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IMPACT OF INDOOR RESIDUAL SPRAYING USING BENDIOCARB 80% ON THE COMPOSITION, ABUNDANCE AND RESTING HABITS OF MALARIA VECTORS IN BAHIR DAR ZURIA DISTRICT, ETHIOPIA

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ABSTRACT: The reduction of malaria-related mortality and morbidity are attributed to the use of indoor residual spraying and long-lasting insecticidal nets. However, data for the measurable impacts of these vector control intervention tools on entomological indices are either unavailable or not sufficient. The impact of indoor residual spraying using bendiocarb 80% on the composition, abundance and resting habits of malaria vectors was assessed. Mosquitoes were sampled from residential houses using pyrethroid spray catches and CDC light traps. Pit shelter collections were also made from pit shelters constructed under shade trees and in close proximity to nearby indoor collecting houses. Vectors were surveyed and recorded before the application of indoor residual spraying. Descriptive and inferential statistics were used for data analyses. A total of 20,243 mosquitoes were collected and 43.8% and 56.2% of them belong to Anopheles and culicines mosquitoes, respectively. *Anopheles arabiensis*, *An. pharoensis* and *An. coustani* were recorded during the study period. *An. arabienesis* was the most abundant species (70.2%), while *An. coustani* was the least abundant (7.9%). Vector population drastically declined after spray. Indoor and outdoor resting habits of *An. arabiensis* were unaffected by the spray. General estimating equation model analyses showed that all effects or explanatory variables and their sublevels played significant role in the build-up of *An. arabiensis* mosquito population, except the number of human hosts that slept the previous night in mosquito collection houses. The results indicated that indoor residual spraying using bendiocarb 80% effectively reduced the abundance of malaria vectors without impacting its resting habits.

Key words/phrases: Anopheles mosquito, Ethiopia, Indoor residual spraying, Mosquito abundance.

INTRODUCTION

Vector control interventions have been suggested after the incrimination of mosquitoes as malaria vectors by Ronald Ross in 1897 and Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli,

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Camillo Golgi and Ettore Marchiafava between 1898 and 1900 (Cox, 2010). Since then, a range of vector control tools including larval control by environmental management and larviciding, and the control of adult mosquitoes using chemical insecticides, house improvement, repellent and biological control have been developed and used. Among these tools developed so far, indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are the primary vector control tools (Curtis, 1996; WHO, 2006; Pluess *et al*., 2010). Indoor residual spraying and LLINs are efficient against vectors of malaria that prefer to bite and rest indoors (Gillies and De Meillon, 1968; Bockarie *et al*., 2009), but less effective for vectors that are biting and resting outdoors (Okumu *et al*., 2013). The efficacy of these intervention tools also depends up on the susceptibility of the vectors to the insecticides used for IRS operation and LLINs impregnation.

In the last decade, reports have indicated that malaria deaths have been reduced by 33% following the massive use of LLINs distribution and IRS coverage (Hemingway, 2014). However, the emergence and spread of resistance against insecticides used for IRS and mosquito net treatment in malaria vector populations have become a growing threat to these insecticide-based vector control operations and to sustain the success achieved so far. Vectors' physiological insecticide resistance to at least one insecticide used for malaria control has been reported from 64 countries (WHO, 2013). Malaria vectors may also challenge the role of IRS and LLINs in malaria control by changing their indoor biting and resting habit to outside of the human homes (Charlwood and Graves, 1987) and by altering their active biting time from late to earlier hours of the night (Yohannes and Boelee, 2012; Gatton *et al*., 2013). These intervention tools could also reduce the abundance of predominant vector species and favour secondary and other potential vectors to boost and maintain disease transmission (Wilikes *et al*., 1996). Thus, assessing the impact of these entomological intervention tools on malaria transmission should be an integral part of any vector control program.

Similarly, IRS and LLINs are the frontline vector control tools in Ethiopia where 70.5% of the households living in areas < 2,000 metres are protected by IRS and LLINs (EMIS, 2015). These insecticide-based indoor intervention tools have been used primarily against *An. arabiensis*, the main malaria vector in the country. *Anopheles pharoensis*, *An. funestus* and *An. nili* are also important vectors (Abose *et al*., 1998). Success stories in the reduction of malaria related mortality and morbidity are attributed to the use

of IRS and LLINs vector control operations which have improved health system. Thus, the country is trying to sustain the achievements made so far and to eliminate malaria from hypo-endemic areas from the country by 2020 by increasing IRS coverage and LLINs distribution and improving case detection and treatment in the health system in general (Adugna Woyessa *et al*., 2013). However, data for the measurable impacts of these frontline vector control intervention tools on entomological indices are insufficient, unavailable or missing.

Therefore, the aim of this study was to assess the impact of indoor residual spraying using bendiocarb on the composition, abundance and resting habits of malaria vectors.

MATERIALS AND METHODS

Description of the study area

A comparative study was carried out in Yegoma Huletu kebele at three villages namely Andassa (N11° 30′ 14.6″, 037° 29′ 27.8″), Tigeza (N11° 29′ 43.6″, 037° 27′ 52.2″), Tikurit (11° 30′ 49.8″, 037° 28′ 02.8″) and in Sebatamit kebele at one village called Tikurit-Dewel (N 11° 31′ 14.2″, E 037° 23′ 52.3″). The area was selected purposively by considering malaria endemicity and associated risk factors for malaria transmission such as the existence of permanent, semi-permanent and temporary vector breeding sites, presence of important malaria vectors, and socio-economic characteristics. The total study area covered about 47.13 km^2. The area has bimodal rainfall patterns, with a long rainy season between June and September, and a short rainy season between March and April. Indoor residual spraying and LLINs are in the first line of defense against *An. arabiensis* (important vector of the study area).

Study design

The study villages were categorized into two study groups, Andassa and Tigeza in one group, named as Andassa; and Tikurit and Tikurit-Dewel in the second group, and named as Tikurit. Grouping was made based on their proximity to each other, while other factors were considered similar. Bendiocarb was sprayed at a rate of 400 mg/m^2 in Andassa in October 5–11/2013 and 2014 to compare the impact between groups and in both study groups in September 4–10/2015 to compare the impact within the same group. Bendiocarb was sprayed after confirming susceptibility of the vector to bendiocarb (0.1%) using WHO susceptibility test procedures. Twenty-four houses (12 houses for PSC and 12 houses for CDC light trap collection)

located nearby mosquito breeding sites were selected and used for mosquito collection. Twelve pit shelters were also constructed with a depth of one metre and width of 1.2 metres under shade trees and nearby pyrethrum spray sheet collection (PSC) mosquito sampling houses. Mosquito sampling was done in the same houses and pit shelters throughout the study period.

Adult mosquito sampling and identification

Mosquito sampling was carried out in the months of September (before the IRS application in 2013 and 2014) and October (one month after intervention), November (two months after intervention) and December (three months after intervention) in the same year. Similar data were collected in September 2015 after IRS application in both Andassa and Tikurit. Adult mosquitoes were collected using PSC, CDC light traps and pit shelter sampling methods. Pyrethroid spray catches and pit shelter collections were carried out from six houses and six pit shelters per study arm, respectively, between 6:30 and 11:00 AM. For indoor mosquito collection, pyrethroid aerosol (Roach killer, M/S Kafr EI Zayat, Egypt with registration No. ET/HHP/130) was applied by one trained spray man on the outside and the investigator from inside of the house simultaneously after room surfaces were covered by white spray sheets. After 12 minutes the spray sheets were taken cautiously out of the house and knocked down mosquitoes were collected using forceps and labelled paper cups covered with white netting. Prior to spraying the aerosols, all food items and animals were taken outside of the house. Mosquitoes resting inside the pit shelters were searched out using torch light, mouth aspirators and paper cups. CDC light trap collections were also made in six houses per study group between 6:00 to 6:30 PM. Traps were hung down from wooden sticks, which were placed across the top of the walls parallel to the foot end of the bed. Mosquitoes that were collected by each sampling method were sorted into species and sexed before being counted. Each species was identified morphologically using keys (Verone, 1962; Gillies and Coetzee, 1987). A PCR molecular technique was employed for sibling species of *An. gambiae* s.l. at CDC laboratory, CDC, Atlanta, Georgia, USA.

PCR based identification of *An. gambiae* complex

DNA extraction

Each mosquito preserved in alcohol (head and thorax for sporozoite infection and the abdomen for species identification) was grinded individually in a tube containing 50 µl of grinding buffer with a sterile blue

knots pestle until no identifiable mosquito parts remained. The pestle was then rinsed with additional 50 µl of grind buffer into the same tube. The tubes were then put in dry bath for 30 minutes to kill nucleases released after grinding the mosquitoes. Thirteen microlitres of 8M potassium acetate was added to each tube while still warm and mixed up by tapping. The tubes were then left on ice for an hour to precipitate out the mosquito parts, other insoluble and proteins denatured by SDS. The solution was micro centrifuged at a maximum speed (13, 2000 rpm) at 4°C for 20 minutes and the supernatants were transferred into the new tubes immediately after the spin without disturbing the precipitate. Two hundred microlitres of 100% ethanol was added to each tube containing the supernatant and left at -20°C for incubation overnight and to precipitate out the DNA. The next day the tubes were micro centrifuged at a maximum speed for 20 minutes to pellet the DNA and two hundred micro litre of 70% ice-cold ethanol added to each tube, micro centrifuged for 5 minutes again. The 70% ethanol was then pipetted out and the DNA pellets dried in air. The DNA pellets were dissolved in 20–50 µl of sterile H_2O depending on the size of DNA pellet and stored at -20° C for short term and -80° C for long term storage.

PCR components and reactions

The mixtures consisted of extracted DNA consisted of Accustar™ II check (a mixture of Taq Polymerase, $MgCl_2$, dNTPs and buffer), primers, biological grade water and DNA template, which made 25 µl in total volume (Table 1). Positive and negative DNA and no template controls using sterile water instead of DNA controls were also used in parallel. Thermal cycling was programed at Bio-Rad PCR machine for 30 cycles with an initial denaturing step at 95°C for 30 seconds, annealing at 60° C for 30 seconds, extension at 72° C for 30 seconds, at 72° C for 5 minutes and held at -12° C for further analyses.

Visualization of amplified DNA

The PCR products were separated by electrophoresis on 2.5% agarose TBE gels, and stained with ethidium bromide. A 1.8 gm of agarose gel was dissolved in 90 ml of buffer and stained by 9 µl of gel red. The solution was microwaved for 2 minutes and cooled by running cold tap water on the outside of heating flask. The solution was then poured into an electrophoresis tray where 14 or 20 well comb was inserted. The comb was removed after the agarose gel was completely cooled. Two microlitres of the PCR product were loaded into each well and run at 70–75 volts for 2½ hours. The amplicons were visualized with an ultraviolet Trans-illumination

gel documentation system (Alpha Imager 2200, San Leandro, California, USA). The predicted DNA bands on the gel were compared to a 100 bp reference ladder.

Data analysis

After entering data in excel spread sheets, they were analyzed using descriptive and inferential statistics. Descriptive statistics was used to show the abundance of indoor and outdoor resting habits of Anopheles mosquitoes across the season and based on categorical variables such as study villages, insecticide spray status, sampling methods, sampling months and years. Bar graphs were used to show total number of mosquitoes recorded against categorical variables.

Table 1. Primers and PCR components used for the identification of *An. gambiae* complex.

Anopheles species ID	**Primer sequence**	**Vol./25 µl rxn**
IMP-UN-F	GCTGCGAGTTGTAGAGATGCG	1 µl
AR-3T-R	GTG TTA AGT GTC CTT CTC CGT C	1 µl
AG-3T-R	GCT TAC TGG TTT GGT CGG CAT GT	1 µl
ME-3T-R	CAA CCC ACT CCC TTG ACG ATG	1 µl
QD-3T-R	GCA TGT CCA CCA ACG TAA ATCC	1 µl
H_2O	Biological grade	5.5 µl
DNA templates	Extracted from mosquitoes	2 µl
Accustar II		12.5 µl
Total volume/well		25 µl

Before performing formal inferential statistics, normality and homogeneity of variance tests were done using Shapiro-Wilk test, and Levene's test. Both tests turned out to be significant, suggesting that the groups to be compared were not normally distributed and they did not have the same variance. Therefore, parametric tests such as ANOVA could not be used. Furthermore, the data generally lacked the third basic assumption of independence because data were temporally correlated. Therefore, Generalized Estimating Equations (GEEs) were employed with reasonable statistical efficiency to analyze the data. The GEE approach is based on the concept of estimating equations and provides a very general approach for analyzing correlated responses.

The GEE with negative binomial distribution was preferred for analysis. The negative binomial distribution is defined by two parameters, the mean and a dispersion parameter, which measures the degree of clumping or aggregation in the distribution (White and Bennetts, 1996). The mean response (mosquito abundance) was modelled as a negative binomial regression model using the explanatory variables such as housing category (two levels, i.e., sprayed and non-sprayed with insecticide), sampling houses

(sprayed and non-sprayed), four sampling periods or months (i.e., September, October, November, December), and sampling methods (CDC light trap, PSC and Pit). The response variable, i.e., number of mosquitoes, for individual houses was assumed to be equally correlated, implying an exchangeable correlation structure. The concept of correlation refers to data collected repeatedly (from September to December) from each house and across years. Data recorded from each house are technically correlated enough because they come from the same house.

RESULTS

Mosquito composition and their abundance

During this study, 20,243 mosquitoes were collected in the main malaria transmission season (September to December) in 2013, 2014 and 2015. Overall, 43.8% (n=8,859) belonged to the different Anophelines, while 56.2% (n=11,384) of them were culicines. *Anopheles arabiensis*, *An. pharoensis* and *An. coustani* were the only Anopheles species identified during the study period. The results of molecular identification using polymerase chain reaction revealed that all Anopheles mosquitoes that were identified as *An. gambiae* s.l morphologically were *An. arabiensis* (Fig. 1). *Anopheles arabienesis* was the most abundant species (70.2%, N=6,215), while *An. coustani* was the least abundant (7.9%, N=696). *Anopheles pharoensis* was the second most abundant malaria vector species next to *An. arabiensis* contributing 22% (n=1,948) of the Anopheles mosquitoes recorded.

Impact of IRS on the abundance of Anopheles mosquitoes

Anopheles arabiensis: the abundance of *An. arabiensis* was high before the application of IRS in 2013 and 2014. Abundance declined in 2015 after IRS application compared to the two-year baseline data that had been recorded before IRS application in both study villages (Fig. 2A). In contrast to non-sprayed village, the number of *An. arabiensis* also declined dramatically after IRS application in 2013 and 2014 in the sprayed village (Fig. 2B). Similar results were also demonstrated in sprayed village following IRS application in October compared with the previous month before IRS application both in 2013 and 2014 (Fig. 2B).

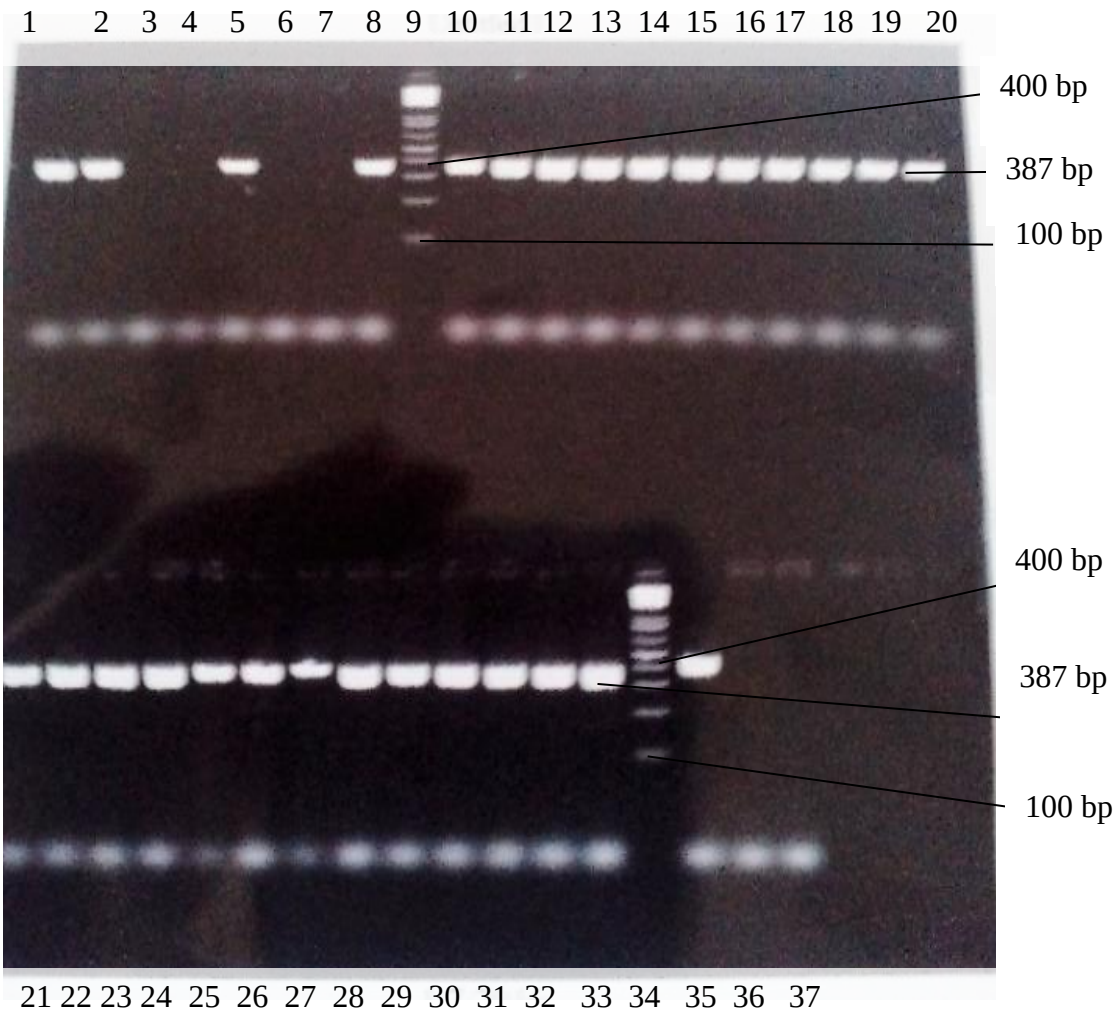

Fig. 1. Gel pictures of *An. gambiae* complex species identified using IMP method. Lane 9 and 34 contain 100 bp marker, lane 1, 2 , 5, 8, 10-20, 21-33 and 35 are bands of *An. arabiensis*, lane 3, 4, 6, 7, 36 and 37 not amplified.

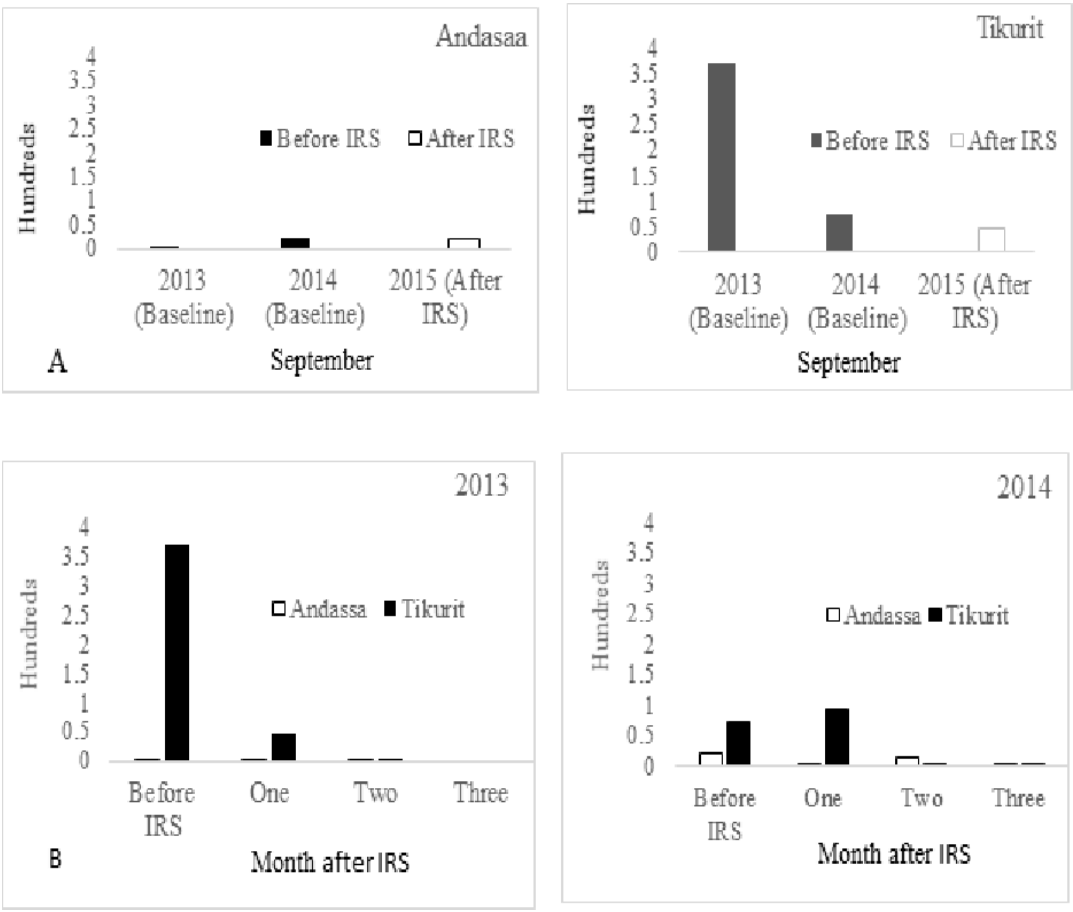

Fig. 2. Bar graphs showing the effect of IRS using bendiocarb on the abundance of adult *An. arabiensis* estimated based on (A) two years baseline data collected in 2013 and 2014 before spray and in 2015 after spray (B) data collected before IRS and one, two and three months after IRS in 2013 and 2014 in Andassa and Tikurit.

Anopheles pharoensis: the impact of IRS on the abundance of *An. pharoensis* was not consistent and strong (Fig. 3A and B) compared to the impact on *An. arabiensis*. Reduction in the abundance of *An. pharoensis* was more pronounced and consistent with time i.e., highest in September and lowest in December (Fig. 3A and B).

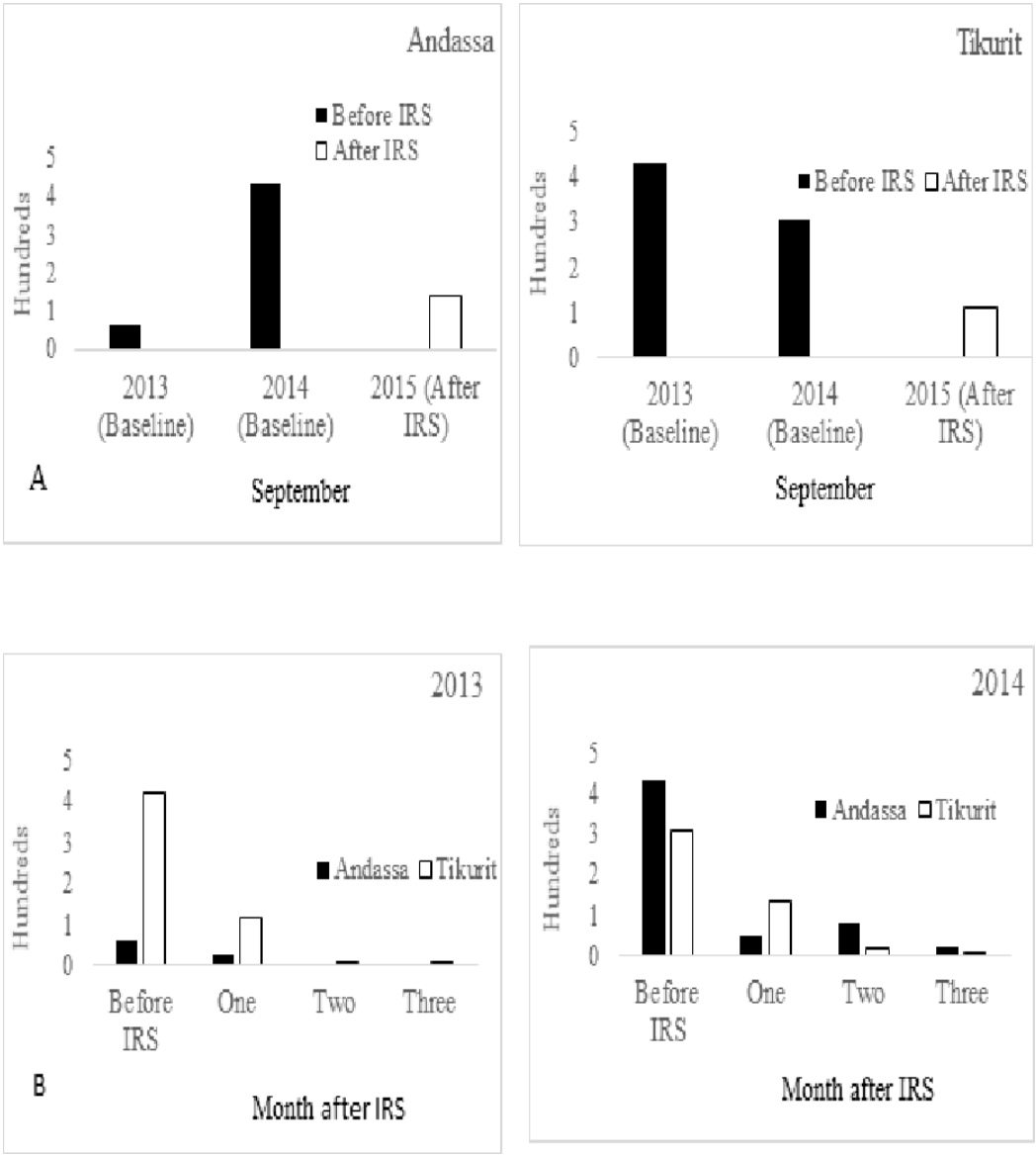

Fig. 3. Bar graphs showing the effect of IRS using bendiocarb 80% on the abundance of adult *An. pharoensis* estimated based on (A) two years baseline data collected in 2013 and 2014 before IRS and 2015 after IRS (B) data collected before IRS and one, two and three months after IRS in 2013 and 2014 in Andassa and Tikurit.

Anopheles coustani: The abundance of *An. coustani* recorded in sprayed village both before IRS and after IRS was negligible to validate the impact of IRS on its abundance. However, in non-sprayed village, the abundance declined after spray in September 2015 compared to the numbers recorded

in September 2013 and 2014 before spray. The abundance of *An. coustani* was also declined in October 2013, but increased in October in 2014 in non-sprayed village when IRS was not applied (Fig. 4B).

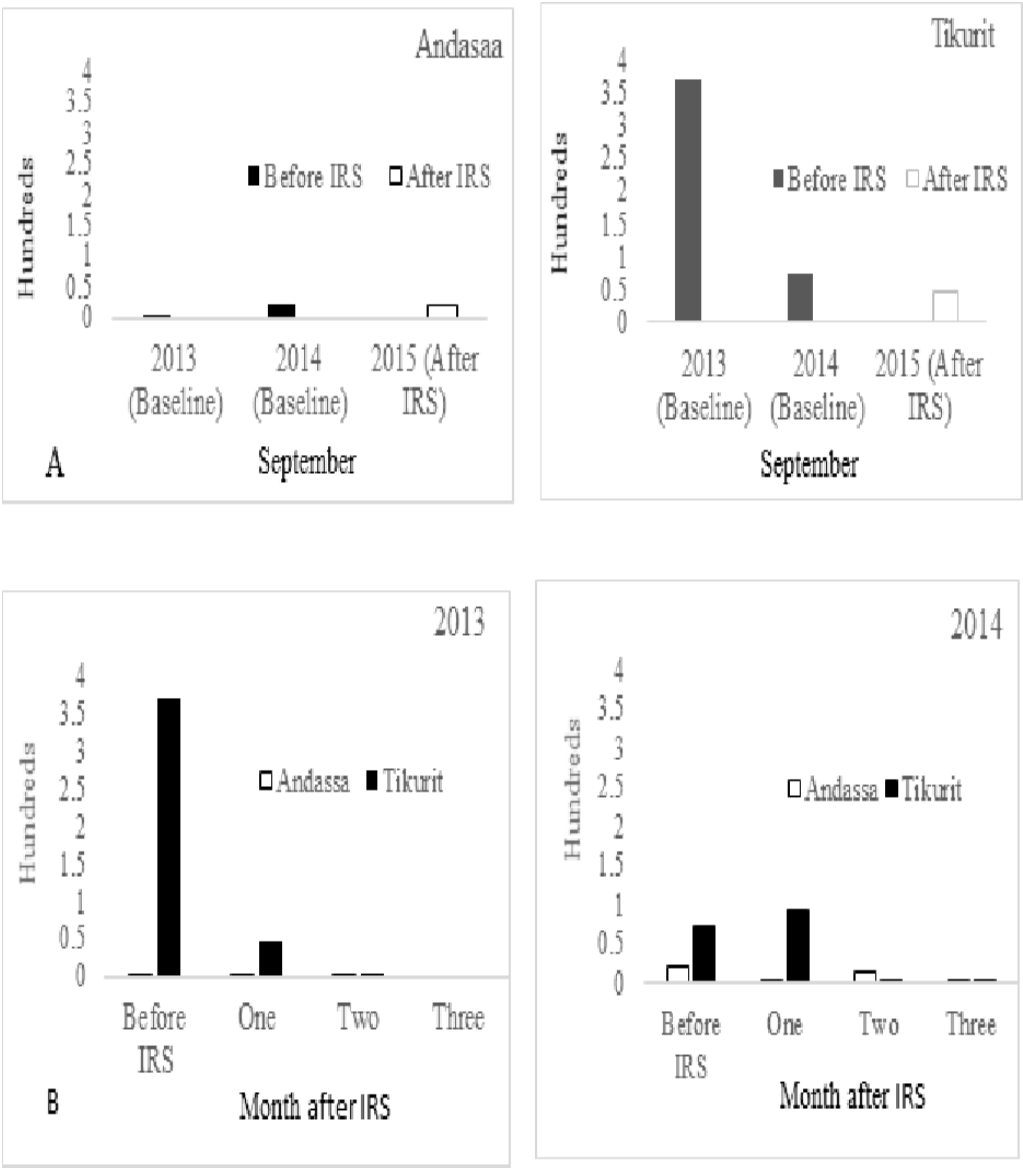

Fig. 4. Bar graphs showing the effect of IRS using bendiocarb 80% on the abundance of adult *An. coustani* estimated based on (A) two years baseline data collected in 2013 and 2014 before IRS and in 2015 after IRS (B) data collected before IRS and one, two and three months after IRS in 2013 and 2014 in Andassa and Tikurit villages.

Impact of IRS on indoor and outdoor resting density of malaria vectors

Anopheles arabiensis: The numbers of outdoor resting *An. arabiensis* captured were too few to compare with the number captured indoor. However, the result indicates that no change was observed in the resting habits of the vector as a result of spraying (Fig. 5A, B and C).

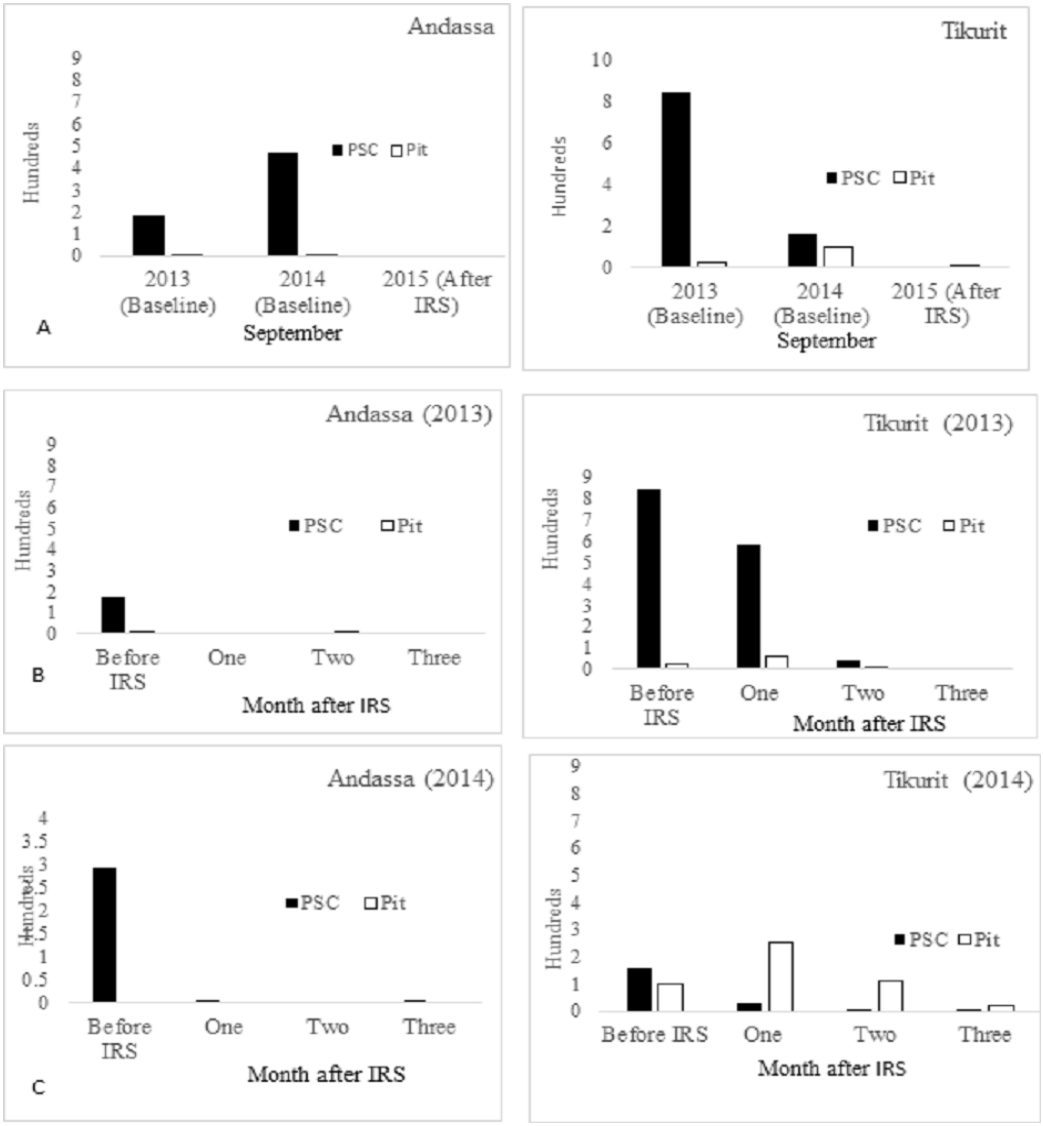

Fig. 5. Bar graphs showing the effect of IRS using bendiocarb 80% on indoor and outdoor density of *An. arabiensis* estimated based on the number of mosquitoes collected using PSC and Pit shelter sampling methods (A) two years baseline data collected in 2013 and 2014 before IRS and in 2015 after IRS; before IRS and one, two and three months after IRS in (B) 2013 and (C) 2014 in Andassa and Tikurit villages.

Anopheles pharoensis: Several indoor resting *An. pharoensis* were recorded only in September 2014 in sprayed village (Fig. 6A and C). However, the numbers of *An. pharoensis* captured in other years either indoor or outdoor were negligible (Fig. 3A, B and C) to do valid analysis and to demonstrate the impact of IRS application on the resting habits of this species.

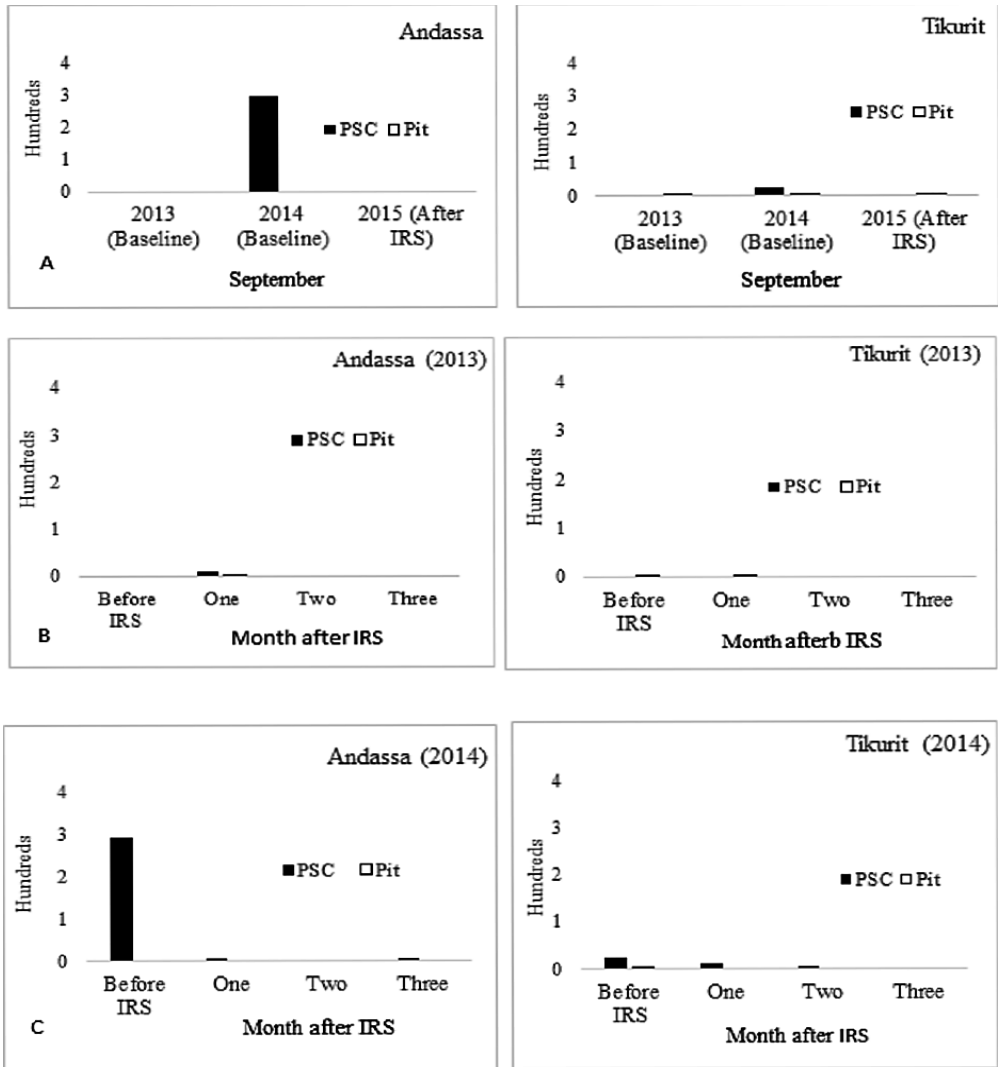

Fig. 6. Bar graphs showing the effect of IRS using bendiocarb 80% on indoor and outdoor density of *An. pharoensis* estimated based on the number of mosquitoes collected using PSC and Pit shelter sampling methods (A) two years baseline data collected in 2013 and 2014 before IRS and in 2015 after IRS; before IRS and one, two and three months after IRS in (B) 2013 and (C) 2014 in Andassa and Tikurit villages.

Anopheles coustani: Few *An. coustani* captured indoor and outdoor by PSC (n=7) and Pit (n=3) during the whole study period so that the numbers were too small to validate the impact of IRS application on the resting habits of this vector species.

Generalized estimating equations

Analyses of maximum likelihood parameter estimate was made using GEE to estimate the effect of explanatory variables including sampling villages, months, methods and hosts (human and cattle) on the number of mosquitoes collected. The parameter estimates table for each species contains parameter estimates, standard errors, confidence intervals, Z scores, and values for the parameter estimates.

Anopheles arabiensis: The parameter estimates table for *An. Arabiensis* is presented in Table 2. All parameter estimates except human hosts significantly contributed for the model ($P<0.05$). That meant all effects or explanatory variables (spraying categories, months, and sampling methods) and their sublevels played significant role in the buildup of mosquito abundance except the number of persons. The role of the number of persons is not strong enough. Because the column in the model matrix corresponding to the parameter for spraying category at non-sprayed villages was found to be linearly dependent, or aliased, with columns corresponding to parameters preceding it in the model, i.e., spraying category at sprayed villages, and therefore PROC GENMOD of SAS assigned it zero for both the parameter estimate and its standard error.

Table 2. Results of analyses of maximum likelihood parameter estimate for *An. arabiensis* abundance against explanatory variables.

Parameter	Levels*	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-square	Pr > Chi-square
Intercept		1	0.7265	0.2236	0.2882	1.1648	10.55	0.0012
Villages	SP	1	-2.5958	0.2379	-3.0620	-2.1296	119.10	<.0001
	NSP	0	0.0000	0.0000	0.0000	0.0000	.	.
Methods	CDC	1	2.0287	0.2332	1.5717	2.4857	75.70	<.0001
	PSC	1	1.2611	0.2724	0.7273	1.7949	21.44	<.0001
	Pit	0	0.0000	0.0000	0.0000	0.0000	.	.
Months	BIRS	1	1.8974	0.2833	1.3422	2.4527	44.86	<.0001
	OML	1	1.0440	0.2576	0.5392	1.5488	16.43	<.0001
	TML	1	-1.1040	0.2861	-1.6648	-0.5433	14.89	0.0001
	THML	0	0.0000	0.0000	0.0000	0.0000	.	.
Persons		1	-0.0602	0.0355	-0.1298	0.0093	2.88	0.0897
Cattle		1	0.0783	0.0344	0.0108	0.1458	5.17	0.0230
Dispersion		1	4.1231	0.3165	3.5027	4.7435		

*Sp=sprayed, NSP=non-sprayed, CDC=CDC light trap, PSC = pyrethroid spray sheet collection, Pit=pit shelter collection, BIRS=before indoor residual spraying, OML=one month later, TML=two months later, THML=three months later

Anopheles pharoensis: Except the sprayed villages category, all explanatory variables played significant (P<0.01) role on the abundance of *Anopheles pharoensis* (Table 3).

Table 3. Results of analyses of maximum likelihood parameter estimates for *An. pharoensis* abundance against explanatory variables.

Parameter	Levels	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > Chi-square
Intercept		1	-5.3332	0.5111	-6.3351	-4.3314	108.87	<.0001
Villages	SP	1	-0.2919	0.2447	-0.7715	0.1878	1.42	0.2330
	NSP	0	0.0000	0.0000	0.0000	0.0000	.	.
Months	B IRS	1	2.2427	0.3012	1.6523	2.8331	55.43	<.0001
	OML	1	1.3870	0.3323	0.7357	2.0383	17.42	<.0001
	TML	1	-1.2177	0.3759	-1.9545	-0.4808	10.49	0.0012
	THML	0	0.0000	0.0000	0.0000	0.0000	.	.
Methods	CDC	1	5.8581	0.4372	5.0012	6.7151	179.52	<.0001
	PSC	1	2.3545	0.4360	1.5000	3.2090	29.17	<.0001
	Pit	0	0.0000	0.0000	0.0000	0.0000	.	.
Persons		1	-0.1177	0.0444	-0.2047	-0.0308	7.04	0.0080
Cattle		1	0.3145	0.0443	0.2278	0.4013	50.49	<.0001
Dispersion		1	3.0761	0.3392	2.4113	3.7408		

*Sp=sprayed, NSP=non-sprayed, CDC=CDC light trap, PSC=pyrethroid spray sheet collection, Pit=pit shelter collection, BIRS=before indoor residual spraying, OML=one month later, TML=two months later, THML=three months later

Anopheles coustani: Except the PSC sampling method and number of persons, all explanatory variables played significant (P<0.01) role on the number of *Anopheles coustani* collected (Table 4).

Table 4. Results of analyses of maximum likelihood parameter estimates for *An. coustani* abundance against explanatory variables.

Parameter	Levels	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-square	Pr > Chi-square
Intercept		1	-5.9966	0.9394	-7.8379	-4.1553	40.75	<.0001
Villages	SP	1	-2.1093	0.4031	-2.8994	-1.3193	27.38	<.0001
	NSP	0	0.0000	0.0000	0.0000	0.0000	.	.
Months	B IRS	1	2.2912	0.4889	1.3330	3.2495	21.96	<.0001
	OML	1	1.1321	0.5401	0.0736	2.1906	4.39	0.0361
	TML	1	-2.6452	0.9169	-4.4423	-0.8481	8.32	0.0039
	THML	0	0.0000	0.0000	0.0000	0.0000	.	.
Methods	CDC	1	5.8708	0.7924	4.3177	7.4239	54.89	<.0001
	PSC	1	0.9462	0.8936	-0.8051	2.6975	1.12	0.2896
	Pit	0	0.0000	0.0000	0.0000	0.0000	.	.
Persons		1	0.0223	0.0593	-0.0940	0.1385	0.14	0.7073
Cattle		1	0.1627	0.0782	0.0094	0.3159	4.33	0.0375
Dispersion		1	4.5936	0.8171	2.9920	6.1951		

*SP stands for sprayed villages, NSP for non-sprayed villages, CDC for CDC light trap, PSC for pyrethroid spray sheet collection, Pit for pit shelter collection, BIRS before indoor residual spraying, OML for one month later, TML for two months later, THML three months late

DISCUSSION

The main aim of indoor residual spraying is to kill and reduce the density of indoor feeding and resting disease vectors (Pates and Curtis, 2005). In the present study, *An. arabiesnsis* is the only sibling species of the *An. gambiae* complex found in this study, which is similar with other studies reported previously (Fettene *et al*., 2013; Meshesha Balkew *et al*., 2010; Delenesaw Yewhalaw *et al*., 2010). The abundance of *An. arabiensis* dramatically declined following IRS application. However, the proportion of indoor and outdoor resting density was not changed throughout the study period indicating that the insecticide did not influence the vector to alter its resting habit. This would be potentially attributed to the less irritant effect of bendiocarb (Padonou *et al*., 2012). Thus, the insecticide was effective to bring an impact on the abundance of *An. arabiensis*. Ansari and Rasdan (2004) reported similar observations on *An. culcifacies* and other mosquitoes. On the other hand, Ossè *et al*. (2012) reported that bendiocarb indoor residual spraying decreased the number of *An. gambiae* s.l resting indoors, while the rate of exophily was higher after intervention. The differences observed between the present and previous studies in exhophily rates might be associated either with the differences in sampling methods used for mosquito collections or the background variations of vector populations or both. In the present study, indoor and outdoor densities of the vector were estimated using PSC and Pit shelter collection methods, respectively, while PSCs and exit window traps were used to estimate endophily rates in the previous study.

The effect of IRS application on the abundance and resting habits of *An. pharoensis* and *An. coustani* was different from *An. arabiensis*, i.e., the impact on *An. pharoensis* was not as strong as it was on *An. arabiensis*, while the abundance of *An. coustani* appeared not to be influenced by IRS application. The difference could be due to the difference in indoor resting habits of these vectors, *An. arabiensis* is more endophagic and endophilic than *An. pharoensis* and *An. coustani*, so that the level of exposure to the insecticide sprayed could vary accordingly. The difference in indoor resting habits of each species could be demonstrated by the number of each species captured indoors by PSC in the present study, i.e., among 2,690 of them collected indoors by PSC, 87% (n=2,341) and 12.7 (n=342) were *An. arabiensis*, *An. pharoensis*, respectively. The number of *An. coustani* captured indoors by PSC were less than 1% (n=7).

A substantial reduction in the composition of Anopheles mosquitoes was seen at IRS villages one month after the intervention, while the proportion was unchanged at non-IRS villages signifying that IRS-induced significant change in the abundance and proportion of vectors. This is consistent with the results of earlier studies documented from East Africa where large scale IRS operations replaced *An. funestus* Gillies by *An. rivulorum* Leeson (Gillies and Smith, 1960). Similar reports from Kenya and Tanzania also reported a significant shift in vector dominance from *An. gambiae* s.s to *An. arabiensis* (Mutuku *et al*., 2011; Russell *et al*., 2011; Zhou *et al*., 2013; Bayoh *et al*., 2010) following the massive scale up of LLIN distribution. The shifts in vector species composition from highly endophilic and endophagic to less endophilic and endophagic species due to successful indoor vector control interventions inevitably alter disease transmission patterns from indoor to outdoor transmission via secondary sources of transmission. Therefore, the need to develop efficient outdoor vector control measures appears to be important (Ferguson *et al*., 2010) against secondary sources of malaria transmission.

CONCLUSION AND RECCOMENDATIONS

The present findings demonstrated that the strategy of indoor residual spraying (IRS) using bendiocarb 80% was effective in the control of *An. arabiensis* without affecting its resting behavior, while the impact was not noteworthy on *An. pharoensis* and *An. coustani*.

Changes in species dominance after the application of the IRS would maintain disease transmission by these secondary sources of transmission, so that the need to study the ecology and behaviour of *An. pharoensis* and *An. coustani* is recommended to develop effective intervention tools other than IRS, which would be suitable for these malaria vector species.

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