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The efficacy of attractive targeted sugar baits in reducing malaria vector abundance in low-endemicity settings of northwest Mali

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Abstract

Background Attractive targeted sugar baits (ATSBs) have the potential to significantly reduce infective female *Anopheles* mosquitoes in arid areas, such as in Northern Mali. Malaria is epidemic in the north due to the limited viability of *Anopheles* species in the desert climate.

The goal of this study was to determine of the effect of ATSB on the number of older female *An. gambiae* and on the number of sporozoite-positive females in villages in northern Mali.

Methods Villages were located in the north of Mali. In this study, 5677 ATSB stations were deployed, two on each home, in ten villages during late July and early August 2019. Ten villages served as controls. After a pre-treatment monitoring period in July, *An. gambiae* populations were monitored again from August to December using CDC-UV light traps, pyrethrum spray catches (PSC), and human landing catches (HLC). Mosquitoes were dissected to estimate their age, while ELISA detected sporozoite positivity. The monthly entomological inoculation rates (EIRs) were calculated for HLC indoors and outdoors. Data from villages were compared using t-tests, while bait station weighted density versus amount of collected females was checked with a Pearson's correlation.

Results A total of 2703 female *An. gambiae* were caught from treated villages, 4582 from control villages, a 41.0% difference. Dissection of 1759 females showed that ATSB significantly reduced the number of older females. The proportion of older females in treated villages was 0.93% compared to 9.4% in control villages. ELISA analysis of 7285 females showed that bait stations reduced the number of sporozoite-positive females. The infective females in treated villages was 0.30% compared to 2.73% in the controls. The greater the density of bait stations deployed, the fewer the older, infective females ($P < 0.05$).

EIRs were low in control villages except in months when *An. gambiae* populations were high. EIRs in ATSB placement villages remained zero. Significant reductions ($P < 0.0001$) in *An. gambiae* males were observed.

Conclusions Bait stations reduced all measures of vector populations in this study. In a low-transmission setting, ATSB has the potential to greatly reduce malaria.

Keywords ATSB, Bait station, *Anopheles gambiae*, Mali, Malaria, Entomological inoculation rate (EIR)

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Background

Malaria remains one of the world's most devastating diseases despite decades of intense research in prevention, therapeutics, and surveillance. In the 2020 World Malaria Report, the World Health Organization (WHO) called for the development of new tools for use towards malaria elimination and the prevention of malaria re-establishment [1]. As a new vector control tool, the attractive targeted sugar bait (ATSB) method has the potential, in conjunction with other methods, such as bed nets, to significantly lower the number of mosquito bites per night and sporozoite-positive *Anopheles* mosquitoes [2], thus reducing the overall entomological inoculation rate (EIR), or number of infective bites of malaria vectors. The ATSB method takes advantage of the mosquito's dependency on sugar meals for survival and employs an attractant (usually floral or fruit scent), a toxin ineffective on mammals such as boric acid, spinosad or dinotefuran, and sugar as a phagostimulant. The mosquito feeds on this mixture sprayed on plants or put into bait stations and dies within 24 h post-ingestion [3, 4]. For the development of ATSB, it was hypothesized that in an arid area nearly devoid of competing blooming plants, the mosquito population could be reduced to zero or at least significantly reduced. Testing of ATSB in the Judean desert proved that this goal could be achieved [5]. ATSB was also tested in low- and high-biting pressure environments in Mali. As expected, in the low-biting pressure environment, mosquito vectors were reduced to near-zero levels [6].

With these promising results, the aim was to evaluate whether a reduction in vectors with ATSB is correlated to a decrease in entomological markers indicative of malaria transmission in an arid environment with low malaria transmission settings. Here, it is demonstrated that the ATSB approach effectively reduced malaria vector abundance and parasite infection rate, leading to a reduction in the entomological inoculation rate (EIR).

Methods

Study site and conditions

The ATSB trial area is located in the Nioro du Sahel region in Northwest Mali, a malaria low-endemicity setting (Fig. 1). The rainy season at the study sites starts in late June or early July and lasts through October. The area experiences reduced annual precipitation with significantly lower anopheline population sizes compared to other parts of the country such as in the south. The region belongs to the arid Sahel climatic zone, with mostly irrigated rice production enhancement projects

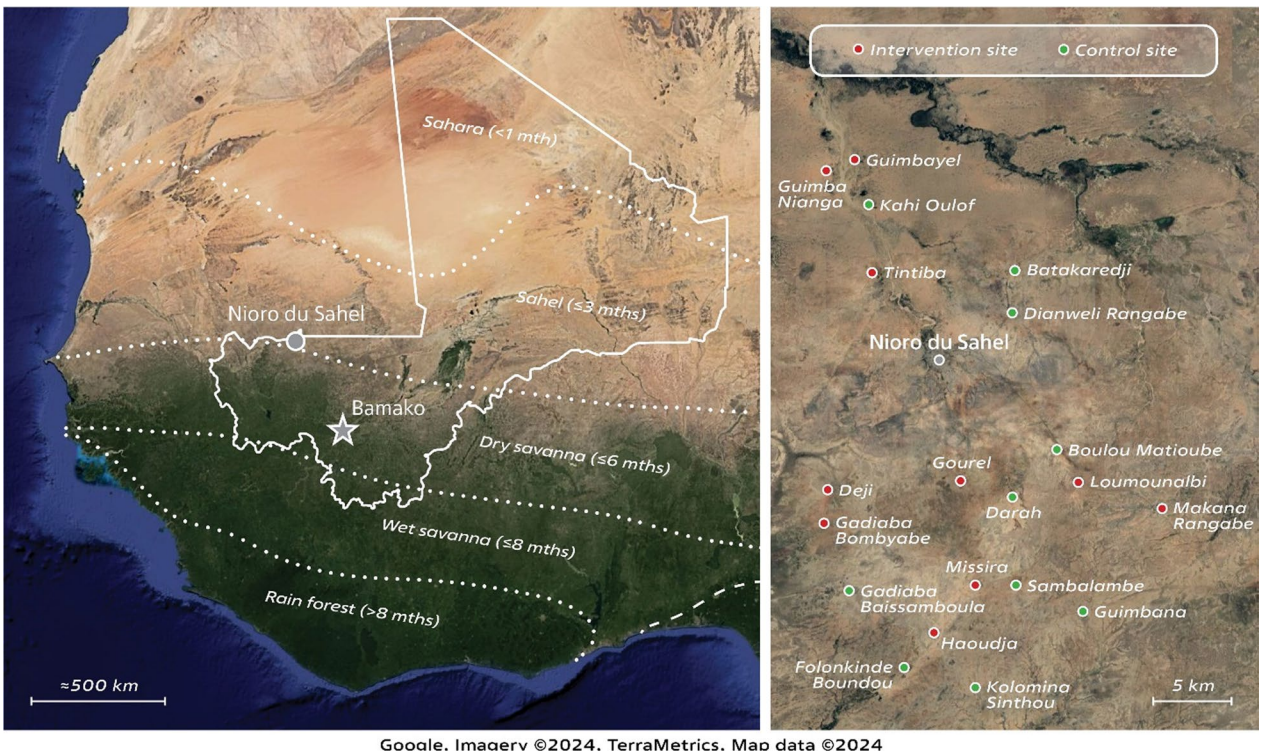

Fig. 1 Right panel—rainfall patterns and vegetation zones in Mali, left panel—location of trial villages. Green circles indicate control villages and red circles indicate treated villages

where malaria epidemiology varies according to water used and agricultural activities. In the Sahel, malaria is markedly seasonal, with more intense transmission during the late wet season and very low transmission during the dry season. This seasonality reflects the availability of suitable breeding sites for mosquitoes, which usually is rain-dependent [7]. Villages in this study, control and treated, were using insecticide-treated bed nets (ITN) as vector control. Participants in this study gave consent to hang bait stations on their homes.

In early 2019, 20 villages in the study area were selected and randomly assigned for either ATSB treatment (ten villages) or untreated control (ten villages). This was done by assigning the villages random numbers from 1 to 20 using the RAND function in excel. Villages 1 to 10 were assigned to be treated while 11 to 20 were designated as controls. The ATSB stations were deployed in late July at a density of two bait stations per sleeping structure, at a height of 1.8 m and according to [6]. The weighted density of bait stations in the villages was determined using the map (Fig. 2), where each square is 100 hectares, to make the following calculation:

$$\text{Weighted Density} = \Big[\left(\#\text{bait stations in } 1^{\text{st}}\text{hectare}\right)^2 + \left(\#\text{bait stations in } 2^{\text{nd}}\text{hectare}\right)^2 + \left(\text{bait stations } 3^{\text{rd}}\text{hectare}\right)^2 + \left(\#\text{bait stations in } 4^{\text{th}}\text{hectare}\right)^2 + \ldots.\Big]$$

divided by total number of bait stations.

Mosquito monitoring

The impact of ATSBs on local mosquito populations was monitored monthly with CDC-UV traps outdoors (10 traps per village per month), pyrethrum spray catches (PSC) in bedrooms (12 rooms per village per month, chosen at random from a pool of volunteers with homes > 10 m apart), and human landing catches (HLC)

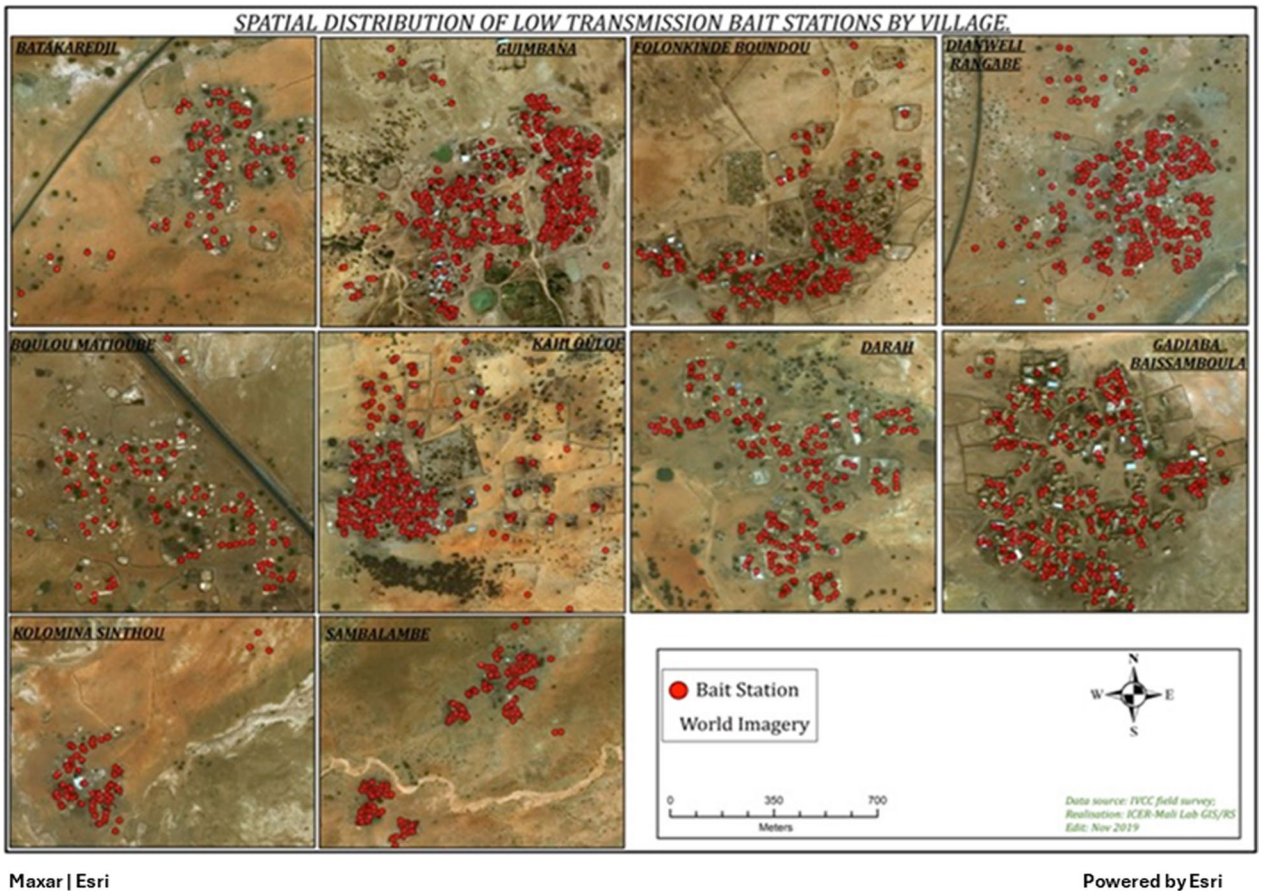

Fig. 2 Satellite geographic information systems (GIS) images of the experimental villages with bait station distribution. Each square represents 100 hectares

indoors and outdoors (2 bedrooms in separate homes, per village per month, chosen at random from a pool of volunteers with homes > 10 m apart). The first round of mosquito monitoring was conducted in late July, just before the first ATSB deployment, and then at the end of the following months until December. For this purpose, the 20 villages were visited once per month.

ATSB composition

The attractive targeted sugar baits contained the active ingredient dinotefuran 0.11% (w/w), 1% (w/w) BaitStab—a product containing antibacterial and antifungal additives (Westham LTD., Israel), 98% is the bait itself which includes: sugar and date syrup-based attractants. Bait stations were Version 1.0, (Supplement 1, Fig) constructed using a white, rectangular plastic frame with the ATSB inside a proprietary, mosquito bite and emanation-permeable, black plastic membrane cover; 100 g of the bait were inserted into the 16 cells covered by the membrane (Westham LTD, Israel (Supplement 1, Fig).

CDC UV light trapping

In each village, trapping was at the approximate center where houses were closer together, and a near-grid pattern could be obtained for good coverage. Ten CDC UV light traps (Model 512, John W. Hock Company, Gainesville, Florida, USA) were set up outdoors at least 10 m apart, in each village. Their location was in a rough grid pattern next to 10 houses (with permission of the owners), about 5.0 m away from the house. Traps were set at 18:00 h and were emptied at 06:00 h. A small square of wet cloth was included in the catch net of the trap to prevent desiccation). Trapping was conducted 1 night per month.

Human landing catches

A protocol for using human volunteers in HLC experiments was developed and carefully followed based on the United States Environmental Protection Agency (EPA) guidelines [8] as well as guidelines from an additional source [9]. Briefly, two local volunteers, one indoor and one outdoor, collected mosquitoes from 18:00 h to midnight and were replaced by two other volunteers from midnight to 06:00 h. Volunteers received full explanations of the study and were tested with rapid diagnostic test (RDT) for malaria prior to mosquito collections. Any volunteers sick with malaria before the study were treated according to the Malian National Malaria Control Programme (NMCP) guidelines and replaced by an alternate volunteer. The volunteers were seated motionless in chairs with an exposed leg extended while observing, collecting, counting, and recording mosquitoes for later identification. Mosquitoes were collected with an entomological hand-vac (Mosquito and sandfly aspirator model 419; John W. Hock Company, Gainesville, Florida USA), which was used to aspirate landing mosquitoes off the human volunteer.

Pyrethrum spray catches

To determine the size of the of the indoor resting mosquito population, PSC was conducted according to established and updated protocols in 12 bedrooms of 12 separate homes per village once a month [2, 10]. Briefly, PSCs were performed at 07:00 by spraying Permethrin for 30–45 s in the room. After 10 min, dead and immobilized mosquitoes were collected.

Age determination

Females collected by HLC and CDC UV light traps (not PSC because fresh, unfed mosquitoes are needed for age grading) were analysed, and the physiological age was determined by dissecting and examining ovaries for the number of past ovipositions in a drop of phosphate-buffered saline under a stereomicroscope at 10× – 100× to expose and count the dilatations in ovarioles [11]. Females were then classified as having undergone either < 3 or ≥ 3 gonotrophic cycles.

ELISA testing

A *Plasmodium falciparum* "sandwich" ELISA was used to test female mosquitoes for sporozoites according to a standard protocol [12]. All female *Anopheles gambiae* sensu lato collected by each mosquito collection method per village per month were processed by ELISA.

Determination of EIR

The EIR, a measure of exposure to infected mosquitoes, is defined as the product of the mosquito landing/biting rate and the sporozoite rate [13]. In this case, the mean monthly entomological inoculation rate was calculated by multiplying the monthly sporozoite rate determined by ELISA.

(for all females tested per village from HLC catches) by the monthly landing rate from control or treated villages. The monthly landing rate is defined as the number of landing females per person per night × 30 nights.

Statistics and data analysis

Mean trap catches (by method and sex), mean number of females with ≥ 3 gonotrophic cycles, and mean number of sporozoite positive females, as well as males, were compared with t-tests to determine significance (taken at $P < 0.05$). Relationship between weighted density and average number of female mosquitoes caught was determined with linear regression (Supplement 2, Fig.) to determine trend lines for each data set followed

by a Pearsons correlation test which determines relationship between each data set. Results are between − 1 to + 1. Statistical tests were performed with GraphPad Prism 8 (La Jolla, California, USA). Reduction in mosquitoes (population, age, and sporozoite positivity) was calculated as 100—(treated site mosquitoes/control site mosquitoes × 100).

Results

Mosquito abundance in ATSB-treated villages

Monthly sampling of female *Anopheles* in the control villages revealed the normal seasonal variability expected in the population throughout the year (Table 1A), coinciding with the rainy and dry seasons.

In the pre-treatment monitoring period of July, the number of female *An. gambiae* was not significantly different between the ATSB and control sites for all trapping methods (t = 3.074, df = 9, P = 0.052; Table 1A). For each trapping method the treated villages experienced a steady decline of trapped females compared to the control, from August through December. The largest number of female *An. gambiae* caught during the pre-treatment period, at both treated and control sites, was seen with the PSC method, which is consistent with the anthropophilic nature of *An. gambiae*. The greatest post-treatment decline at treated sites was in the CDC UV light trap catches, where the number of female *An. gambiae* was decreased by 71.48% July immediately after ATSB treatment, to August (Table 1A).

Table 1A presents the total number of monthly trapped female *An. gambiae*, the number of females with ≥ 3 gonotrophic cycles (older females), and the number of sporozoite-positive females. More mosquitoes were captured by all trapping methods in control villages in September, October, and November than in ATSB-treated villages. The total number of female *An. gambiae* caught by all methods in ATSB-treated villages was reduced by 41% (t = 2.621, df = 16.63, P = 0.018), compared to control villages, while the numbers of older and sporozoite positive mosquitoes were decreased by 97% (t = 8.884, df = 9, P < 0.0001) and 82% (t = 6.329, df = 10.01, P < 0.0001), respectively.

To break it down by collection method, the placement of ATSB in villages resulted in lower numbers of female *An. gambiae* caught by CDC UV light traps by 69% compared to the control (t = 1.973, df = 18.00, P = 0.044), the number of older females by 97% (t = 2.121, df = 18.00, P = 0.046) and the number of sporozoite positive females by 89% (t = 2.000, df = 18.00; P = 0.005).

The number of *An. gambiae* females captured by HLC outdoors was lower by 68% in ATSB-treated villages compared to control villages (t = 1.009, df = 10.24, P = 0.004). The numbers of older and sporozoite + mosquitoes were both lower by 100%. Similarly, the total number of females caught by HLC indoors was lower by 63% (t = 1.570, df = 11.62, P = 0.04336, while the numbers of older and sporozoite positive mosquitoes were lower by 94% (t = 1.622, df = 9.187, P = 0.039 and 100% (t = 1.000, df = 9.000, P = 0.043), respectively.

The number of females collected by PSC in ATSB-treated villages was lower by 30% (t = 4.533, df = 18.00, P = 0.0003) compared to control villages, while the number of sporozoite positive mosquitoes was lower by 78% (t = 2.351, df = 18.00, P = 0.030).

For male mosquitoes (Table 1B), significantly fewer were caught with CDC UV light traps at the ATSB-treated sites than at the control sites (Df = 5, $P < 0.0001$), whereas the number of male mosquitoes caught by PSC did not differ significantly between the ATSB and control sites (Df = 5, $P = 0.112$).

EIRs in ATSB-treated and control villages

Using the number of bites/person/per month and the sporozoite infection rate (IR), the monthly EIRs were calculated for the indoor and outdoor HLCs (Table 2). Using the indoor HLC, the monthly EIRs in control villages were mostly zero except for August and October, whereas EIRs in ATSB-treated sites remained zero in all months. Outdoors, EIRs in the control villages were mainly zero except for October and November, whereas in treated villages, EIRs were zero for all months.

The impact of ATSB weighted density on mosquito abundance

The Pearson's correlation number determines the relationship between datasets. If the r number is negative, there is a negative correlation between datasets, ie: the number of bait stations increases, while the number of females decreases. If the number is positive, the number of bait stations increases and the number of females increases. If the number is zero, there is no relationship. The total numbers of female *An. gambiae* caught by CDC UV light traps, indoor and outdoor HLC, and PSC per village for the duration of the study were significantly lower in villages with a higher weighted density of ATSB stations (r = − 0.656, P = 0.039; Table 3). Yet, the impact of ATSB weighted density on sporozoite positive mosquitoes was difficult to analyze since the number of sporozoite positive samples was very low: zero in most ATSB villages throughout the study except for Batakaredji, which had a single sporozoite positive sample caught by the CDC UV light trap and 4 by PSC (Table 3), 2 by PSC in Boulou Matioube, and 1 additional in Gadiaba Baissamboula. Similarly, only 3 dissected females showed ≥ 3 gonotrophic cycles, all from Batakaredj.

Table 1 Total and average amount of monthly caught (A) female *Anopheles gambiae* s.l., total and mean number of females ± SEM with 3 or more gonotrophic cycles, and the total and average number of sporozoite positive females ± SEM, (B) monthly total and average caught males ± SEM

A	Treatment		Total trap catch—all villages							≥3 Gonotrophic cycles						
Trap Method			**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Total**	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Total**
CDC-UV	Treated	Total	14	75	114	51	13	3	270	0	1	1	1	0	0	3
		Mean	1.4	7.5	11.4	5.1	1.3	0.3		0	0.1	0.1	0.1	0	0	
		SE	0.50	1.34	4.28	1.61	0.91	0.30		0.00	0.13	0.13	0.13	0.00	0.00	
	Control	Total	11	263	308	170	84	41	877	0	13	36	37	12	0	98
		Mean	1.1	26.3	30.8	17	8.4	4.1		0	1.3	3.6	3.7	1.2	0	
		SE	0.49	3.50	8.13	5.03	2.83	1.37		0.00	0.55	1.06	1.35	0.54	0.00	
HLC—outdoors	Treated	Total	0	19	18	15	3	0	55	0	0	0	0	0	0	0
		Mean	0	1.9	1.8	1.5	0.3	0		0	0	0	0	0	0	
		SE	0	0.5044249	0.6960204	0.4013865	0.1527525	0		0	0	0	0	0	0	
	Control	Total	0	44	66	49	10	5	174	0	0	4	5	1	0	10
		Mean	0	4.4	6.6	4.9	1	0.5		0	4.4	6.6	4.9	1	0.5	
		SE	0.00	1.42	4.14	0.66	0.21	0.22		0.00	1.42	4.14	0.66	0.21	0.22	
HLC—indoors	Treated	Total	0	56	25	18	3	1	103	0	1	0	0	0	0	1
		Mean	0	5.6	2.5	1.8	0.3	0.1		0	0.1	0	0	0	0	
		SE	0.00	2.60	0.65	0.55	0.15	0.10		0	0.1	0	0	0	0	
	Control	Total	0	79	92	83	14	12	280	0	2	5	7	2	1	17
		Mean	0	7.9	9.2	8.3	1.4	1.2		0	0.2	0.5	0.7	0.2	0.1	
		SE	0.00	3.43	5.27	1.16	0.31	0.36		0.00	0.13	0.40	0.21	0.13	0.10	
PSC	Treated	Total	31	1036	1062	124	12	10	2275	ND	ND	ND	ND	ND	ND	ND
		Mean	3.1	103.6	106.2	12.4	1.2	1		ND	ND	ND	ND	ND	ND	
		SE	0.84	49.71	22.62	1.98	0.55	0.68		ND	ND	ND	ND	ND	ND	
	Control	Total	46	1289	1602	235	66	13	3251	ND	ND	ND	ND	ND	ND	ND
		Mean	4.6	128.9	160.2	23.5	6.6	1.3		ND	ND	ND	ND	ND	ND	
		SE	1.5790292	35.889475	29.635864	5.0094355	1.5648926	0.3		ND	ND	ND	ND	ND	ND	
ND—not done					Total (all trap methods):				2703							4

Trap method	**Treatment**		**ELISA +**						
CDC-UV	Treated	Total	Jul	Aug	Sept	Oct	Nov	Dec	Total
		Mean	0	0	1	0	0	0	1
		SE	0	0	0.1	0	0	0	
	Control	Total	0.00	0.00	0.10	0.00	0.00	0.00	
		Mean	0	2	6	1	0	0	9
		SE	0	0.2	0.6	0.1	0	0	

Table 1 (continued)

Trap method	Treatment		ELISA +						
HLC—outdoors	Treated	Total	0	0.1721326	0.2108185	0.1290994	0	0	
		Mean	0	0	0	0	0	0	0
		SE	0	0	0	0	0	0	
	Control	Total	0	0	0	0	0	0	
		Mean	0	0	1	1	0	0	2
		SE	0	0	0.1	0.1	0	0	
HLC—indoors	Treated	Total	0	0	0.1	0.1	0	0	
		Mean	0	0	0	0	0	0	0
		SE	0	0	0	0	0	0	
	Control	Total	0	0	0	0	0	0	
		Mean	0	1	0	1	0	0	2
		SE	0	0.1	0	0.1	0	0	
PSC	Treated	Total	0	0.1	0	0.1	0	0	
		Mean	0	6	1	0	0	0	7
		SE	0	0.6	0.1	0	0	0	
	Control	Total	0	0.3399346	0.1	0	0	0	
		Mean	0	19	9	3	1	0	32
		SE	0	1.9	0.9	0.3	0.1	0	
ND—not done			0	0.4818944	0.2333333	0.1527525	0.1	0	
									8

B	Treatment		Total catch—all villages						
Trap method			**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Total**
CDC-UV	Treated	Total	15	54	83	1	0	0	153
		Mean	1.5	5.4	8.3	0.1	0	0	
		SE	0.40	1.11	1.73	0.10	0.00	0.00	
	Control	Total	12	195	144	7	3	0	361
		Mean	1.2	19.5	14.4	0.7	0.3	3.056626	
		SE	0.326599	3.034066	2.856571	0.260342	0.152753	0	

Table 1 (continued)

B	Treatment		Total catch—all villages						
Trap method			**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Total**
PSC	Treated	Total	6	215	282	125	1	0	629
		Mean	0.6	21.5	28.2	12.5	0.1	0	
		SE	0.6	14.349797	10.103685	3.3772112	0.1	0	
	Control	Total	5	96	255	68	10	0	434
		Mean	0.5	9.6	25.5	6.8	1	0	
		SE	0.31	3.56	11.36	1.06	0.56	0.00	
								Total	1577

Table 2 Human landing catches with monthly entomological inoculation rates calculated

A	Indoor treated sites					Indoor control sites				
Month	**Monthly BP**	**#Tested**	**#Infected**	**Infection rate (IR)**	**EIR**	**Monthly BP**	**#Tested**	**#Infected**	**Infection rate (IR)**	**EIR**
Jul	0	0	0	0	0	0	0	0	0.0000	0
Aug	840	56	0	0	0	1185	79	1	0.0127	15
Sept	375	25	0	0	0	1380	98	0	0.0000	0
Oct	270	18	0	0	0	1245	83	1	0.0120	15
Nov	45	4	0	0	0	210	14	0	0.0000	0
Dec	15	1	0	0	0	180	12	0	0.0000	0
B	**Outdoor treated sites**					**Outdoor control sites**				
Month	**Monthly BP**	**#Tested**	**#Infected**	**Infection rate (IR)**	**EIR**	**Monthly BP**	**#Tested**	**#Infected**	**Infection rate (IR)**	**EIR**
Jul	0	0	0	0	0	0	0	0	0.0000	0
Aug	285	19	0	0	0	660	44	0	0.0000	0
Sept	270	18	0	0	0	990	66	0	0.0000	0
Oct	225	15	0	0	0	735	49	1	0.0204	15
Nov	45	3	0	0	0	150	10	1	0.1000	15
Dec	0	0	0	0	0	75	6	0	0.0000	0

(A) Indoor HLC, (B) Outdoor HLC

BP: (number of bites per month/2 volunteers)*30 nights; IR: (# infected/number tested); EIR: (BP*IR)

Discussion

After the development of ATSBs, they have been extensively field-tested in the Mediterranean climate around Jerusalem and the arid mosquito habitat of the Jordan Valley, Israel [4, 5, 14–16]. At the end of the dry season in the arid region of southern Israel, ATSB sprayed around sewage ponds was found to significantly reduce *Culex pipiens* 5 to 8 per trap at the experimental site compared to ~ 60 per trap at the control site throughout the study [3]. In 2 small oases in a barren desert area in southern Israel, ATSB treatment collapsed the *Anopheles sergentii* and *Aedes caspius* populations within days. Compared to the control site, the *An. sergentii* and *Ae. caspius* populations were reduced to < 1/10 and 1/3 of their original sizes, respectively [5]. In the current study, the female *An. gambiae* population in an arid region of Mali was lower by 69% in ATSB deployment areas over 6 months compared to control areas (Table 1A). This result further highlights the potential for the ATSB method to be applied for control in other arid areas.

The use of ATSB stations outdoors has also been associated with major reductions in EIR. In a 2020 study [2], within 10 km of the Niger river, in an area where mosquitoes have more access to vegetation, the monthly entomological inoculation rate (EIR) was calculated for HLCs at indoor and outdoor sites. The EIR was high during the rainy season and low during the dry season and ATSB was effective during both, but more so during the dry season. In that study, at indoor control sites, the EIR was as low as zero during the dry season in April, May and December but as high as 70.71 in September (wet season). Post-intervention, EIRs were reduced to zero for all dry season months. Exceptions were wet season months of July, August, September and October with the highest EIR of 10.71 in September. At outdoor control sites, the highest EIR was 57.93, lowest zero and this was reduced to zero in all dry season months. Exceptions were wet season months of August, September and October (highest was 6.45). In the current study, EIR was 15 at the indoor control sites in August and October and at the outdoor control sites in October and November. In the ATSB treatment villages, EIR remained zero for all months at both indoor and outdoor sites (Table 2). These results confirm the impact of ATSB on reducing entomological indicators of malaria such as EIR in different ecological and endemicity settings.

It is worth noting that ATSB significantly reduced female mosquitoes with ≥ 3 gonotrophic cycles, as revealed by all trapping methods, suggesting an overall reduction of the adult mosquito age (Tables 1 and 3). Female anopheline mosquitoes require a 10–18-day growth cycle after feeding on human blood infected with malaria parasites before transmitting the parasites [17]. This means that only female *Anopheles* ≥ 10 days old may effectively transmit malaria. Also, a mosquito that has gone through three blood feeding/egg laying cycles

Table 3 Weighted density and total annual numbers of female *An. gambiae* mosquitoes caught by each trapping method, per village and the subset of those that are infected (sporozoite positive) or the number of those that have undergone ≥ 3 gonotrophic cycles

WD	Experimental villages	Population density				ELISA +				3 + Gonotrophic cycles			
		CDC Traps	HLC outdoors	HLC indoors	PSC	CDC Traps	HLC outdoors	HLC indoors	PSC	CDC Traps	HLC outdoors	HLC indoors	PSC
53.08	GUIMBANA	19	8	4	99	0	0	0	0	0	0	0	ND
51.34	GADIABA BAISSAMBOULA	51	5	9	75	0	0	0	1	0	0	1	ND
51.24	KAHI OULOF	17	1	28	72	0	0	0	0	0	0	0	ND
44.15	DARAH	14	3	3	107	0	0	0	0	0	0	0	ND
41.79	FOLONKINDE BOUNDOU	29	4	5	171	0	0	0	0	0	0	0	ND
34.41	BOULOU MATIOUBE	26	14	14	336	0	0	0	2	0	0	0	ND
29.37	SAMBALAMBE	28	3	5	85	0	0	0	0	0	0	0	ND
28.19	DIAWELI RANGABE	22	10	12	348	0	0	0	0	0	0	0	ND
27	KOLOMINA SINTHOU	31	4	11	178	0	0	0	0	0	0	0	ND
23.17	BATAKAREDJI	33	3	12	804	1	0	0	4	3	0	0	ND
	Total	**270**	**55**	**103**	**2275**	**1**	**0**	**0**	**7**	**3**	**0**	**1**	ND
WD	**Control villages**	**Population density**					**ELISA +**				**3 + Gonotrophic cycles**		
		CDC Traps	**HLC outdoors**	**HLC indoors**	**PSC**	**CDC Traps**	**HLC outdoors**	**HLC indoors**	**PSC**	**CDC Traps**	**HLC outdoors**	**HLC indoors**	**PSC**
N/A	DEJI	144	7	21	319	1	0	0	2	17	0	0	ND
N/A	GADIABA BOMBYABE	91	18	23	542	0	0	1	6	10	1	3	ND
N/A	GOUREL	68	18	28	84	2	1	0	0	10	1	2	ND
N/A	HAOUDJA	69	54	63	256	1	1	0	1	5	3	5	ND
N/A	MISSIRA	63	7	13	394	1	0	0	5	6	1	0	ND
N/A	TINTIBA	91	6	5	88	2	0	0	1	9	1	1	ND
N/A	MAKANA RANGABE	93	7	22	335	1	0	0	4	14	0	2	ND
N/A	LOUMOUNALBI	51	12	66	421	0	0	1	3	1	1	2	ND
N/A	GUIMBAYEL	123	31	19	640	1	0	0	8	17	2	1	ND
N/A	GUIMBA NIANGA	84	14	20	172	0	0	0	2	9	0	1	ND
	Total	877	174	280	3251	9	2	2	32	98	10	17	ND

WD: Weighted density; N/A: Not applicable; ND: Npt done

is more likely to be infected than a younger one and therefore capable of transmitting malaria. Thus, reducing these females should result in a general decrease in malaria transmission.

The current study has offered important entomological evidence showing the effects of ATSB, as a new malaria intervention, in reducing the *Anopheles* population, older females of ≥ 3 gonotrophic cycles, sporozoite positive females, and, ultimately, the EIRs (Tables 1, 2, 3). As the world looks to expand the tools in the fight against malaria [18–20], ATSB may prove an important method to fill the current gap in vector control, once its epidemiological impact is demonstrated.

Strengths and limitations

The current study adds to the body of evidence that ATSB application reduces important entomological markers, such as the number of older, more dangerous females. It is also demonstrated that the greater the weighted density of ATSB stations a village, the greater the decline in these entomological markers. This study confirms the effectiveness of ATSB treatment in areas where transmission is already low.

This study is limited in that it did not directly measure the influence of ATSB on malaria prevalence and incidence. As ATSB has proved to be effective in reducing the *An. gambiae* population in this type of ecosystem, more extensive trials can be planned to establish its role as a new vector control intervention in different malaria endemicity settings. The current study would have benefitted from more monitoring days per month and data collection over multiple seasons, however this would require considerable effort and funds as villages are remote and dispersed.

Conclusions

This study demonstrates ATSB's potential to significantly reduce and eliminate infective malaria vectors in arid areas with low mosquito population size and sporozoite positivity rates. Therefore, the preponderance of evidence from previous studies in addition to this suggest ATSB is a viable tool to be added to the current malaria control arsenals.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions
GCM YS and JCB conceived and designed the study. AJ, MMT, and TAP performed data analysis and interpretation and drafted the original manuscript. GCM, AMP, ASK, MMT, SFT, AS, RY, JV, NS, MD, PG, SD, and EER supervised and carried out the fieldwork. LC, and RX assisted in drafting the manuscript. AJ carried out statistical analyses.

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Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All the work involving the use of human volunteers in experimental homes and HLCs was approved by the ethical review committee of the University of Sciences Techniques and Technology (IRB 2015/107/CE/FMPOS).

Competing interests
The authors declare no competing interests.

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