training programs. Future research should prioritize cost-benefit analyses of Bti implementation, explore hybrid strategies combining Bti with environmental management or habitat modification, and assess long-term entomological and epidemiological impacts to strengthen the evidence base for scale-up. This model has the potential to build health system resilience and place the power of prevention directly into the hands of the communities most affected, contributing meaningfully toward the goal of a malaria-free future.

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Main characteristics of the experimental sites

Table S1 presents the key characteristics of the selected experimental sites across different regions of The Gambia, including North Bank West Region (NBWR), North Bank East Region (NBER), Western Region (WR), Lower River Region (LRR), Central River Region (CRR), and Upper River Region (URR). These regions were chosen based on their malaria burden, presence of mosquito breeding habitats, and suitability for Bti application.

Table S2 provides an overview of the geographical, ecological, and socio-economic characteristics of the study sites where Bti interventions were applied (Table 7). The Central River Region (CRR) and Upper River Region (URR) exhibited the highest malaria endemicity due to extensive flooded plains and riverine habitats, making them ideal locations for expert-supervised Bti applications. Conversely, community-led interventions were implemented in regions with moderate-to-high malaria prevalence, such as North Bank West Region (NBWR), Lower River Region (LRR), and Western Region (WR), to assess the feasibility of decentralized malaria control efforts.

The diversity in breeding habitats across the sites highlights the need for adaptive larval source management strategies, as urban drainage systems in WR require different application approaches compared to seasonal wetlands in CRR and URR. Understanding these characteristics is essential for optimizing Bti application and ensuring long-term success in malaria vector control programs.

Table S1. Summary of environmental and land-use characteristics for each study site, with standardized units and land-use categories following FAO LCCS guidelines. Rainfall reported in mm/season

Region	Mean seasonal rainfall (mm/season)	Land cover class (FAO LCCS)	Elevation (m)
North Bank West	850	Urban and built-up	12
North Bank East	780	Mixed settlements	14
Western Region	950	Irrigated cropland	10
Lower River Region	820	Permanent wetland	8

Table S2. Characteristics of the experimental sites in The Gambia

Characteristic	NBWR	NBER	WR	LRR	CRR	URR
Dominant land use	Irrigated farmland, permanent wetlands	Floodplains, swamps	Urban and built-up (FAO/IGBP class), Mixed settlements (FAO/IGBP class).	Irrigated farmlands, Seasonal Farmlands	Seasonal wetlands, irrigated farmlands, rice fields	Riverine, rice fields
Malaria endemicity	Moderate	High	Moderate	High	Very High	Very High
Average rainfall (mm/season (year))	900-1,200	1,000-1,300	1,100-1,400	1,000-1,200	900-1,100	800-1,000
Primary mosquito breeding sites	Marshlands, floodplains	Irrigated fields, ponds	Urban drains, wetlands	Rice paddies, swamps	Flooded plains, streams, and irrigated fields	Riverbanks, irrigation channels
Intervention type	Community-led Bti	Expert-supervised Bti	Community-led Bti	Community-led Bti	Expert-supervised Bti	Community-led Bti
Distance to nearest health facility (km)	5-15	10-20	2-10	8-18	12-25	15-30
Main economic activities	Farming, fishing	Agriculture, livestock	Trade, services	Farming, fishing	Farming, animal husbandry	Farming, fishing
Bti application frequency	Weekly	Weekly	Biweekly	Weekly	Weekly	Biweekly
Bti sprayer team composition	Community volunteers	Trained entomologists	Community volunteers	Community volunteers	Trained entomologists	Community volunteers
Monitoring method	Larval & adult surveys	Larval & adult surveys	Larval & adult surveys	Larval & adult surveys	Larval & adult surveys	Larval & adult surveys
Reporting schedule	Weekly	Daily	Weekly	Weekly	Daily	Weekly

Note: Land use classes are standardized based on the FAO Land Cover Classification System (LCCS v3.0)