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ENTOMOLOGICAL MONITORING OF THE PMI AIRS PROGRAM IN NORTHERN GHANA

2015 ANNUAL REPORT

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1. ACRONYMS

AIRS	Africa Indoor Residual Spraying
b/p/n	bites/person/night
BYD	Bunkpurugu-Yunyoo District
CS	Circumsporozoite antigens
DA	District Assembly
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked immunosorbent assay
EMD	East Mamprusi District
HLC	Human Landing Catch
IRS	Indoor Residual Spraying
IRD	Indoor Resting Density
kdr	Knockdown Resistance
KD	Kumbungu District
LLIN	Long-lasting Insecticide-treated bed nets
MBR	Man Biting Rate
m/r	Mosquitoes/room
NMIMR	Noguchi Memorial Institute for Medical Research
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction Restriction Fragment Length Polymorphism

PM	**Pirimiphos Methyl**
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SND	Savelugu-Nanton District
TD	Tolon District
TKD	Tolon-Kumbungu District
TML	Tamale Metropolis
USAID	United States Agency for International Development
WHO	World Health Organization
WMD	West Mamprusi District

EXECUTIVE SUMMARY

BACKGROUND AND METHODS

The President's Malaria Initiative (PMI) Africa Indoor Residual Spraying (AIRS) Project conducted monthly entomological surveys across five sentinel districts in Ghana between January and December 2015. The sentinel districts included two indoor residual spraying (IRS) districts (Bunkpurugu-Yunyoo District (BYD) and Kumbungu District (KD), two IRS withdrawn districts (Tolon District (TD) and Savelugu Nanton District (SND), and Tamale metropolis which has never been sprayed. The PMI AIRS Project reintroduced IRS in KD during the 2015 IRS campaign because malaria transmission had increased after IRS withdrawal in 2013. Using the human landing and pyrethrum spray collection methods, the project collected mosquitoes from the sentinel sites to assess the effect of IRS on entomological indices of malaria transmission across all sites. The project also conducted World Health Organization (WHO) wall bioassay tests to determine the decay rate of sprayed insecticide and tube tests for susceptibility.

RESULTS & DISCUSSION

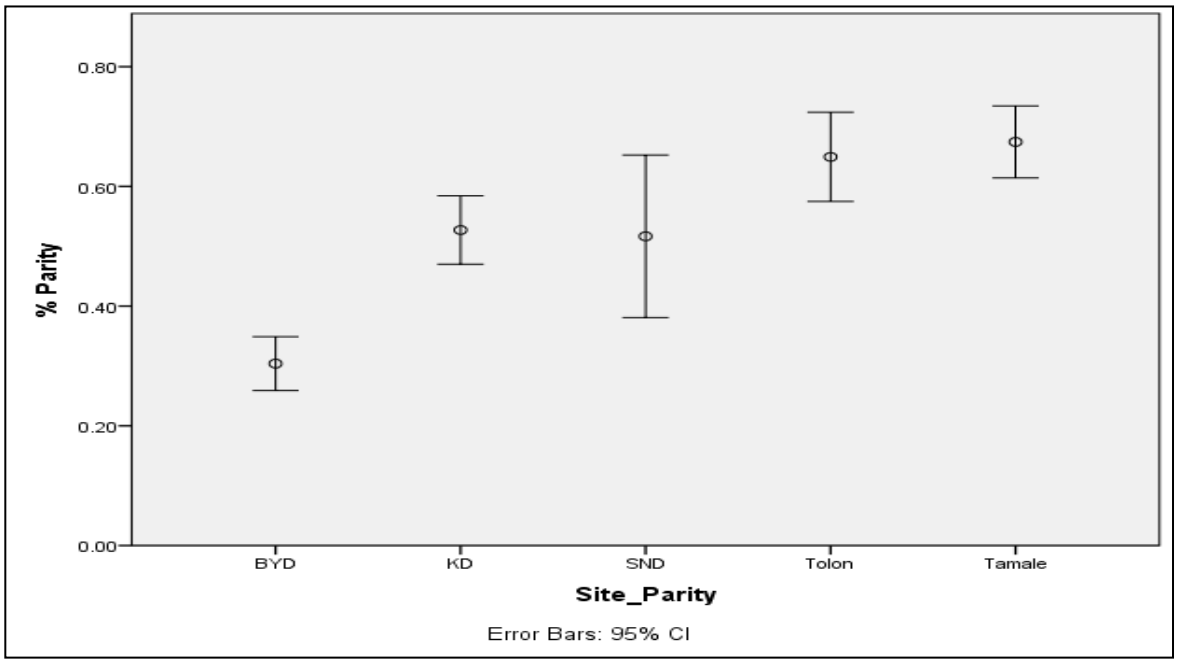
Vector Species Composition & Seasonality: *An. gambiae* s. l. was the most abundant species in all the study sites, comprising 96.6% (33,494) of the total 34,657 *Anopheles* collected. The project identified all the *An. gambiae* s.l. (198) analyzed by Polymerase Chain Reaction (PCR) as *An. gambiae* s.s. The project did not detect any *An. arabiensis* in the samples analyzed. *Anopheles coluzzii* constituted 62.1% (123), whereas *An. gambiae* Giles made up only 37.8% (75) of the samples. The project team observed a strong correlation between *An. gambiae* s.l. biting rates and indoor resting densities (IRD), and the mean rainfall, suggesting that the risk of malaria transmission was highly dependent on rainfall patterns.

Parity rates: Results from the ovary dissections showed significantly lower longevity of vector species in IRS districts (KD and BYD) compared to the unsprayed districts (TD and Tamale). The mean proportion of parous (parity rates) *An. gambiae* s.l. for the two IRS districts – BYD (26.4%) and KD (53.1%) were significantly ($p<0.05$) different from the mean parity rates for Tamale metropolis (68.3%) and TD-KD (65%) (Figure1). The parity rate for BYD was significantly lower than parity rate for SND (51.2%), where the project withdrew IRS in 2013.

Residual life of sprayed Insecticide: Monthly wall bioassays conducted after spraying to assess the residual efficacy of the sprayed insecticide showed that the sprayed insecticides remained efficacious in killing local vectors up to seven months. The project monitored the decay rate until percentage mortalities in tests remained below the 80% threshold.

Insecticide Susceptibility/Resistance: *An. gambiae* s.l. in the tested site were resistant (35% - 89% mortality) to the pyrethroids (alpha-cypermethrin 0.05% and deltamethrin 0.05%). Local vectors were resistant (87%) to bendiocarb 0.1% in Gbullung (under KD) but susceptible in Kumbungu Town to both bendiocarb and propoxur 0.1%. Vectors were fully susceptible (98-100%) to pirimiphos-methyl in all IRS communities. The project also documented high pyrethroid resistance intensity to 1x, 2x and 5x concentrations of alpha-cypermethrin in SND, while only 1x and 2x resistance was detected in KD. Results from synergist assays suggest a role of mono-oxygenases in the resistance of *An. gambiae* s.l. from Tarikpaa to the pyrethroids tested (alphacypermethrin and deltamethrin). However, resistance to the insecticides was only partially abolished, and hence it is not the only mechanism involved for insecticide resistance in the area. The project is conducting further biochemical assays for confirmation of these observations.

FIGURE 1: MEAN PARITY FOR AN. GAMBIAE S.L. COLLECTED FROM IRS INTERVENTION DISTRICTS AND UNSPRAYED DISTRICTS



Sporozoite Rates and Entomological Inoculation Rate: BYD had the lowest sporozoite positive infections (0.46%) as compared to KD (1.25%), SND (1.11%), TD (1.0%), and Tamale (1.10%). The seemingly high infection rate in KD (comparable to Tamale) could be due to the previous IRS two-year withdrawal, which might have resulted in the development of a gametocyte pool from which the few parous/older *An. gambiae s.*1. were being infected. The project recorded relatively high outdoor sporozoite rates compared to indoor in all the IRS and Non-IRS districts with the exception of Tamale (unsprayed district). Transmission was highly seasonal with the greatest proportion of sporozoite positive infections occurring between the rainy months of June and October. The high sporozoite rate in KD resulted in high EIRs of infective bites/man/year (ib/m/y) in that district. Malaria transmission intensity was lowest in BYD.

The team estimated the EIR, which measures the risk of exposure to malaria, to be 0.01 infective bites/person/night (ib/p/n) for BYD, 0.12 ib/p/n for KD, 0.04 ib/p/n for SND, 0.06 ib/p/n for TD, and 0.312 ib/p/n for TML. This translates to 0.83 infective bites/man/year (ib/m/yr), 26.21ib/m/yr, 14.65 ib/m/yr, 12.99 ib/m/yr and 8.51ib/m/yr, respectively (Table 10). Monthly trends of transmission showed that transmission was highly seasonal in both IRS and non-IRS districts

CONCLUSION

IRS has significantly maintained transmission at low levels in BYD. The re-introduction of IRS in KD seems to have contributed to the significant reduction in parity rates in the district as compared to Tamale and TD. Lower EIR was maintained in the IRS district BYD. This effect could be attributed to the impact of pirimiphos-methyl in killing high proportions of the older female *An. gambiae* s.s. and *An. coluzzii* mosquitoes that rest in the rooms. This also confirms that the local vector species in the IRS areas are still highly susceptible (98-100%) to pirimiphos-methyl used for the 2015 IRS operations. However, there was an increase in malaria transmission intensity in SND, probably as a result of the withdrawal of IRS in 2015.

2. INTRODUCTION

Abt Associates' currently implements the PMI AIRS Project in collaboration with the Ghana National Malaria Control Program. In 2015, AIRS Ghana implemented IRS in five districts: East Mamprusi (EMD), BYD, West Mamprusi (WMD), Mamprugu Moaduri, and KD, but withdrew from Savelugu Nanton District. To assess the impact of the IRS intervention on vector transmission indices, the project conducted entomological surveys through the year (2015) to:

1. Assess the quality of the IRS operation and evaluate the residual efficacy of the sprayed Actellic 300 CS formulation (Pirimiphos-methyl CS, an organophosphate insecticide)
2. Identify the species of malaria vectors in the targeted districts
3. Assess the vector density, behavior, and seasonality
4. Determine the susceptibility of local vector species to the WHO-recommended insecticides for IRS and identify mechanisms of resistance if resistance is detected
5. Assess malaria transmission indices in the sentinel sites.

The AIRS entomology team worked closely with the Ghana Health Service and District Assemblies to implement all planned field activities. AIRS Ghana also partnered with the Noguchi Memorial Institute for Medical Research (NMIMR) for support in advanced molecular evaluations. This report focuses on all entomological monitoring activities the project carried out between January and December 2015.

3. ACTIVITIES

3.1 MOSQUITO COLLECTIONS

3.1.1 STUDY AREA

In 2015, the project used five districts as entomological monitoring districts. For the study, the project team considered TD and KD as two separate districts since Tolon-Kumbungu District (TKD) split into two administrative districts in 2012. In 2015, KD represents sprayed districts and TD represents unsprayed but with a history of IRS. We used 14 corresponding communities under the five districts as sentinel sites (including three control sites) for the entomological monitoring activities (Figure 1).

Below, we present the districts and their corresponding communities selected for the entomological surveillance.

TABLE 1: ENTOMOLOGICAL MONITORING SITES

Districts	Communities/ Sentinel Sites	Insecticide spray history					
		2008-2010	2011	2012	2013	2014	2015
BYD	Bunbuna, Yunyoo, Nasuan, and Sanbiruk	NSp	ACy	ACy	PM	PM	PM
KD	Gbullung and Gupanerigu	DM	ACy	ACy	NSp	NSp	PM
SND	Diare, Nanton, and Tarikpaa (IRS was withdrawn in 2015)	DM	ACy	PM	PM	PM	NSp
TD	Dimabi and Woribugu (IRS was withdrawn in 2013)	DM	ACy	ACy	NSp	NSp	NSp
Tamale Metropolis (TML)	Kulaa, Tugu, and Yong (comparison communities with no history of IRS)	Control	Control	Control	Control	Control	Control

NSp= Not sprayed; DM= Deltamethrin; ACy= Alphacypermethrin; PM= Pirimiphos Methyl

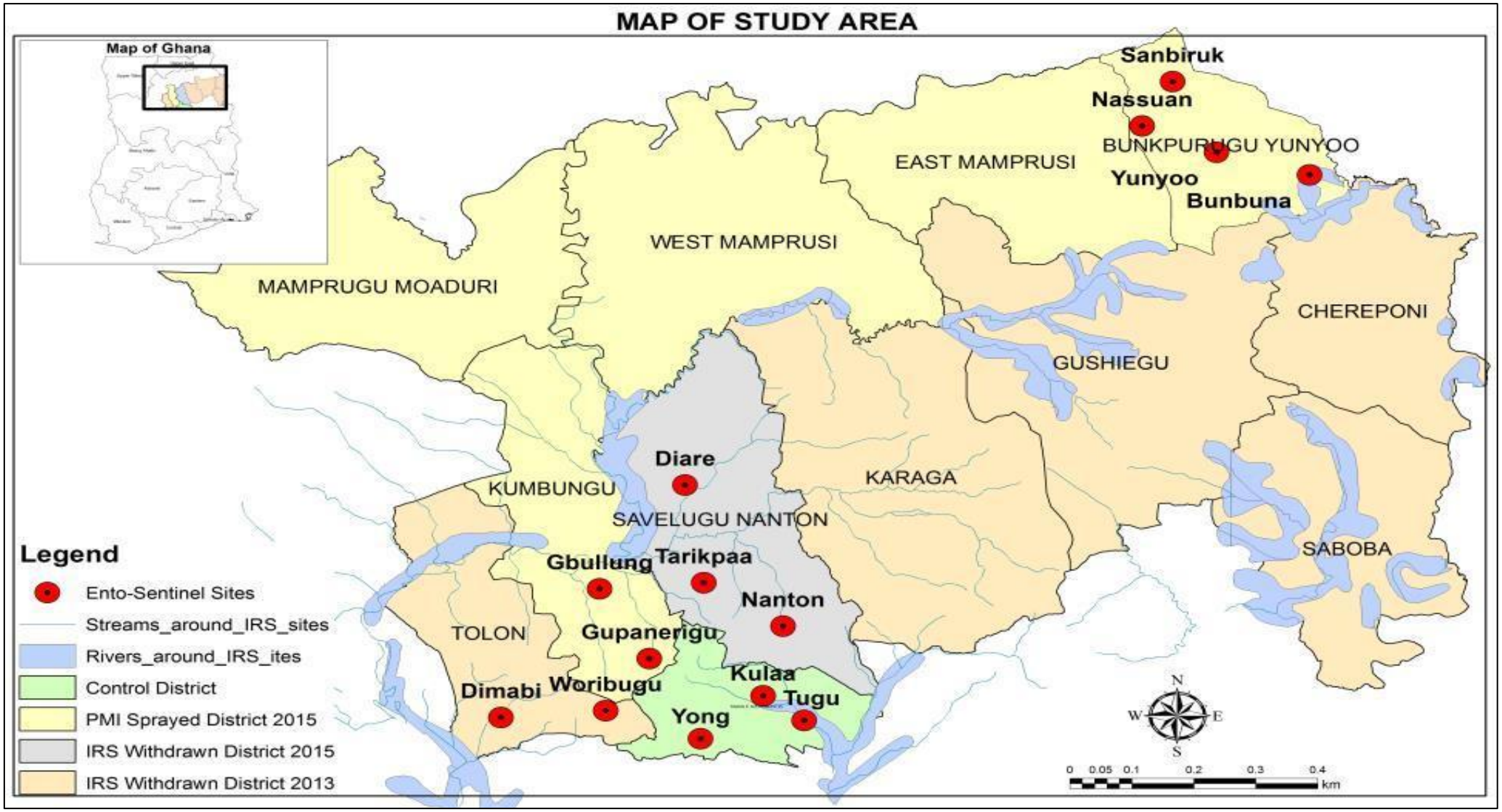
Based on insecticide susceptibility and residual efficacy test results, the project used the organophosphate pirimiphos-methyl (Actellic 300CS, at 1g/m^2) to spray all of the beneficiary districts (including KD and BYD) for the 2015 IRS campaign, which began April 14, 2015, and ended May 23, 2015.

The project carried out entomological monitoring in SND to monitor changes in malaria transmission indices that could arise because the district was not scheduled to be sprayed in 2015. The rural communities under the TML selected as sentinel sites have never received IRS, so they continued to serve as control sites for comparison.

3.2 METEOROLOGICAL DATA

The project obtained mean daily rainfall data from the Ghana Meteorological Services weather stations in Savelugu Nanton and Tamale as well as Savannah Agriculture Research Institute weather station in Nyankpala (TD).

FIGURE 2: MAP OF GHANA SHOWING THE PMI IRS DISTRICTS AND ENTOMOLOGICAL MONITORING SITES



3.2.1 SENTINEL SITES ADULT MOSQUITO SURVEYS

The project carried out mosquito collections in the five sentinel districts to assess and understand the effect of IRS on species composition, density, behavior, longevity, and entomological inoculation rates of the local vectors in the areas where spraying took place. It also used the collections to make comparisons with other unsprayed communities. The project carried out the pre- and post-spray mosquito collections using the Human Landing Catch (HLC) and Pyrethrum Spray Collection (PSC) methods (WHO, 2013) to collect mosquitoes from the sentinel sites. The team conducted collections four times each month, beginning in January 2015 and ending in December 2015.

The project team conducted HLCs using eight trained mosquito collectors in each community. The collectors worked in two teams of four, in two houses each night. In each house, two collectors worked indoors while the other two worked outdoors, taking a total of four nights to evaluate eight compounds in the community per month.

The team used the PSCs to determine indoor resting mosquito species and their densities. The team conducted collections the next morning (between 6:00 a.m. and 7:00 a.m.) in different rooms/structures than those used for the HLCs the previous night. The project team surveyed a total of eight rooms for each community every month.

We used the taxonomic keys of Gillies and Coetzee (Gillies and Coetzee, 1987) to identify mosquitoes collected from the HLCs and PSCs. The project team dissected proportions (approximately one-third) of unfed mosquitoes from the HLCs to assess their parity rates by observing the degree of coiling in the ovarian tracheoles (Detinova, 1969). The team preserved mosquito samples morphologically identified as *Anopheles gambiae* s.l. in 1.5 ml Eppendorf tubes with desiccants for further analyses at the NMIMR laboratory.

3.3 ASSESSMENT OF SPRAY QUALITY AND RESIDUAL EFFICACY

As part of the PMI AIRS Project quality check standard, the team conducted standard WHO cone bioassays (WHO, 2013) to test the quality of work by the different spray teams and to evaluate the residual life of the sprayed insecticide (Actellic 300 CS) using both the 'Kisumu' strain and wild *An. gambiae* s.l. reared from the field. The team conducted tests on three main types of sprayed surfaces: mud from traditional houses, cement from modern houses, and wood from the doors and windows.

3.3.1 QUALITY ASSURANCE OF THE IRS PROGRAM

The team conducted quality assurance tests in eight communities:

- Bogupaligu, Gbullung, and Gupanerigu in KD
- Naa Nori in EMD
- Guabuliga in WMD
- Nanponti-Bauk, Bunbuna, and Yunyoo in BYD

To remove bias arising for spray operator efficiency, the project selected houses sprayed by different spray operators from different spray teams for the test.

In each community, the team selected four houses (two with cement wall surfaces and two with mud wall surfaces) for the assessment of the quality of spray on the predominant surface types (cement and mud). To obtain information about the performance of the sprayed insecticide on wood surfaces, we conducted cone bioassays on the wooden doors or windows of each room selected for the cone bioassay.

In Bogupaligu, Gupanerigu, and Gbullung (Kumbungu), we conducted wall bioassays using both laboratory raised Kisumu strain *An. gambiae* s.s. and wild female adults of *An. gambiae* s.l. reared from larvae collected from project districts. All the mosquitoes used for the bioassays were two to five days old. In Naa Nori, Guabuliga, Nanponti-Bauk, Bunbuna, and Yunyoo we conducted wall bioassays using the Kisumu strain only. The team had difficulty collecting enough wild mosquito larvae from these areas and raising them for the wall bioassays because the areas had not received sufficient rain and breeding habitats were scarce at the time of larval collections and the bioassays. To assess the spray quality on the different wall surfaces in each room, we tested three walls of the room by fixing the cones at a height of about 1.5 m on each wall. The team carried out three cone bioassays in each sampled house together with one assay on the wooden door or window using 10 adult female mosquitoes per cone.

The project team conducted one control cone assay for every four bioassay tests by fastening cardboard on unsprayed surfaces and exposing the control mosquitoes to the cardboard, but also to conditions similar to exposed mosquitoes. To avoid the possibility of the control mortality increasing due to the airborne effect of the Actellic 300 CS formulations, we set the control tests up in unsprayed structures with fairly similar conditions (relative humidity and temperature) as the tested rooms.

The team fastened the cone exposure chamber to the selected spot on the surface to be tested with tape. We introduced 10 mosquitoes into the chamber and left them exposed on the surface for 30 minutes. At the end of the exposure period, the team collected the mosquitoes and transferred them to paper cups. We then recorded the number of mosquitoes knocked down at the end of the exposure period (30 minutes) and again at 60 minutes.

The team brought the mosquitoes to the AIRS Ghana entomology laboratory where we maintained the temperature and relative humidity at 25°C-29°C and 75% to 85%, respectively. We gave the mosquitoes a 10% sugar solution on cotton pads during the 24 hour holding period. The team counted the dead and live mosquitoes after 24 hours and calculated the mortalities. We corrected the mortalities using Abbott's formula if the control mortalities were between 5% and 20%, but the team discarded the tests and repeated if control mortalities exceeded 20%.

The team also assessed the airborne effect of Actellic 300 CS during the bioassays by placing mosquitoes in uncontaminated paper cups in the sprayed rooms and monitoring knockdown rates and 24 hour mortality.

3.3.2 Residual Efficacy of Actellic 300 CS

The project team conducted follow-up bioassays to assess the residual efficacy of pirimiphos-methyl sprayed across all sites using susceptible Kisumu colonies from the AIRS insectary and the insectary of the Navrongo Health Research Center, as well as wild *An. gambiae* collected from Bogupaligu, Gupanerigu, and Gbullung communities. AIRS Ghana conducted bioassays from May 2015 through December 2015. Both the spray quality and residual efficacy were indirectly estimated from the percentage mortality of the exposed mosquitoes from the WHO cone bioassay on the different types of sprayed surfaces (mud, wood, and cement).

The project assessed the airborne effect of Actellic 300 CS during the bioassays from T0 (one week after spraying) up to T4 (four months after spraying), by placing mosquitoes in uncontaminated paper cups in the sprayed rooms and monitoring knockdown rates and 24 hour mortality.

AIRS Ghana presents the results for the tests in the results section below.

3.4 Insecticide Susceptibility Tests

To maintain the efficacy of the IRS program and to assess the susceptibility status of the local vector species to insecticides recommended for IRS, the team conducted insecticide susceptibility tests across selected sentinel sites in the IRS beneficiary districts. The team also conducted these tests to guide the selection of insecticides to be used in the 2016 IRS campaign.

The team carried out susceptibility tests across communities in five districts in the Northern region: Bunbuna and Yunyoo (BYD); Kumbungu and Gbullung (KD); Nanton and Tarikpaa (SND); Dimabi and Woribugu (TD); Kulaa and Tugu (TML); and in Nalerigu (EMD).

3.4.1 Mosquito Collection

The team collected larvae and pupae of *Anopheles* mosquitoes from breeding sites in and around established sentinel communities and reared them to adulthood for susceptibility tests. The team made every effort to collect larvae and pupae from various breeding sites so that the mosquitoes tested will be fully representative of the vector population in the area. The team morphologically identified mosquitoes at an adult stage and selected only *Anopheles gambiae* s.l. for the susceptibility test.

3.4.2 WHO Bioassay Test Procedure

The team used sugar fed female *Anopheles gambiae* s.l. mosquitoes between two and five days old for the insecticide susceptibility tests, exposing them to WHO approved diagnostic doses of selected insecticide impregnated papers using the WHO tube method (WHO, 2013). The team tested the following insecticides.

- Pyrethroids: alpha-cypermethrin 0.05% and deltamethrin 0.05%
- Carbamates: bendiocarb 0.1% and propoxur 0.1%;
- Organophosphate: pirimiphos-methyl 0.25% and fenitrothion 1%
- Organochlorine: DDT 4%

Procedure:

- Set up four test replicates and two controls for each insecticide tested to assess the susceptibility of the local *Anopheles gambiae* s.l.
- Aspirated a total of 20 – 25 female *An. gambiae* s.l. mosquitoes in batches of at most 10 from mosquito cages into the holding tubes (lined with clean white sheets) to give six replicate (four tests and two controls) samples. The team held the mosquitoes for one hour before the test began. The team removed any damaged or weakened mosquito at the end of the pre-exposure holding time.
- Introduced mosquitoes into the exposure tubes lined with specific insecticide impregnated papers (as listed above) or oil impregnated control papers for a period of one hour (60 minutes). We scored knockdown rates of the insecticides at 10, 15, 20, 30, 40, 50 and 60 minutes during the one hour exposure period.
- Transferred mosquitoes back to holding tubes at the end of the one hour exposure period with a pad of cotton-wool soaked in 10% sugar solution placed on the mesh-screen end of the holding tubes. The team made another count after 60 minutes and at 80 minutes whenever the observed knockdown rate measured less than 80% of the mosquitoes in the holding tube.
- Maintained mosquitoes in the holding tubes for 24 hours (the recovery period).
- Maintained temperature and relative humidity during the exposure period and the recovery period for each test at 25 °C ± 2 °C and 80% ± 10% relative humidity.

- Counted and recorded the number of dead mosquitoes at the end of recovery period (i.e. 24 hours post-exposure).
- Transferred mosquitoes to individual, clearly labeled tubes (placing dead and live mosquitoes into separate tubes) for storage. Transferred mosquitoes on completion of the susceptibility test. Placed mosquitoes that survived after the 24 hour holding period in cryo-tubes, stored in liquid nitrogen, and transported them to NMIMR labs for further supplementary testing.

3.4.2.1 Interpretation of Susceptibility Test Results

- Scored mortality after the 24-hour holding period (i.e. count of the number of dead mosquitoes in both the exposure and the control tubes). When control mortality was greater than 5% but less than 20%, the team corrected the observed mortality using Abbots formula. If the control mortality was above 20%, the team discarded the tests and conducted a repeat test.
- Evaluated the susceptibility levels of *Anopheles gambiae* s.l. on the basis of the WHO criteria of test mortality (WHO, 2013); 98-100% mortality after 24 hours indicates susceptibility. A mortality of less than 98% suggests the existence of resistance and further investigation is needed. If the observed mortality (corrected if necessary) measures between 90% and 97%, the presence of resistant genes in the vector population must be confirmed, but if mortality is less than 90% then the vector population is resistant.

3.4.3 CDC Bottle Bioassays

3.4.3.1 Resistance Intensity Rapid Diagnostic Test

The team measured the intensity of pyrethroid resistance in *An. gambiae* s.l. from three sentinel sites (Tarikpaa, Gbullung and Kumbungu) using a simplified version of the Centers for Disease Control and Prevention (CDC) bottle bioassay (Brogdon and Chan, 2010).

- Four, pre-measured vials were provided by CDC, Atlanta, each containing alpha-cypermethrin at concentrations of 1x, 2x, 5x and 10x. The team diluted these vials in acetone and applied them to 250 ml bottles. Alphacypermethrin was tested because of the long history of its usage in IRS and also for ITNs distributed the previous years.
- Added four replicates of 500 µl of acetone to each insecticide vial, and washed off into a 50ml graduated falcon tube. The falcon tube was topped up to the 50ml mark. The team stored the prepared insecticide solutions in a refrigerator at 4 °C until use.
- Prepared the control bottle by adding 1ml of acetone into a 250ml Wheaton bottle and coated as described by Brogdon and Chan (2010). The team then coated four different test bottles with one milliliter of different concentrations of the prepared insecticides solution to get one bottle each of 1x, 2x, 5x and 10x insecticide concentration.
- Introduced between 20 and 25 mosquitoes into the four replicates with different concentrations.
- Ran a control bottle (coated with acetone only) alongside the tests and then recorded the knockdown rate at 15 minute intervals until all mosquitoes died in each bottle.

3.4.3.2 Synergist Assays

The team exposed *An. gambiae* s.l. populations from Tarikpaa which showed resistance to the pyrethroids alpha cypermethrin and deltamethrin to the effect of 1x piperonyl butoxide (PBO), a synergist found to inhibit oxidase activity.

- Prepared two bottles to run the synergist assays. One bottle coated with 1 ml of acetone served as a synergist-control bottle (without synergist) and the second bottle coated with 1ml of the PBO stock solution served as the synergist-exposure bottle.

- Introduced about 125 mosquitoes into the synergist control bottle, and then introduced another batch of 125 mosquitoes from the same population into the synergist coated bottle. The team held both setups for one hour. After the one hour holding period, the team transferred the mosquitoes into two holding cartons, one for the synergist-control mosquitoes and another for the synergist-exposed mosquitoes.
- Ran two CDC bottle bioassays, using one set of insecticide-coated bottles (one control and four test bottles) for the synergist-control mosquitoes, and another set (one control and four test bottles) for the synergist-exposed mosquitoes. The team monitored the number of dead or alive mosquitoes at 15 minute intervals as per the CDC bottle bioassay protocol. The team then compared data for the two populations of test mosquitoes (mosquitoes exposed to synergist before test and mosquitoes not exposed).

3.5 Advanced Molecular Evaluations

The NMIMR and AIRS Ghana held discussions and agreed on a scope of work for entomological surveillance by NMIMR to support the 2015 IRS activities, which included PCR identifications, sporozoite detection using ELISA, and detection of resistance mechanisms in the malaria vectors. The expected outcomes of these molecular evaluations are indicated below.

1. **Transmission indices**: to determine sporozoite rates and entomological inoculation rates (EIR).
2. **Identification to species** (molecular identification): to determine the members of the *An. gambiae* complex and molecular forms.
3. **Detection of mechanisms of insecticide resistance**: to use molecular techniques to determine the frequency of the knockdown resistance *(kdr)* and Ace-1 gene, as well as other mechanisms of resistance.

3.5.1 Methodology

3.5.1.1 Vector Collection Methods

The team collected adult mosquitoes of the *An. gambiae* complex and *An. funestus* using HLC and PSC methods. The project team used HLCs to determine the biting rates as well as transmission indices including EIR.

3.5.2 Vector Species Identification

The project team morphologically identified the mosquitoes using the taxonomic keys of Giles and Coetzee (1987). The team then identified samples of the *An. gambiae* s.l. into sibling species using ribosomal DNA-polymerase chain reaction (PCR) (Scott *et. al*., 1993) and into molecular forms following a PCR- restriction fragment length polymorphism (RFLP) procedure described by Fanello *et. al.* (2002).

3.5.2.1 Circumsporozoite Evaluation

The NMIMR team sorted the head and thorax of all samples and tested for the presence of circumsporozoite antigens (CS) of *Plasmodium falciparum* using enzyme-linked immunosorbent assay (ELISA) described by Wirtz *et al*. (1985). The NMIMR team used the ELISA tests to assess the parasite infection rate in the local mosquito vectors.

3.5.2.2 Kdr and Ace-1 Genotype Test

The NMIMR team used the conventional PCR technique described by Martinez Torres et al, 1998 and real time PCR described by Chris Bass et al, 2007 to detect the presence of West Africa kdr gene and the Ace-1 mutation in the local *An. gambiae* s.l. vectors using the protocol described by Weill et al, 2003.

3.5.3 ANALYSIS OF DATA

The project estimated the following parameters for the important *Anopheles* vector species (*An. gambiae* s.l. and *An. funestus* group):

- Man biting rate (MBR) = the total number of vectors collected/number of collectors X number of nights of capture
- Sporozoite rates = the proportion of *Anopheles* found positive for the presence of circumsporozoite proteins
- EIR calculated by the formula:
    - EIR=daily human biting rates X sporozoite rates
    - Annual EIR = Sum of monthly EIRs

4. RESULTS

4.1 SPECIES COMPOSITION AND VECTOR SEASONALITY

4.1.1 SPECIES COMPOSITION

4.1.1.1 MORPHOLOGICAL IDENTIFICATION

Through HLC and PSC collection, the AIRS Ghana team morphologically identified the *Anopheles* species *An. gambiae* s.l., *An. funestus, An. nili, An. pharoensis* and *An. rufipes* during the project period. The team found *An. gambiae* s.l. to be the predominant species across all sites, constituting 96.64% of the total number (34,657) of *Anopheles* collected (Table 1). The other species, *An. funestus, An. nili, An. pharoensis* and *An. rufipes,* constituted 0.71%, 1.62%, 1.01% and 0.02% of the collection respectively. Most of the *An. funestus* collected from both PSCs and HLCs were collected from KD (IRS district) and TD (non-IRS district), (Table 2).

Of the total *Anopheles* mosquitoes collected during the period (January to December 2015), the team collected 33,636 by HLCs, while they collected 1,021 by PSCs (Table 1).

TABLE 2: TOTAL NUMBER OF MOSQUITOES COLLECTED BY HLC AND PSC FROM ALL SENTINEL SITES

Type of *Anopheles* mosquito	HLC		PSC		TOTAL	
	N	%	N	%	N	%
An. gambiae s.l.	32,526	96.70%	968	94.81%	33,494	96.64%
An. funestus	196	0.58%	49	4.80%	245	0.71%
An. nili	563	1.67%	0	0.00%	563	1.62%
An. pharoensis	347	1.03%	2	0.20%	349	1.01%
An. rufipes	4	0.01%	2	0.20%	6	0.02%
Total	33,636		1,021			34,657

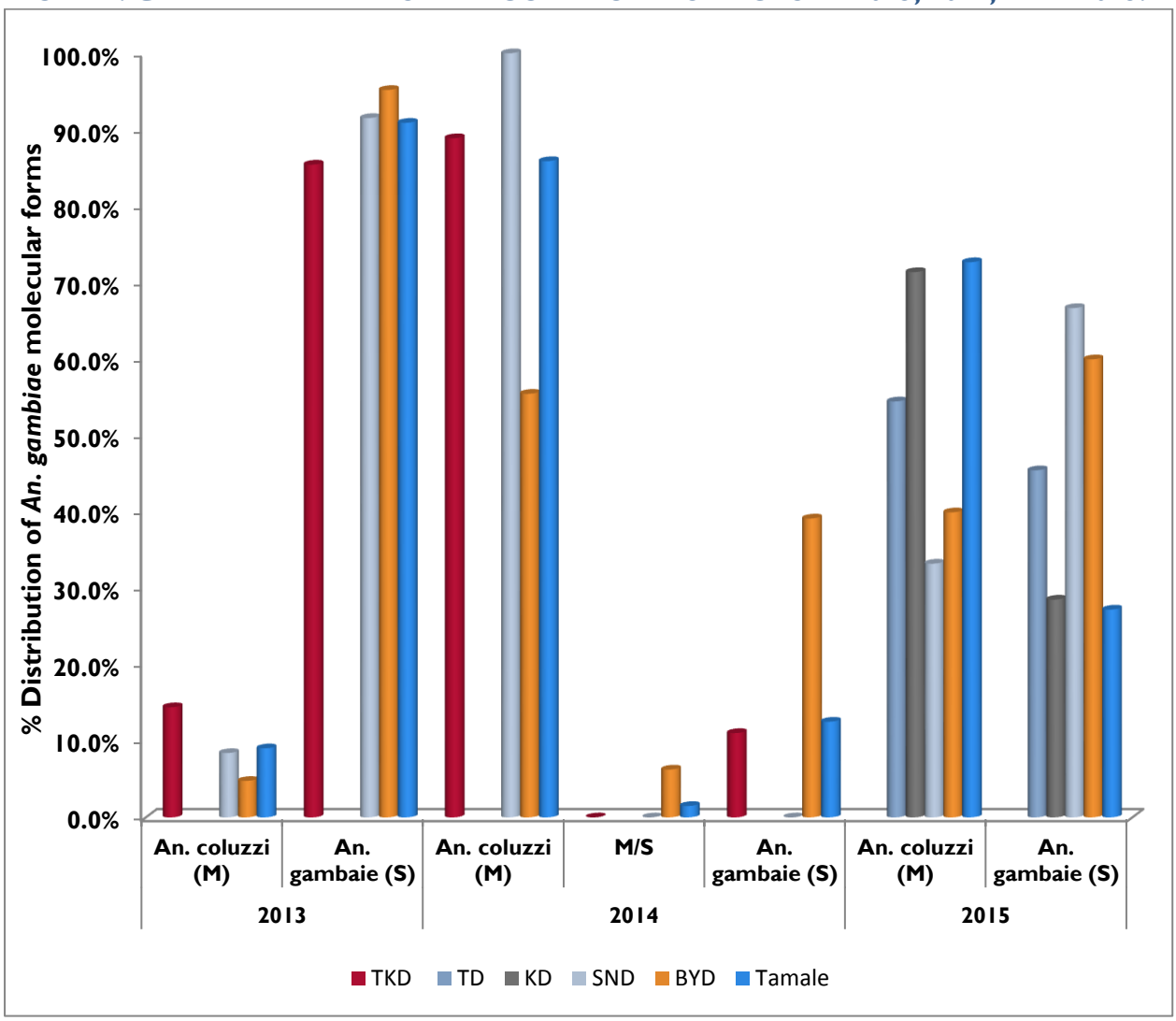
TABLE 3: TOTAL NUMBER OF *ANOPHELES* SPECIES COLLECTED BY HLC AND PSC FROM BYD, KD, SND, TD AND TML

Anopheles species	BYD	KD	SND	TD	Tamale	TOTAL
An. gambiae s.l.	3,460	7,227	4,212	4,664	13,931	33,494
An. funestus	29	115	6	71	24	245
An. nili	91	91	19	12	350	563
An. pharoensis	155	41	46	6	101	349
An. rufipes	4	0	0	1	1	6
Total (%)	3,739	7,474	4,283	4,754	14,407	34,657
	(10.8%)	(21.6%)	(12.4%)	(13.7%)	(41.6%)	

4.1.1.2 PCR ANALYSIS

In all, 198 (35 from BYD, 35 from KD, 18 from SND, 11 from TD, and 99 from TML) specimens were analyzed. NMIMR analyzed *An. gambiae* s.l. by PCR into sibling species (Scott et al, 1993) and M (*An. coluzzii*) and S molecular forms (Coetzee, *et al*., 2013). The team identified all 198 as *An. gambiae* s.s. *An. coluzzii* (M) constituted 62.1% (123 out of 198) samples analyzed, with *Anopheles gambiae* Giles (S-form) making up only 37.8% (75) of the samples. Unlike in 2014, the team did not find any hybrid forms in the samples analyzed (Figure 2).

FIGURE 3[1]: DISTRIBUTION OF THE *AN. COLUZZII* (M) AND S MOLECULAR FORMS OF *AN. GAMBIAE* IN THE IRS AND CONTROL DISTRICTS IN 2013, 2014, AND 2015.



[1] Tolon and Kumbungu were combined as TKD IN 2013 and 2014

4.1.2 VECTOR SEASONALITY

4.1.2.1 BITING RATE

The mean biting rates for *An. gambiae* s.l. (the predominant species collected from all sites) are presented in Figures 3-6, and Table 3 below. Figure 3 also includes mean monthly rainfall data recorded during the period. The abundance of *An. gambiae* s.l. collected from BYD, KD, SND, and TML were strongly correlated to the mean rainfall (69.3mm). The coefficients of correlation were 0.765, 0.684, 0.575, 0.670 and 0.752 for BYD, KD, TD, SND and TML, respectively (Table 3). Figures 4 and 5 show the mean monthly indoor and outdoor biting rates for *An. gambiae* s.l. for all study sites.

Comparatively, the average monthly biting rates recorded for TML (control) and KD (IRS) were higher than those recorded for BYD (IRS) and SND and TD (non IRS) districts. The average MBRs recorded for *An. gambiae* s.l. during the period were 2.22 bites per man per night (b/m/n), 9.34 b/m/n, 3.45 b/p/n, 5.90 b/p/n and 12.18 b/m/n for BYD, KD, SND, TD and TML, respectively (Table 3). The biting rates recorded for BYD, SND, and TD increased marginally ($p>0.05$) when compared to 2014 biting rates. Biting rates of *An. gambiae* s.l. in KD and TML decreased by about 23% and 14% respectively. However, these reductions were non-significant ($p>0.05$).

TABLE 4: MEAN MBRS AND INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L. COLLECTED BY HLC AND PSC, ALL SENTINEL SITES

Study Site	Mean biting rate (b/p/n)	Mean Room density (mosquitoes/room)	Total Number of *An. gambiae* s.l.	Rainfall Correlation r^2
IRS				
BYD (IRS)	2.22	0.20	3,460	0.765*
KD (IRS)	9.34	1.24	7,227	0.684*
Non-IRS				
SND (IRS withdrawn)	3.45	0.84	4,212	0.670*
TD (IRS withdrawn)	5.90	0.68	4,664	0.575
TML (Non-IRS)	12.18	1.04	13,931	0.752*

[2] *Pearson correlation is significant at the 0.05 level (2-tailed), Mean rainfall = 69.3mm

FIGURE 4: MEAN MBR OF *AN. GAMBIAE* S.L. COLLECTED BY HLC FROM SENTINEL SITES IN BYD, KD, SND, TD AND TML, JANUARY - DECEMBER 2015

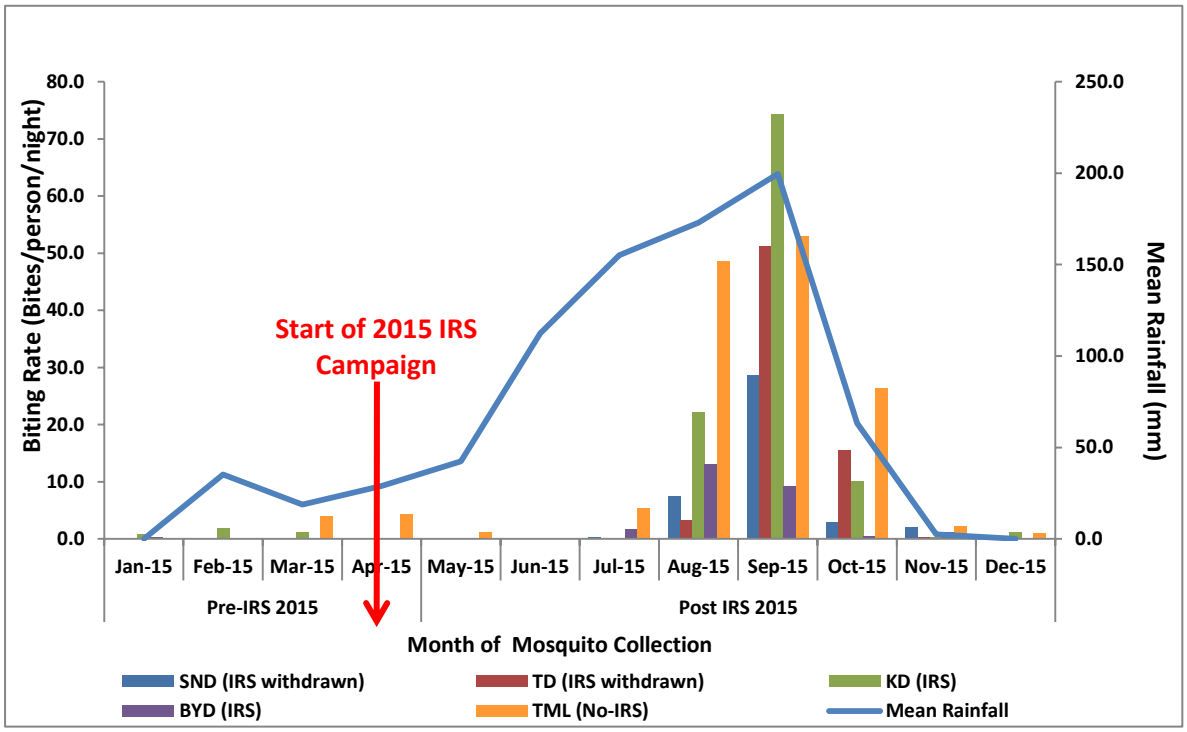


FIGURE 5: MEAN INDOOR MBR OF *AN. GAMBIAE* S.L. COLLECTED BY HLC FROM SENTINEL SITES IN BYD, KD, SND, TD AND TML, JANUARY - DECEMBER 2015

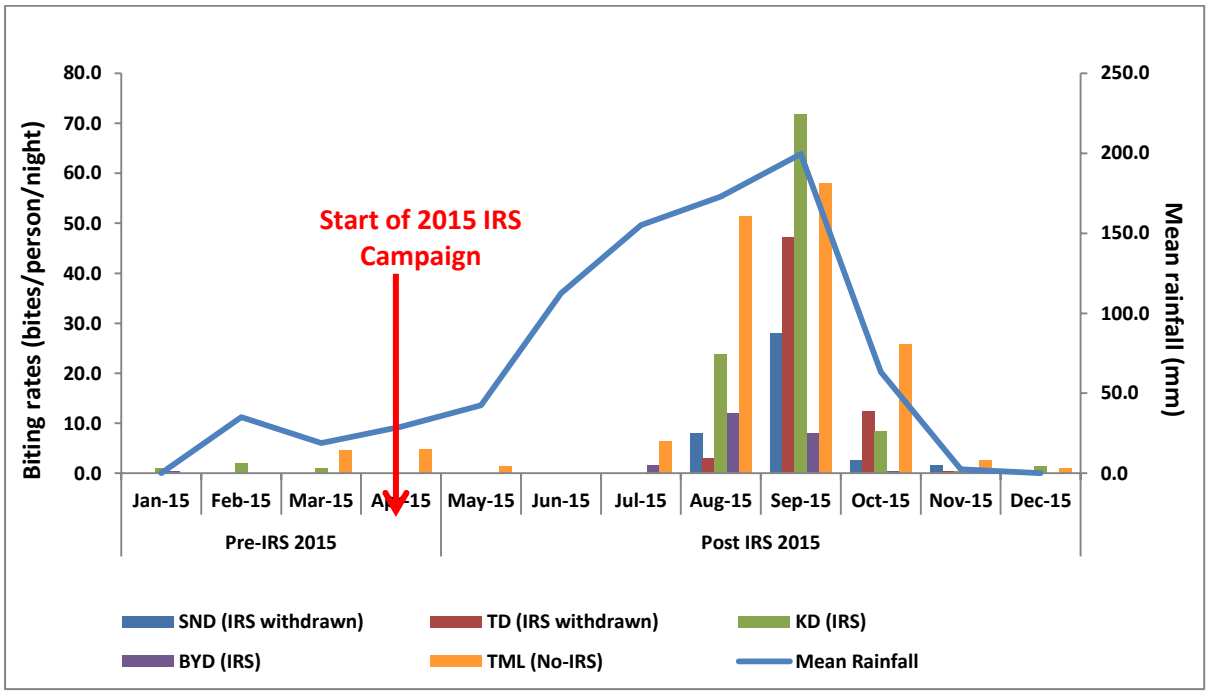


FIGURE 6: MEAN OUTDOOR MBR OF *AN. GAMBIAE* S.L. COLLECTED BY HLC FROM SENTINEL SITES IN BYD, KD, SND, TD AND TML, JANUARY - DECEMBER 2015

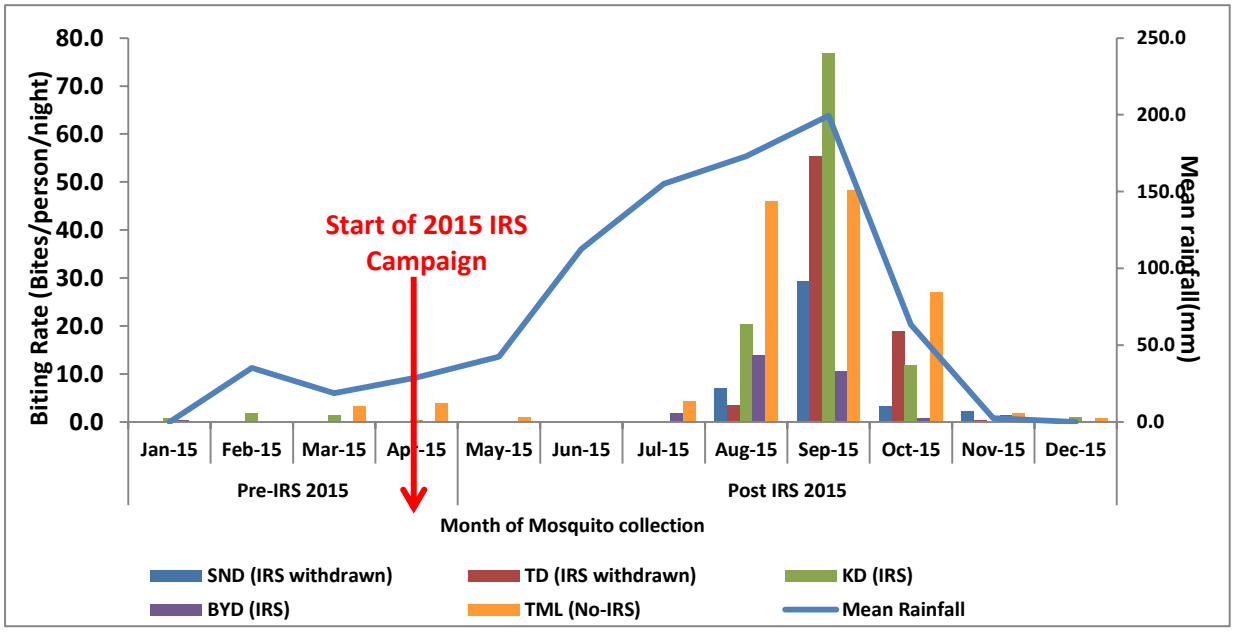
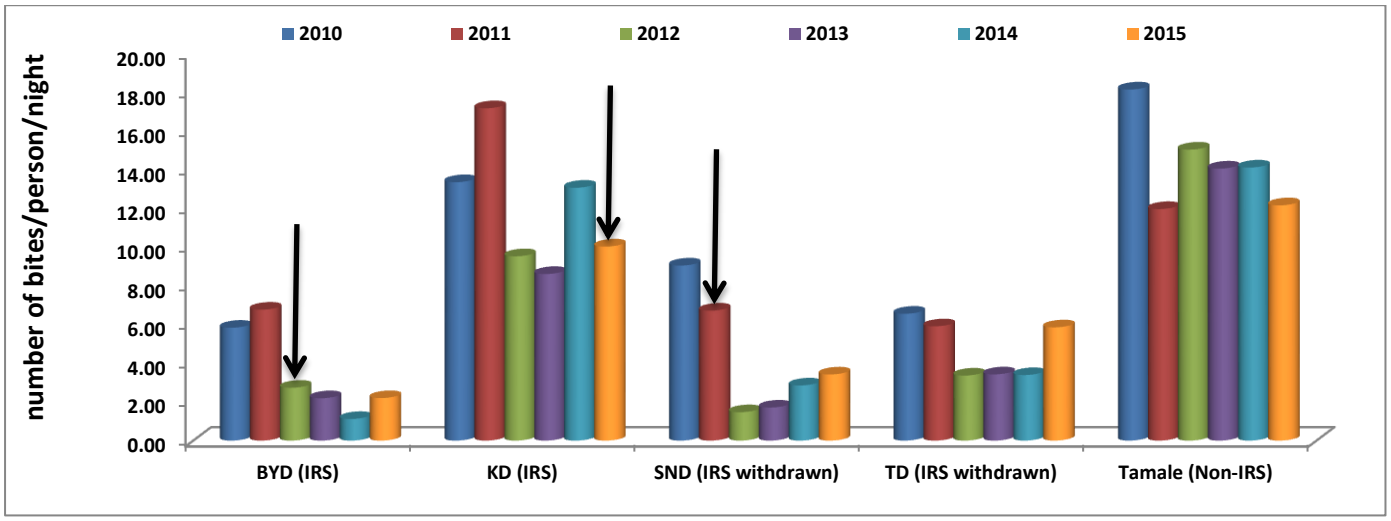


FIGURE 7[3]: YEARLY COMPARISON OF MEAN MBR OF *AN. GAMBIAE* S.L. FOR ALL SENTINEL SITES, 2010 - 2015



Note: Black arrows indicate introduction of PM

4.1.2.2 INDOOR RESTING DENSITIES OF VECTORS

The mean number of *An. gambiae* s.l. mosquitoes per room, or indoor resting density (IRD), recorded for the reporting period (January to December 2015) was 0.20, 1.14, 0.84, 0.68, and 1.04 mosquitoes/room (m/r) for BYD, KD, SND, TD and TML, respectively as demonstrated in Table 4 and Figure 7. The figure also includes data on mean monthly rainfall recorded during the period. The mean monthly IRD increased with the onset of the rains across all sites. The graph for comparison of yearly IRDs for 2010 to 2015 shows a decreasing trend, with IRDs remaining depressed since 2013 in BYD, the district that has been continuously under IRS (Figure 7). A comparison of IRDs recorded in 2015 with IRDs for 2014 reveal a non-significant ($p>0.05$) increase in IRDs for *An. gambiae* s.l. in BYD, KD, and SND. However, in TML the IRD of *An. gambiae* s.l. was significantly lower in 2015; 2.13m/r in 2014 vs 1.04 m/r in 2015 ($t_{(574)}=2.546$, $p= 0.011$). Similarly the IRDs of *An. gambiae* s.l. in TD was also lower in 2015, but the reduction was not statistically significant ($p>0.05$). The team recorded relatively low IRDs for *An. funestus* across all sites, except in TD where the IRD was slightly higher.

TABLE 5: TOTAL NUMBER OF *AN. GAMBIAE* S.L. COLLECTED BY PSC AND THEIR GONOTROPHIC STAGES

Study Site	Unfed	Blood fed	Half Gravid	Gravid	Total	Total # of mosquitoes /room[4]

[3] **Districts and Insecticides sprayed** :
2010: SND & TKD - Pyrethroid (Deltamethrin);
2011: BYD, SND & TKD - Pyrethroid (Alphacypermethrin);
2012: BYD & TKD – Pyrethroid (Alphacypermethrin), SND sprayed organophosphate (Pirimiphos-methyl).
2013: BYD& SND - organophosphate (Pirimiphos-methyl) IRS was withdrawn from TKD in 2013.
2014: BYD& SND - organophosphate (Pirimiphos-methyl) TKD was not sprayed in 2014.
2015: BYD& KD - organophosphate (Pirimiphos-methyl) TD and SND were not sprayed in 2015

ITN Distribution: Door to Door- July 2012, School based October 2014, ANC & CHC- 2015

[4] Total number of rooms surveyed in the PSC for 2015 - SND and TML: 288 rooms; BYD: 384 rooms; TD: 192 rooms and KD:176 rooms.

An. gambiae s.l.							
BYD (IRS)	14	59	4	0	**77**	8.0%	0.20
KD (IRS)	58	140	7	14	**219**	22.6%	1.14
SND (IRS withdrawn)	14	178	9	41	**242**	25.0%	0.84
TD (IRS withdrawn)	11	103	7	10	**131**	13.5%	0.68
TML (Non-IRS)	47	195	13	44	**299**	30.9%	1.04
An. funestus							
BYD (IRS)	1	3	0	0	**4**	8.2%	0.01
KD (IRS)	0	3	0	0	**3**	6.1%	0.02
SND (IRS withdrawn)	6	7	0	0	**13**	26.5%	0.05
TD (IRS withdrawn)	1	17	1	3	**22**	44.9%	0.11
TML (Non-IRS)	1	6	0	0	**7**	14.3%	0.02
Total					**1,012**		

FIGURE 8: MEAN IRD OF *AN. GAMBIAE* S.L. COLLECTED BY PSC FROM SENTINEL SITES IN BYD, KD, SND, TD AND TML, JANUARY - DECEMBER 2015

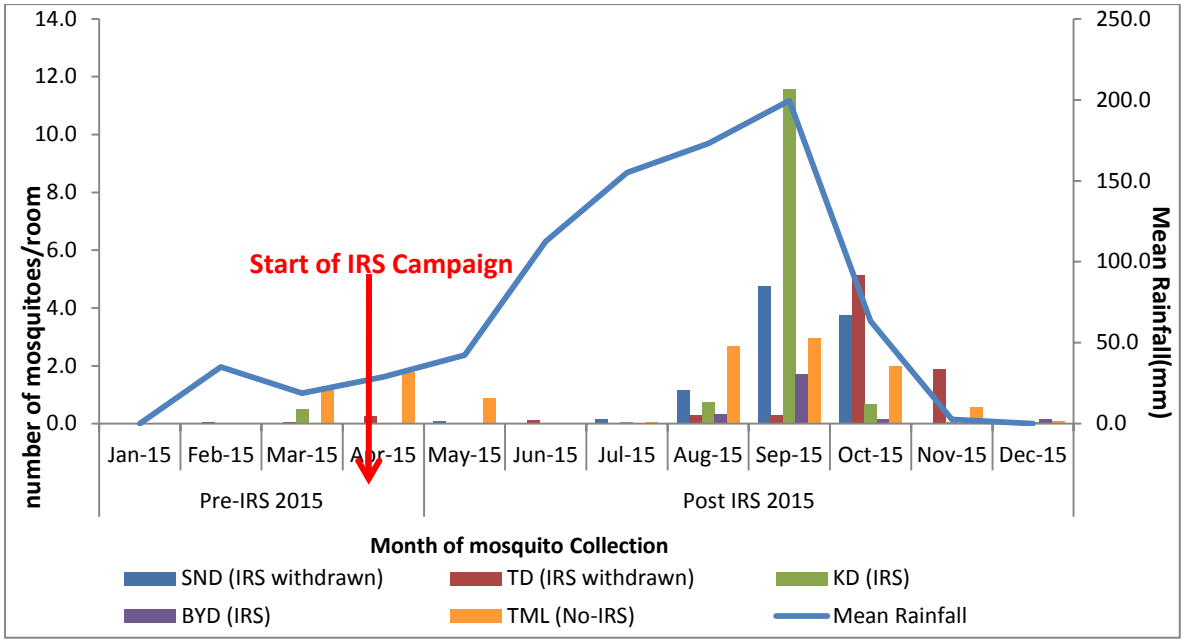
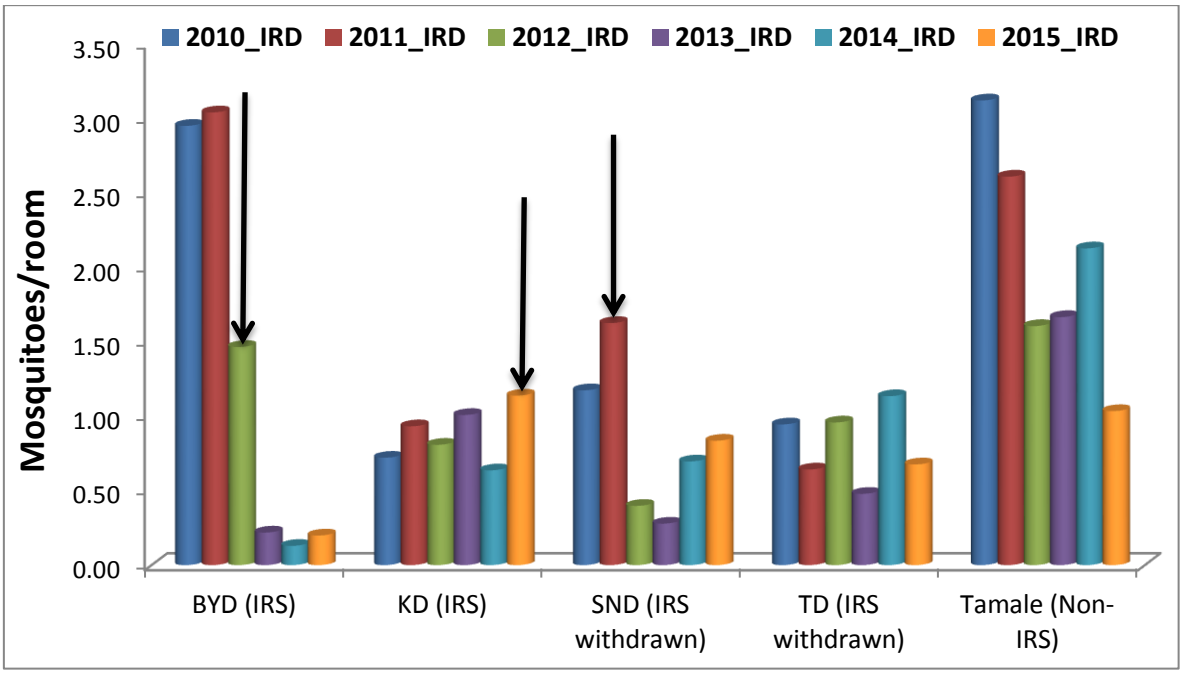


FIGURE 9[5]: YEARLY COMPARISON OF MEAN IRD OF *AN. GAMBIAE* S.L., ALL SENTINEL SITES, 2010 – 2015



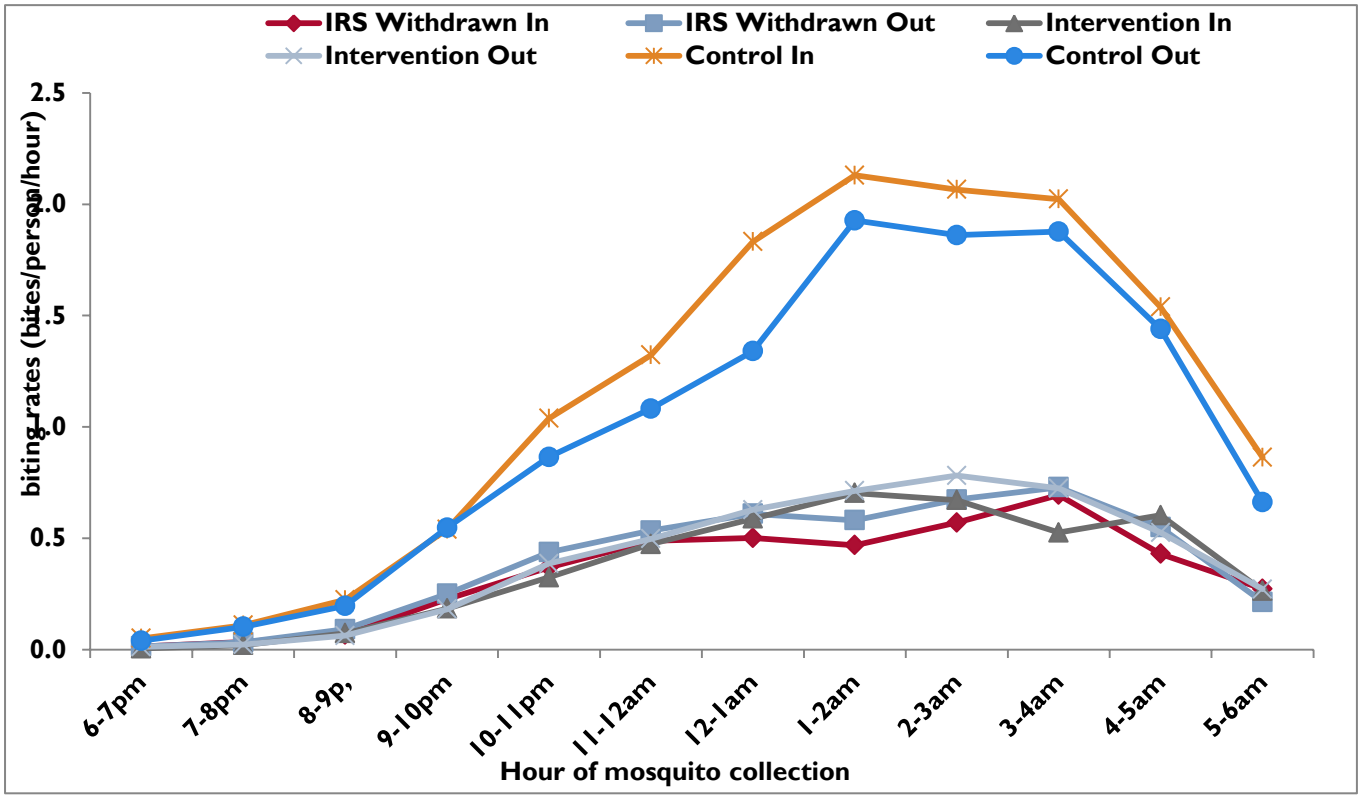
Note: Black arrows indicate introduction of PM

4.2 FEEDING TIME

Figure 9 shows the biting cycle of *An. gambiae* s.l. (the predominant vector species) collected between January and December 2015. The team observed that in IRS intervention districts (KD and BYD), IRS withdrawn districts (TD and SND), as well as in the unsprayed district, both indoor and outdoor biting activity started from 6:00 p.m. and gradually rose from 8:00 p.m. The majority of bites occurred between 11:00 p.m. and 5:00 a.m. The team found that the densities of mosquitoes biting during these peak times were higher for the unsprayed district than the IRS districts and IRS withdrawn districts.

[5] **Districts and Insecticides sprayed** :
2010: SND & TKD - Pyrethroid (Deltamethrin);
2011: BYD, SND & TKD - Pyrethroid (Alphacypermethrin);
2012: BYD & TKD – Pyrethroid (Alphacypermethrin), SND sprayed organophosphate (Pirimiphos-methyl).
2013. BYD& SND - organophosphate (Pirimiphos-methyl) IRS was withdrawn from TKD in 2013.
2014. BYD& SND - organophosphate (Pirimiphos-methyl) TKD was not sprayed in 2014.
2015. BYD& KD - organophosphate (Pirimiphos-methyl) TD and SND were not sprayed in 2015
ITN Distribution: Door to Door- July 2012, School based October 2014, ANC & CHC- 2015

FIGURE 10: HOST SEEKING BEHAVIOR OF *AN. GAMBIAE* S.L. COLLECTED INSIDE AND OUTSIDE OF SPRAYED ROOMS



4.3 FEEDING LOCATION

The team observed variations in indoor biting and outdoor biting densities of *An. gambiae* s.l. between the IRS and non-IRS sites (Table 5). The project team collected very low numbers of mosquitoes during the pre-IRS period (January to April 2015). We collected most of the *An. gambiae* s.l. (from BYD, KD and SND) outdoors, except for in TD. However, these differences in indoor/outdoor biting rates did not significantly differ ($p>0.05$) (Table 5) during the pre-IRS period.

The biting rates increased between May and October 2015 (rainy months) which also coincided with the post-IRS period. The team recorded higher outdoor biting rates for BYD, KD, SND and TD. The outdoor biting rates in BYD and TD, 3.54 b/p/n and 9.79 b/p/n respectively, were significantly higher than the indoor biting rates of 2.92b/p/n for BYD and 7.90b/p/n for TD ($p<0.05$). The differences in indoor/outdoor biting rates during the post- IRS period in KD and SND were not statistically significant ($p>0.05$). In TML, the indoor biting rates were significantly higher than outdoor biting rates during both pre- and post-IRS periods.

TABLE 6: PRE AND POST IRS MEAN INDOOR AND OUTDOOR BITING RATES OF *AN. GAMBIAE* S.L., HLC, ALL SENTINEL SITES, 2015

Sentinel Site	Indoor biting rate	Outdoor biting rate	Endophagic index	Exophagic index	χ^2	P value
Pre-IRS						
BYD (IRS)	0.14	0.15	0.49	0.51	0.01	**0.907**
KD (IRS)	0.97	1.02	0.46	0.54	0.87	**0.352**

SND (IRS withdrawn)	0.01	0.01	0.50	0.50	0.00	**1.000**
TD (IRS withdrawn)	0.02	0.01	0.75	0.25	1.00	**0.317**
TML (Non-IRS)	2.35	1.88	0.56	0.44	10.42	**0.001***
Post-IRS						
BYD (IRS)	2.92	3.54	0.45	0.55	30.55	**0.000***
KD (IRS)	13.25	13.77	0.49	0.51	2.60	**0.107**
SND (IRS withdrawn)	5.07	5.27	0.49	0.51	1.53	**0.216**
TD (IRS withdrawn)	7.90	9.79	0.45	0.55	51.51	**0.000***
TML (Non-IRS)	18.34	16.10	0.53	0.47	55.93	**0.000***

***** Differences in mean indoor/outdoor biting rates is statistically significant at 0.05 level

4.4 INSECTICIDE SUSCEPTIBILITY

4.4.1 WHO TUBE TEST RESULTS

Tables 6 and 7 and Figures 10 and 11 below present the results of the WHO tube test conducted for the following insecticides WHO recommends for use in IRS: alpha-cypermethrin 0. 5%, deltamethrin 0.05%, bendiocarb 0.1%, propoxur 0.1%, pirimiphos-methyl 0.25%, fenitrothion 1%, and DDT 4%.

The results of the WHO susceptibility tests indicated that *Anopheles gambiae* s.l. from both IRS and non-IRS districts were susceptible to pirimiphos-methyl and fenitrothion (with mortalities ranging between 98% and 100%), except in Woribugu and Kulaa where the mosquitoes showed possible resistance to pirimiphos-methyl (94%-97% and 95-97.5% respectively). The two areas, Woribugu and Kulaa, are from TD, from where IRS was withdrawn after the 2012campaign, and TML, a control site that has never been sprayed respectively. *Anopheles gambiae* s.l. was resistant to DDT, deltamethrin, and alpha-cypermethrin across all the sites.

The team observed indications of vector species resistance to bendiocarb in some of the sites in both sprayed (Gbullung) and unsprayed communities (Kulaa). However *Anopheles gambiae* s.l. from Tarikpaa and Kumbungu were susceptible to both bendiocarb and propoxur. Figure 9 shows the insecticide susceptibility/resistance maps for all the study sites.

TABLE 7: SUMMARY OF WHO INSECTICIDE RESISTANCE TEST RESULTS, *AN. GAMBIAE* S.L. TESTED AGAINST PYRETHROIDS AND ORGANOCHLORINE IN 2015

District	Community	Pyrethroids		Organochlorine
		Alpha cypermethrin 0. 5%	Deltamethrin 0.05%	DDT 4%
East Mamprusi *(IRS)*	Nalerigu	89.00(100)* R		
Kumbungu *(IRS)*	Gbullung	55.00(100) R	46.00(100) R	50.00(100)
	Kumbungu	67.00(100) R	75.00(100) R	26.00(100)
Savelugu Nanton *(IRS withdrawn after 2014)*	Nanton	50.00(100) R	77.00(100) R	56.25(80)
	Tarikpaa	55.00(100) R	35.00(100) R	20.00(100)
Tolon *(IRS withdrawn in 2012)*	Dimabi	84.00(100) R	63.75(80) R	
	Woribugu	66.00(100) R		42.00(100)
Tamale Metropolitan *(No-IRS)*	Kulaa	75.81(186) R		

*Numbers in parenthesis represent number of mosquitoes exposed.

TABLE 8: SUMMARY OF WHO INSECTICIDE RESISTANCE TEST RESULTS, *AN. GAMBIAE* S.L. TESTED AGAINST ORAGANOPHOSPHATES AND CARBAMATES IN 2015

District	Community	Organophosphates		Carbamate	
		Pirimiphos methyl	Fenithrothion	Bendiocarb	Propoxur
Bunkpurugu Yunyoo *(IRS)*	Bunbuna	100.0(100)* S			
	Yunyoo	98.00(100) S			
East Mamprusi *(IRS)*	Nalerigu	100.0(100) S			
Kumbungu *(IRS)*	Gbullung	99.00(200) S		87.00(100) R	
	Kumbungu	99.50(200) S		100.00(100) S	100.00(100)
Savelugu Nanton *(IRS withdrawn after 2014)*	Nanton	100.0(100) S		69.44(180) R	
	Tarikpaa	100.0(100) S	100.00(100)	100.00(100) S	99.00(100)
Tolon	Dimabi	98.00 (100) S		97.00(100) PR	
	Woribugu	95.50(200) PR		93.00(100) PR	
Tamale Metropolis (No-IRS)	Kulaa	96.37(193) PR		83.00(100) R	
	Tugu	99.00 (100) S			

*Numbers in parenthesis represent number of mosquitoes exposed.

FIGURE 11: SUSCEPTIBILITY STATUS OF *AN. GAMBIAE* S.L. IN AIRS GHANA ENTOMOLOGICAL SENTINEL SITES

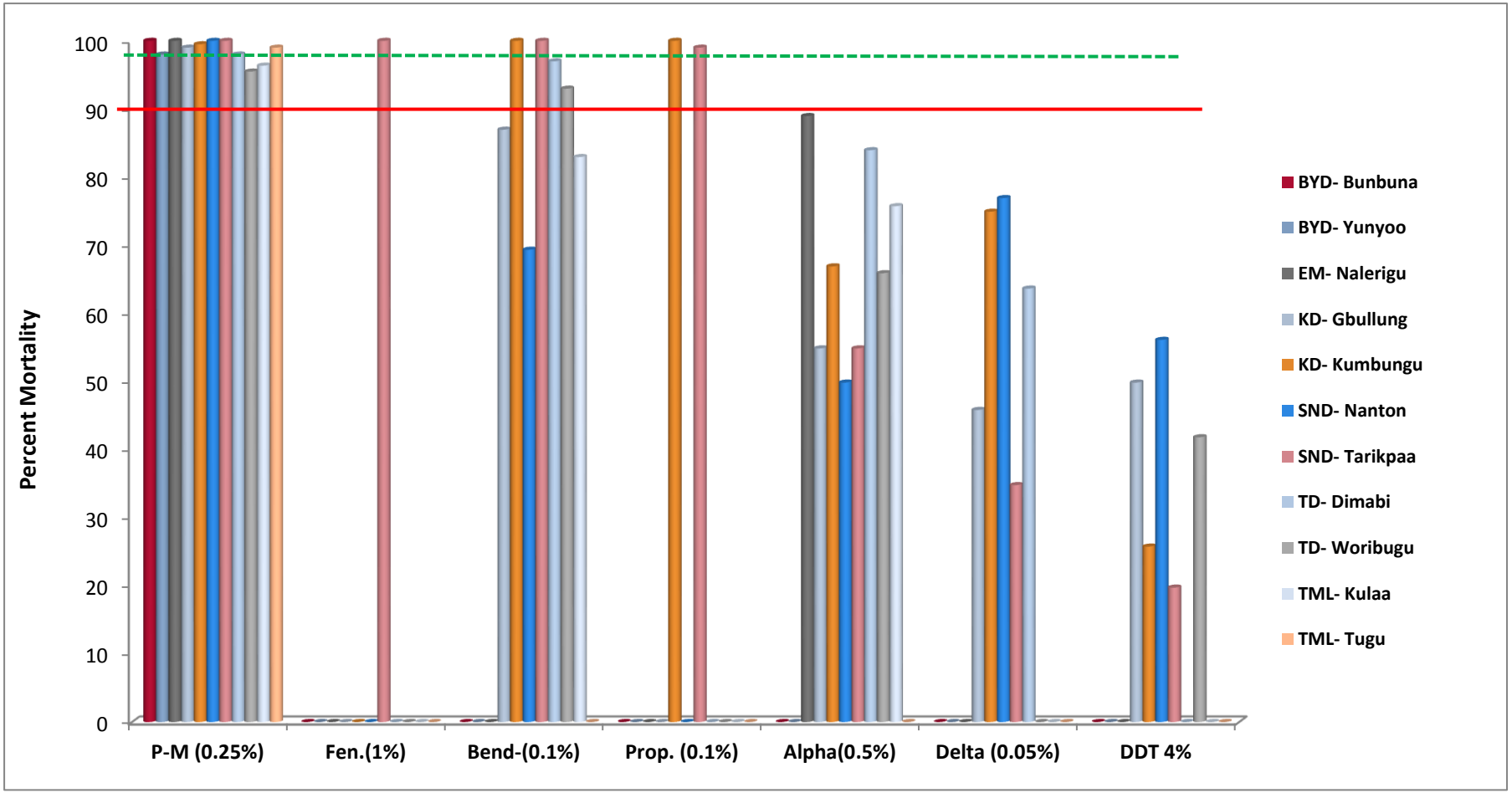
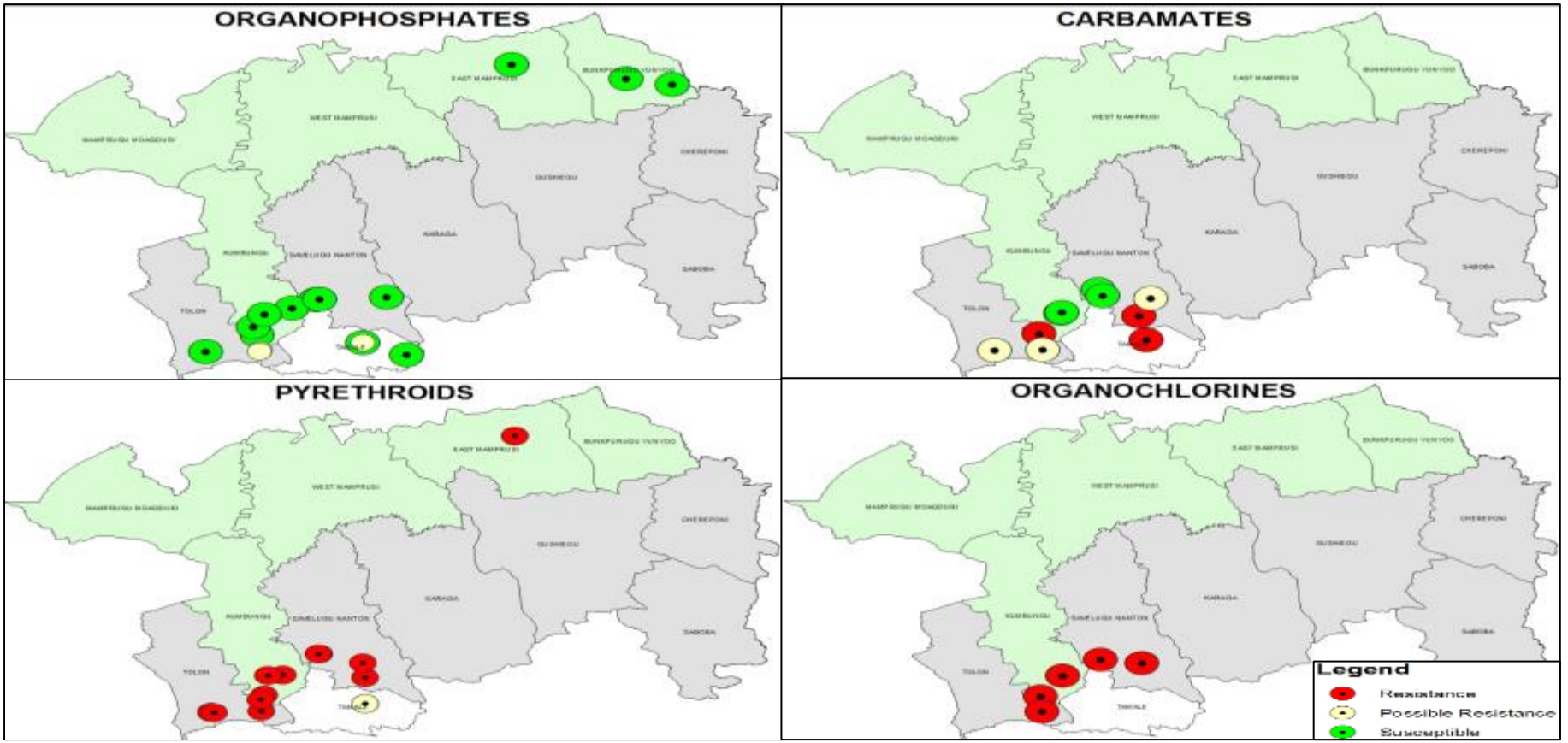


FIGURE 12: INSECTICIDE SUSCEPTIBILITY MAP FOR *AN. GAMBIAE* S.L. IN THE AIRS GHANA ENTOMOLOGICAL SENTINEL SITES



4.5 Resistance Mechanism

4.5.1 Target Site Resistance

4.5.1.1 Frequency of the Knockdown Resistance (kdr-west) and Ace-1 Gene

The NMIMR team investigated two target-site gene mutations in *An. gambiae* s.s. mosquitoes. The first, which consisted of a leucine–phenylalanine substitution at amino acid position 1014, is widespread in West Africa (hitherto called *kdr-w*) and is responsible for pyrethroid and DDT resistance. On the other hand, *Ace-1* gene is responsible for organophosphate and carbamate resistance.

The NMIMR team analyzed a total of 198 *An. gambiae* s.s samples for their *kdr-w* and *Ace-1* status. The *kdr-w* molecular analysis showed that the homozygous resistant variant alleles (RR) were present in samples from all sites but were relatively higher in BYD, followed by TD and SND (Figure 12). The NMIMR team found relatively high numbers of susceptible homozygote alleles (SS) in the samples from KD and TML in 2015. More *An. coluzzii* were harboring the *kdr-w* alleles than in the S-form (Figure 13).

Figure 14 shows the distribution of *kdr* resistance gene in the population of *An. gambiae* s.s. from 2011 to 2015 in all the sites. The NMIMR team detected higher frequencies of susceptible (SS) alleles in 2015 in most of the sites than in 2014.

FIGURE 13: FREQUENCY OF KDR-W GENE IN *AN. GAMBIAE* S.S. FROM THE IRS AND NON-IRS AREAS, 2015

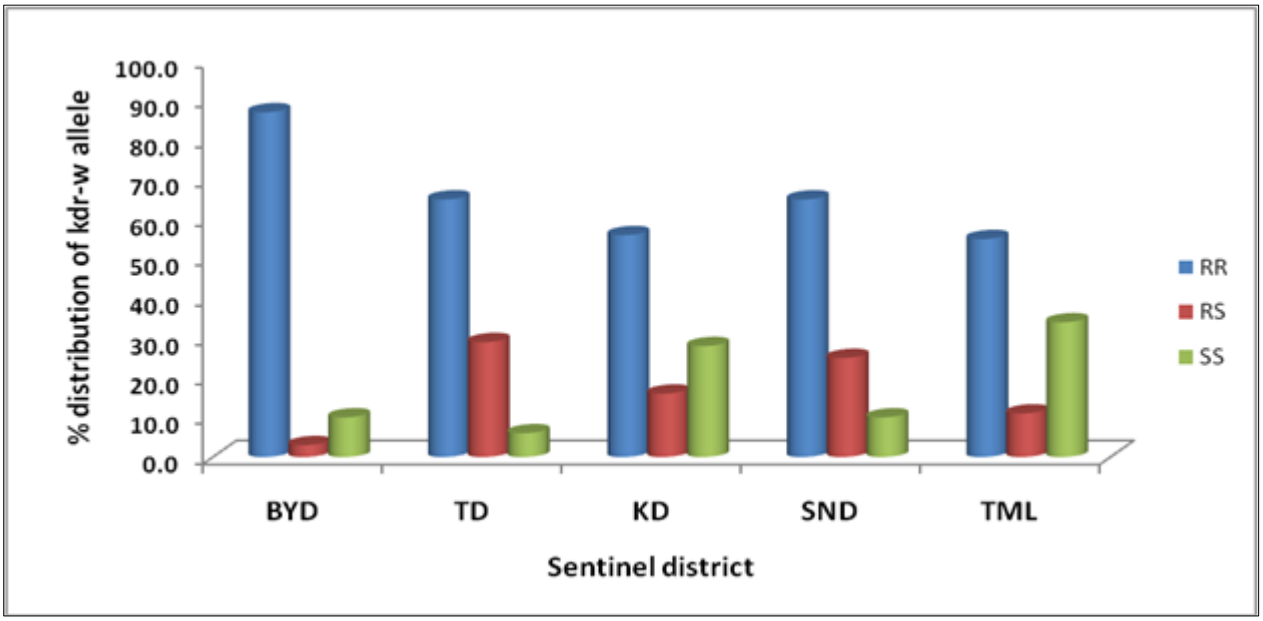


FIGURE 14: YEARLY TRENDS IN THE DISTRIBUTION OF KDR-W ALLELE IN MOLECULAR FORMS OF *AN. GAMBIAE* S.S., IRS AND NON-IRS AREAS, 2013 - 2015

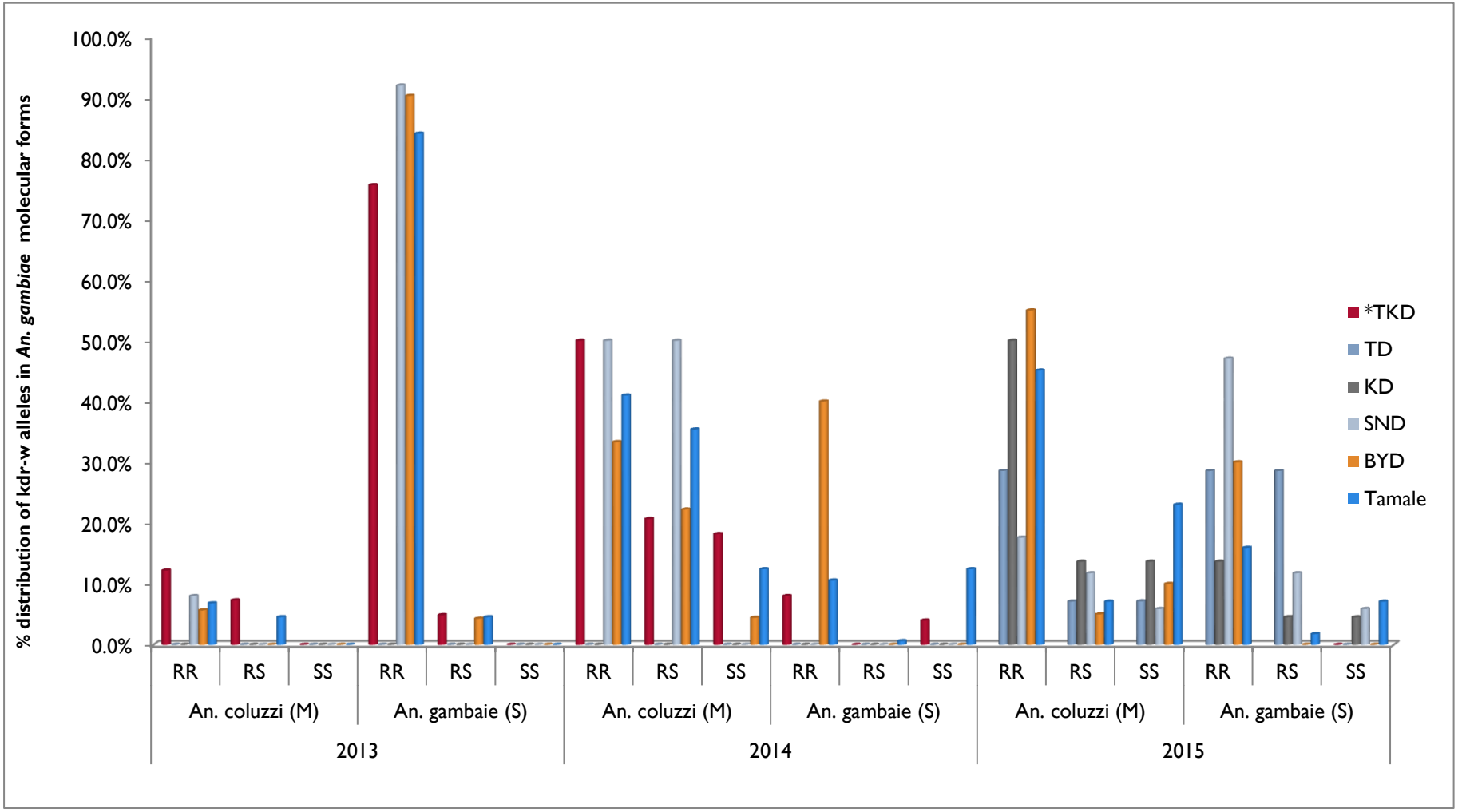
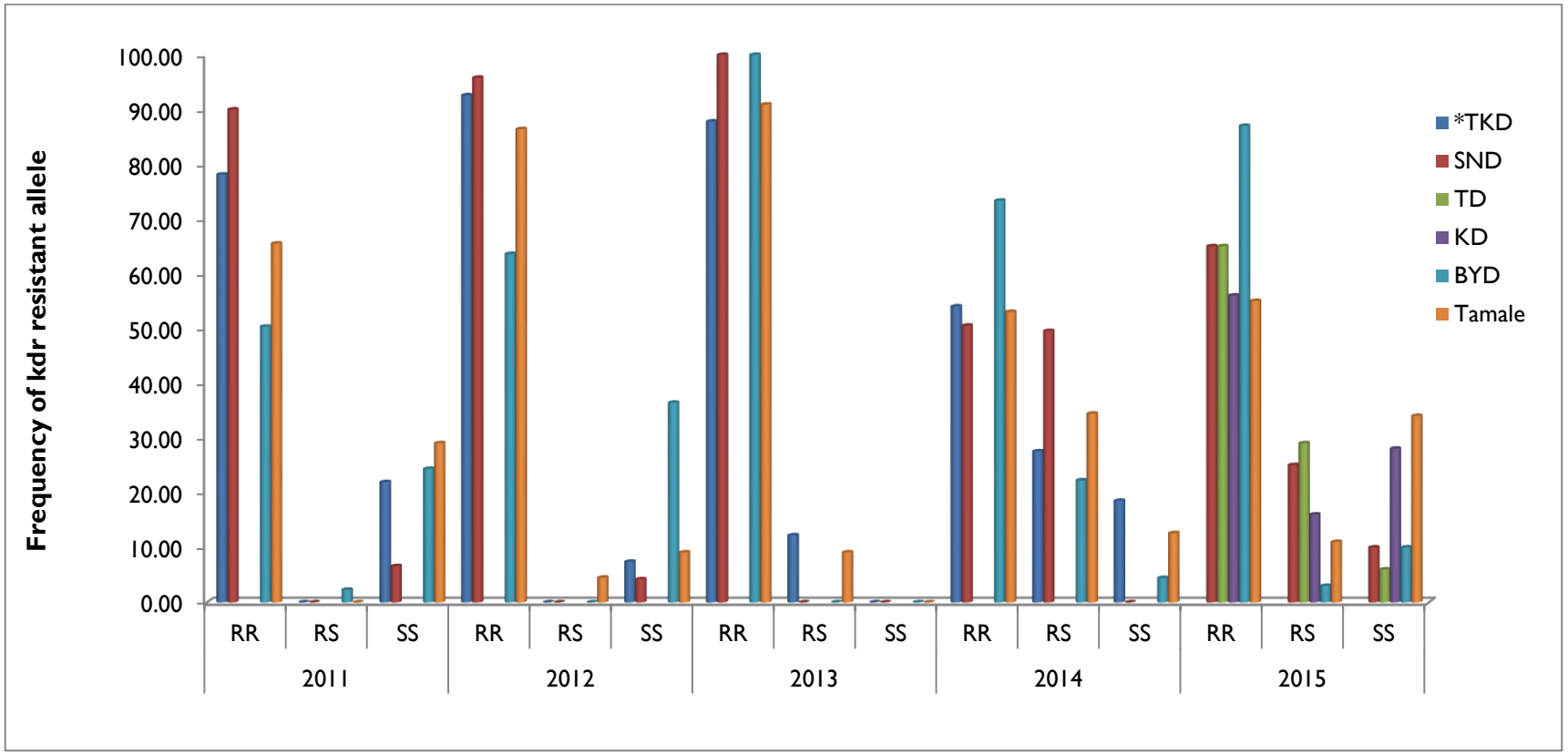


FIGURE 15[6]: YEARLY TRENDS IN THE FREQUENCY OF *KDR* GENE IN *AN. GAMBIAE* S.S., IRS AND NON-IRS AREAS

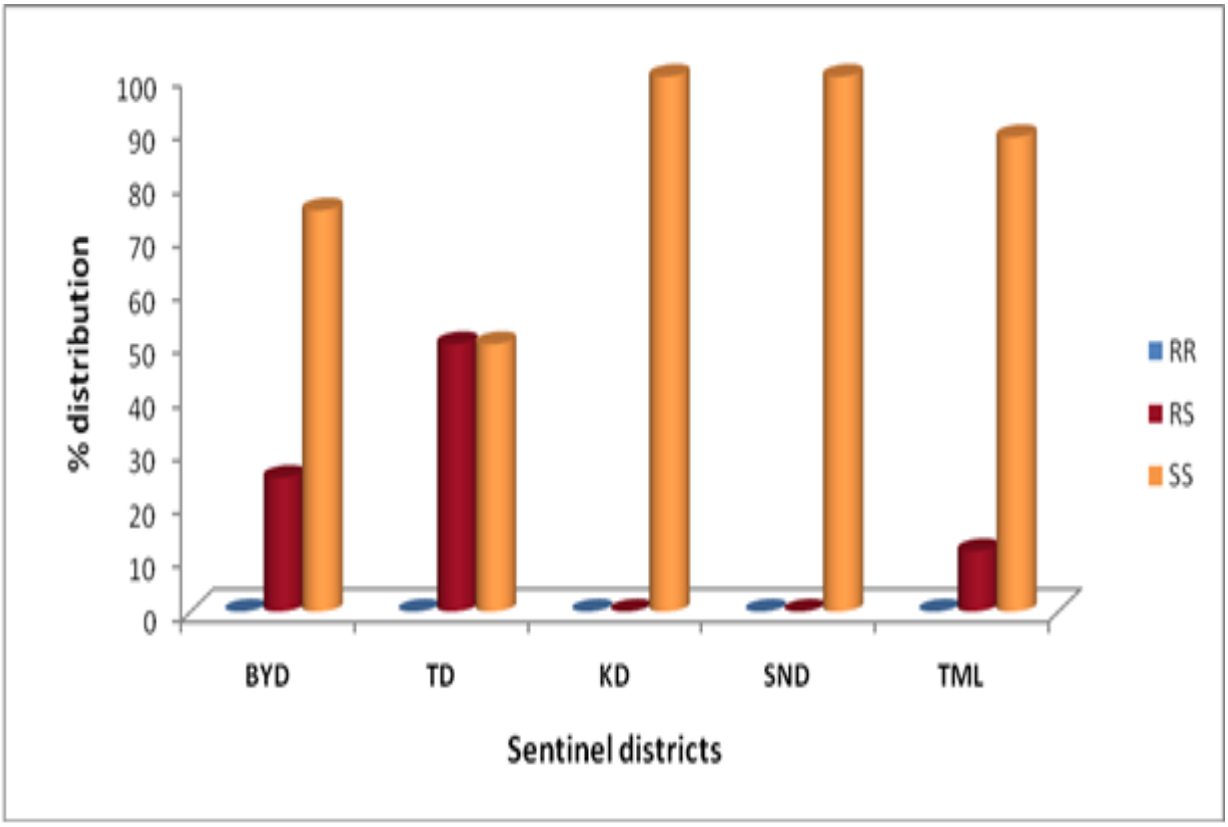


[6] **Districts and Insecticides sprayed** :
2010: SND & TKD - Pyrethroid (Deltamethrin);
2011: BYD, SND & TKD - Pyrethroid (Alphacypermethrin);
2012: BYD & TKD – Pyrethroid (Alphacypermethrin), SND sprayed organophosphate (Pirimiphos-methyl).
2013. BYD& SND - organophosphate (Pirimiphos-methyl) IRS was withdrawn from TKD in 2013.
2014. BYD& SND - organophosphate (Pirimiphos-methyl) TKD was not sprayed in 2014.
2015. BYD& KD - organophosphate (Pirimiphos-methyl) TD and SND were not sprayed in 2015

ITN Distribution: Door to Door- July 2012, School based October 2014, ANC & CHC- 2015

The results of the *Ace-1* gene analysis also showed that the NMIMR team found nearly 100% homozygote susceptible alleles (SS) in *Anopheles* from KD and SND (Figure 15). NMIMR found relatively higher heterozygote (RS) resistant alleles in TD (50%) and BYD (25%) than in TML (11.3%), SND (0%), and KD (0%). NMIMR did not detect any homozygous (RR) resistant alleles in the samples tested.

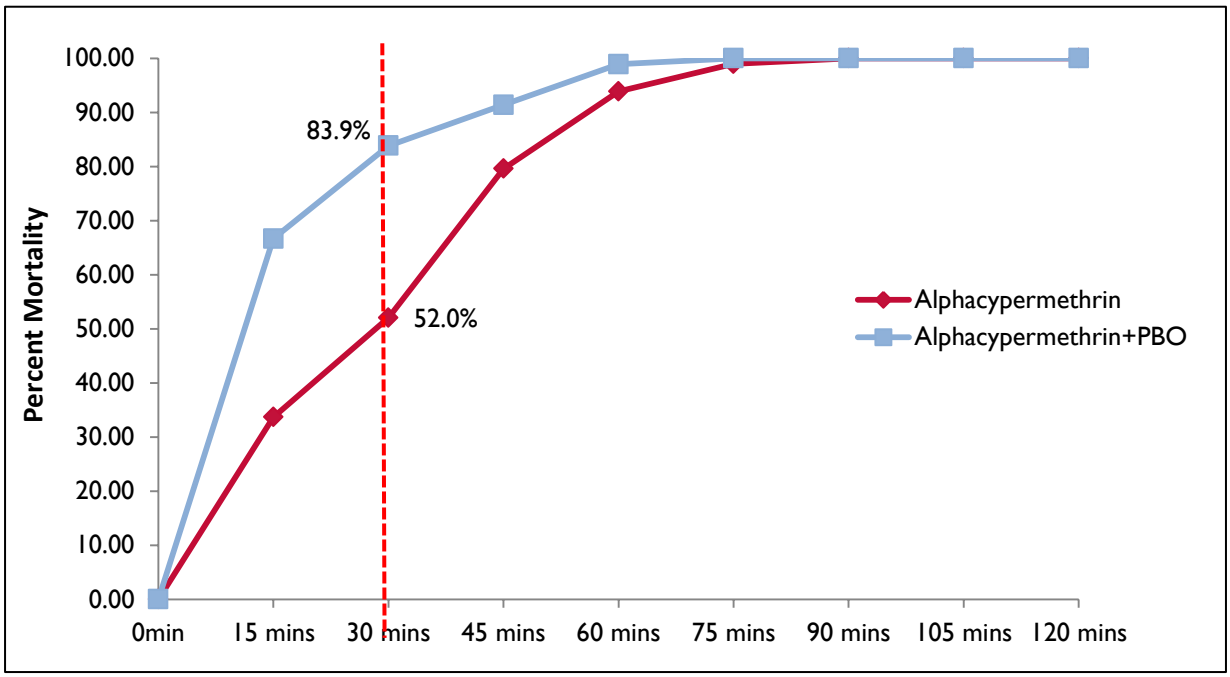
FIGURE 16: FREQUENCY OF ACE-1 GENE IN *AN. GAMBIAE* S.S., IRS AND NON-IRS AREAS, 2015



4.5.1.2 DETECTION OF MECHANISMS OF RESISTANCE

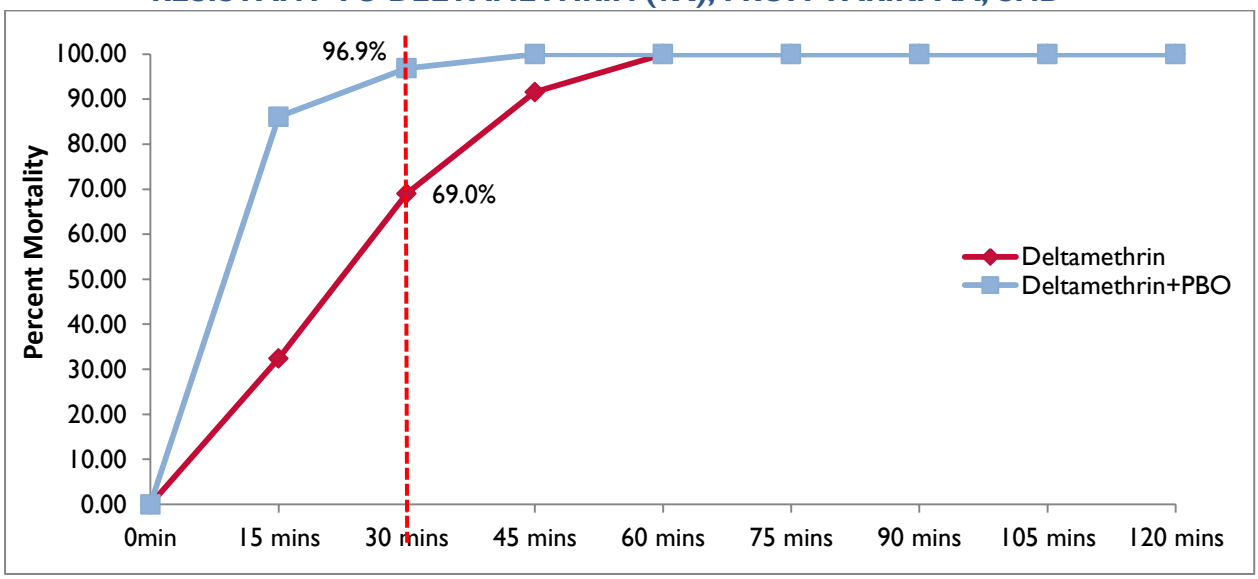
Results from the synergist assays conducted on *An. gambiae* s.l. populations from Tarikpaa which showed resistance to the pyrethroids are presented in Figures 14 and 15. The findings show that exposure to PBO resulted in an increase in susceptibility to alpha-cypermethrin and deltamethrin in *An. gambiae* s.l. from Tarikpaa. The mortality rate increased from about 52% to about 84% (p=0.0077) (Figure 16) in mosquitoes exposed to PBO before testing them against alpha-cypermethrin. Similarly, the deltamethrin test also showed the mortality rate increased from 69% to about 97% (p=0.0298) (Figure 17).

FIGURE 17: EFFECTS OF SYNERGIST ON *AN. GAMBIAE* S.L. POPULATIONS RESISTANT TO ALPHA-CYPERMETHRIN (1X) FROM TARIKPAA, SND



Red line indicate susceptible threshold

FIGURE 18: EFFECTS OF SYNERGISTS ON *AN. GAMBIAE* S.L. POPULATIONS RESISTANT TO DELTAMETHRIN (1X), FROM TARIKPAA, SND

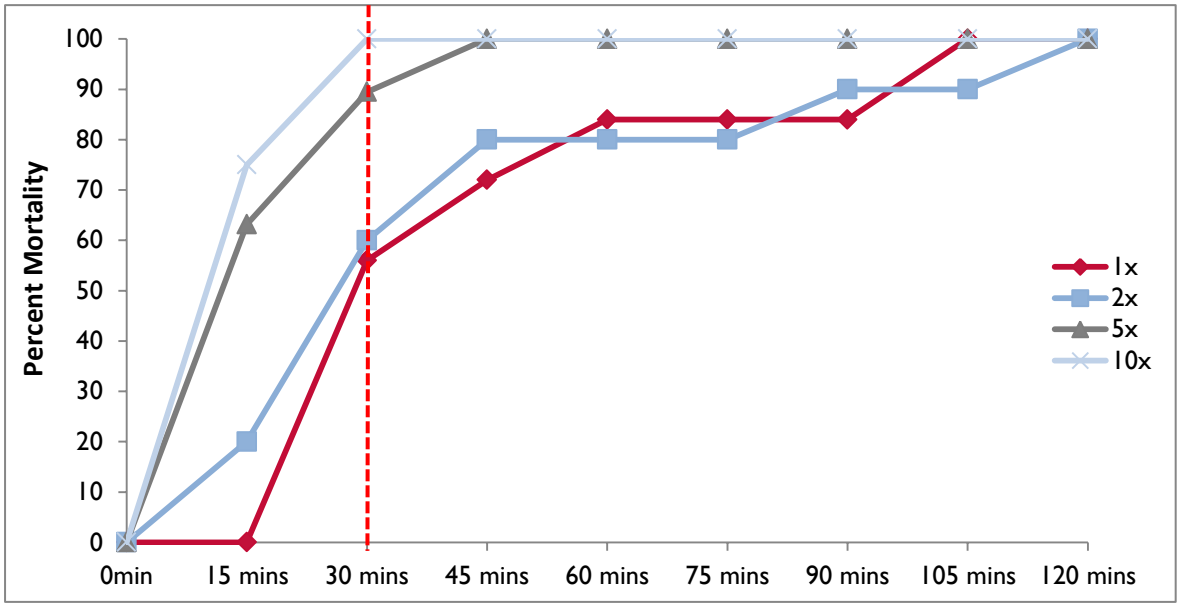


Red line indicate susceptible threshold

4.5.2 PYRETHROID RESISTANCE INTENSITY ASSAY

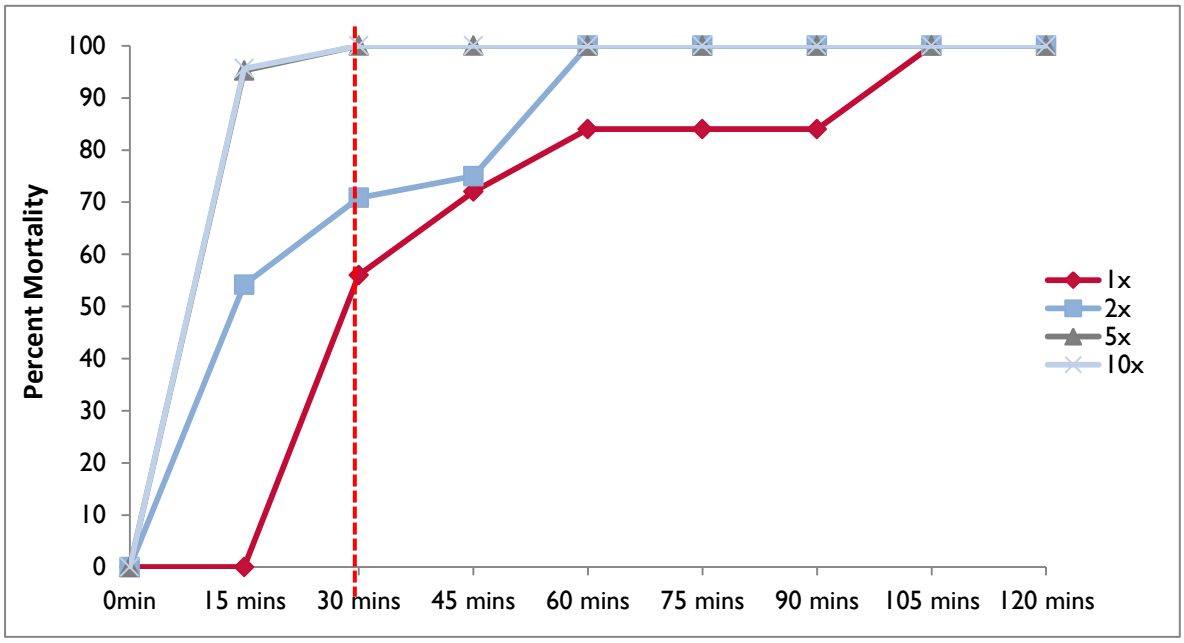
Using the CDC bottle bioassay guidelines recommended threshold, the results (Figure 18 - 20) indicate that, *An. gambiae* s.l. mosquitoes from Gbullung and Kumbungu were resistant to 1x and 2x diagnostic doses of alpha-cypermethrin, but were susceptible to the 5x and 10x doses. However, the AIRS team found that *An. gambiae* s.l. from Tarikpaa was highly resistant to 1x, 2x and 5x doses but only susceptible to the 10x dose.

FIGURE 19: INTENSITY OF RESISTANCE TO ALPHA-CYPERMETHRIN IN *AN. GAMBIAE* S.L., TARIKPAA, SND, 2015.



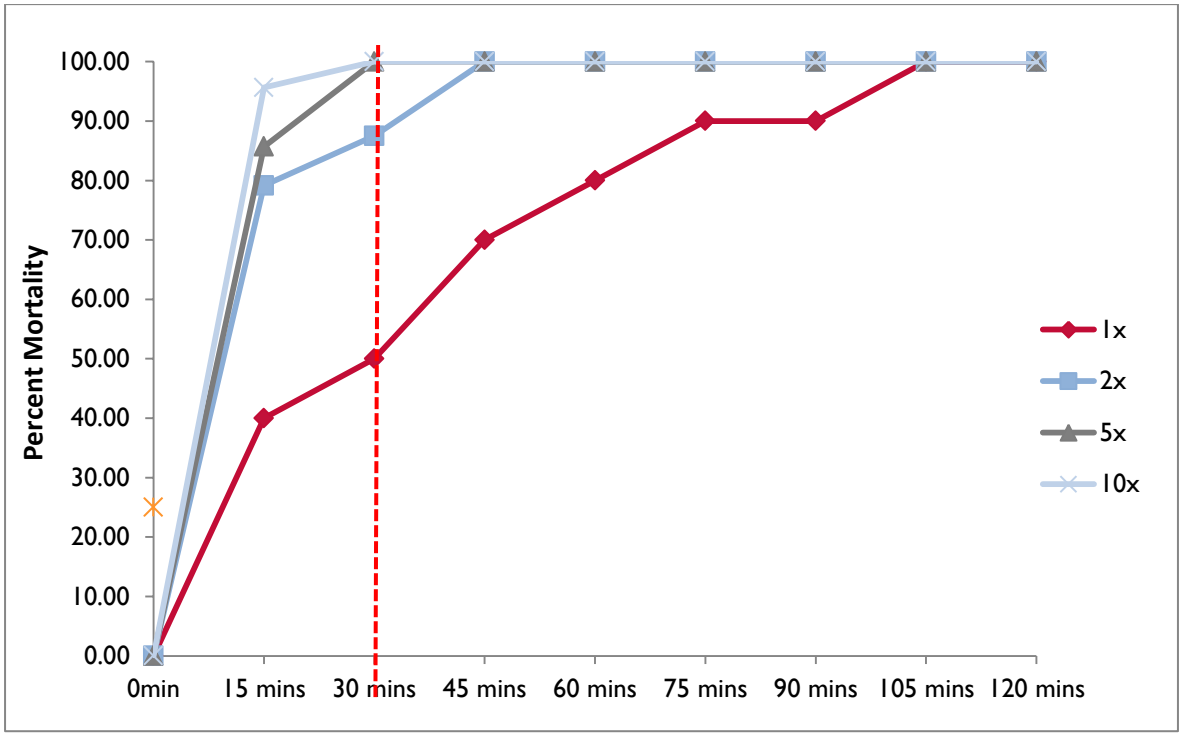
Red line indicate susceptible threshold

FIGURE 20: INTENSITY OF RESISTANCE TO ALPHA-CYPERMETHRIN IN *AN. GAMBIAE* S.L., GBULLUNG, KD, 2015.



Red line indicate susceptible threshold

FIGURE 21: INTENSITY OF RESISTANCE TO ALPHA-CYPERMETHRIN IN *AN. GAMBIAE* S.L., KUMBUNGU, KD, 2015.



Red line indicate susceptible threshold

4.6 CONE BIOASSAYS ON SPRAYED SURFACES

4.6.1 SPRAY QUALITY ASSESSMENTS

The results for the spray quality test are presented in Figure 21 below. The 24-hour mortalities for all the tests conducted on all surfaces were 100% in all the communities evaluated. Control mortalities ranged between 0% and 5%. As a result, the team did not calculate a correction for the mortalities recorded.

4.6.2 RESIDUAL EFFICACY OF SPRAYED INSECTICIDES ON SPRAYED SURFACES

Figures 22 to 30 below show the decay rate of the sprayed insecticide, Actellic 300CS on different wall surfaces. The sprayed insecticide lasted between six and eight months depending on the type of sprayed surface. Based on the WHO Pesticide Evaluation Scheme recommended threshold of 80%, the sprayed insecticide lasted about seven months (82% mortality) on cement surfaces for tests with the Kisumu strain and about six months for the tests using wild *An. gambiae* s.l. However, on the wooden surfaces (doors and windows) Actellic 300 CS lasted up to eight months (80.6% mortality) for the cone bioassays with the Kisumu strain and about seven months (79.1% mortality) for bioassays performed with wild *An. gambiae* s.l. collected from the IRS sites.

4.6.3 AIRBORNE EFFECT OF ACTELLIC 300CS

Figure 31 shows the airborne effect of Actellic 300CS on the laboratory raised Kisumu strain of *Anopheles gambiae* s.s. and wild collected female adults (reared from larvae) of *An. gambiae* s.l. of known ages (about two to five days old). Results indicate that pirimiphos-methyl showed an airborne effect on mosquitoes, especially in the first few weeks T0-T1 after the spraying. However, there was no significant airborne effect after one month, while exposed mosquito mortality to the sprayed walls was still 100% (Figures 22-30).

FIGURE 22: PERCENTAGE MORTALITY OF *ANOPHELES GAMBIAE* FROM SPRAY QUALITY CONE WALL BIOASSAYS, ONE TO TWO DAYS AFTER SPRAY, 2015

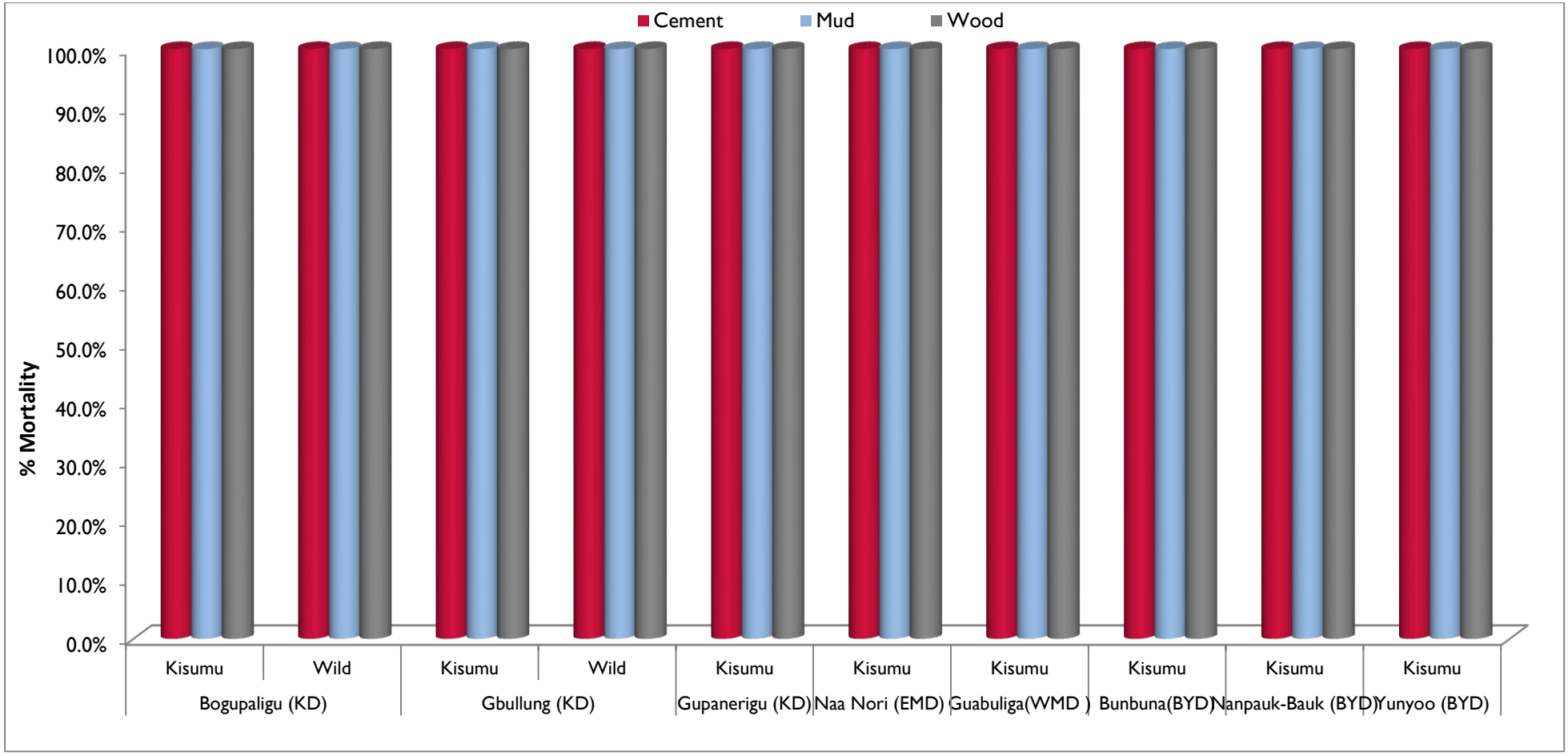


FIGURE 23: MEAN PERCENTAGE MORTALITY OF *AN. GAMBIAE* 'KISUMU' STRAIN AND WILD *AN. GAMBIAE* S.L. ON CEMENT, MUD, AND WOOD SURFACES, CONE WALL BIOASSAYS, ALL SITES, APRIL - DECEMBER 2015.

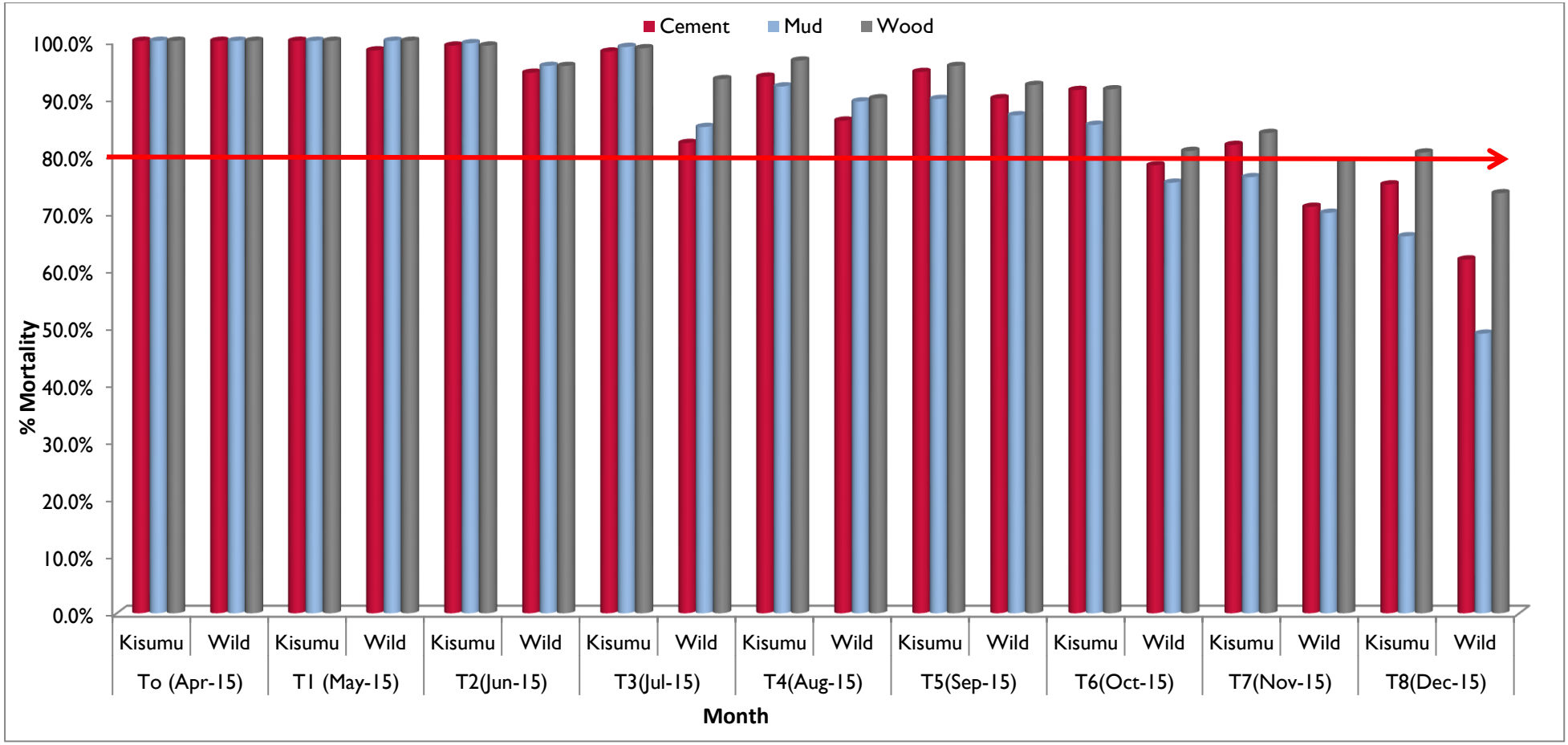


FIGURE 24: DECAY RATE OF PIRIMIPHOS-METHYL SPRAYED ON DIFFERENT WALL SURFACES IN BOGUPALIGU, KD: RESULTS OF WALL BIOASSAY USING KISUMU STRAIN AND WILD *AN. GAMBIAE* S.L.

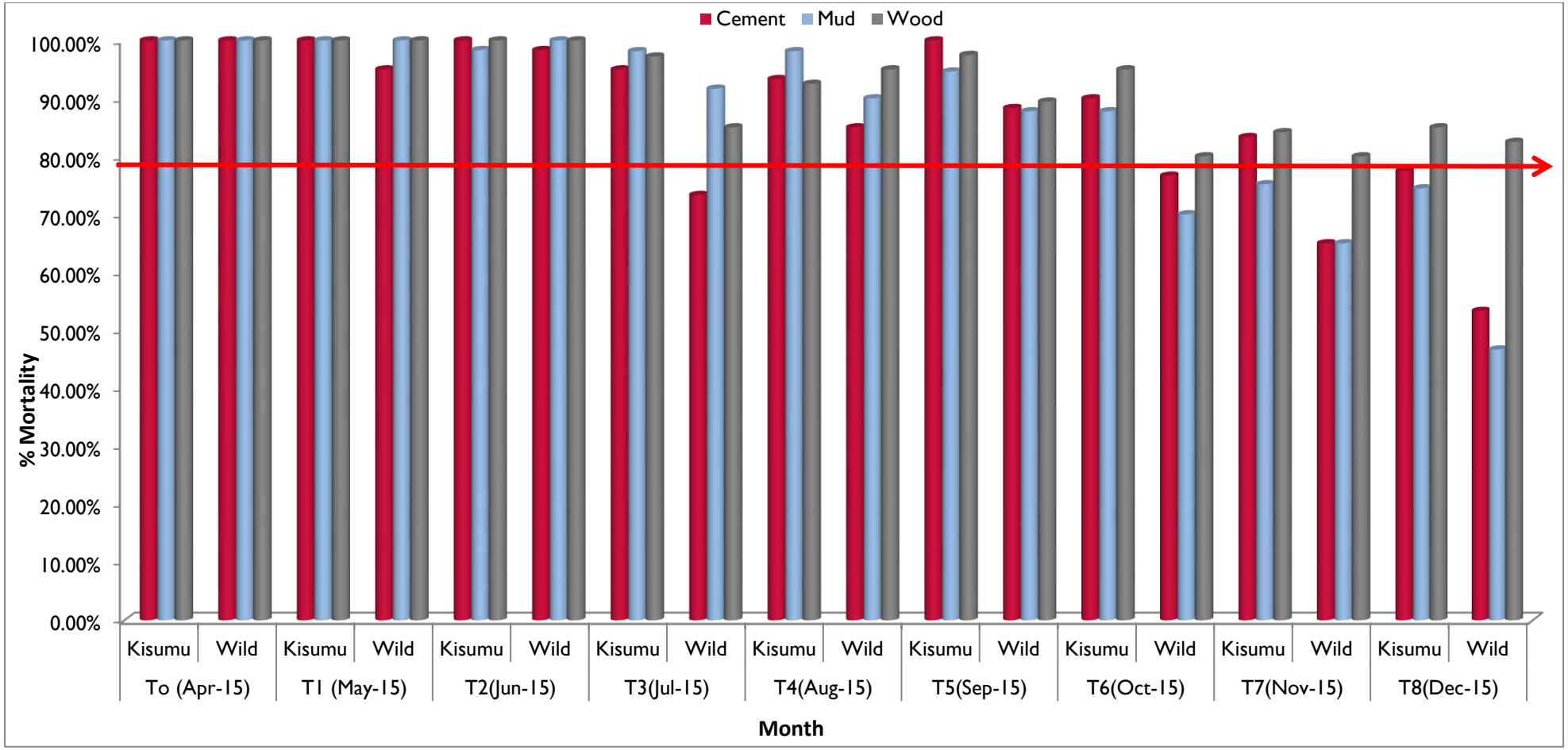


FIGURE 25: DECAY RATE OF PIRIMIPHOS-METHYL SPRAYED IN DIFFERENT WALL SURFACES IN GBULLUNG, KD: RESULTS OF WALL BIOASSAY USING KISUMU STRAIN AND WILD *AN. GAMBIAE* S.L.

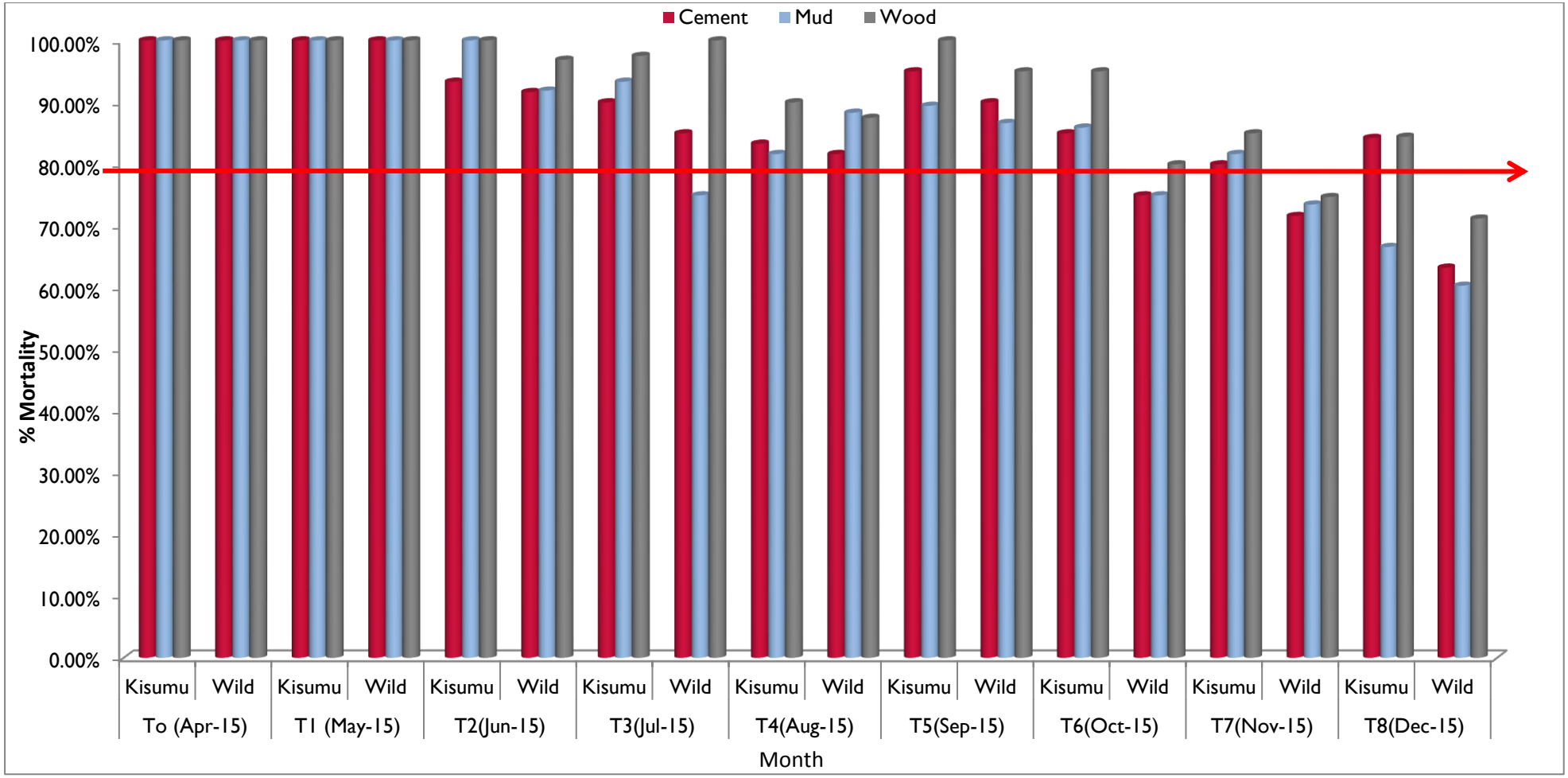


FIGURE 26: DECAY RATE OF PIRIMIPHOS-METHYL SPRAYED IN DIFFERENT WALL SURFACES IN GUPANERIGU, KD: RESULTS OF WALL BIOASSAY USING KISUMU STRAIN AND WILD *AN. GAMBIAE* S.L.

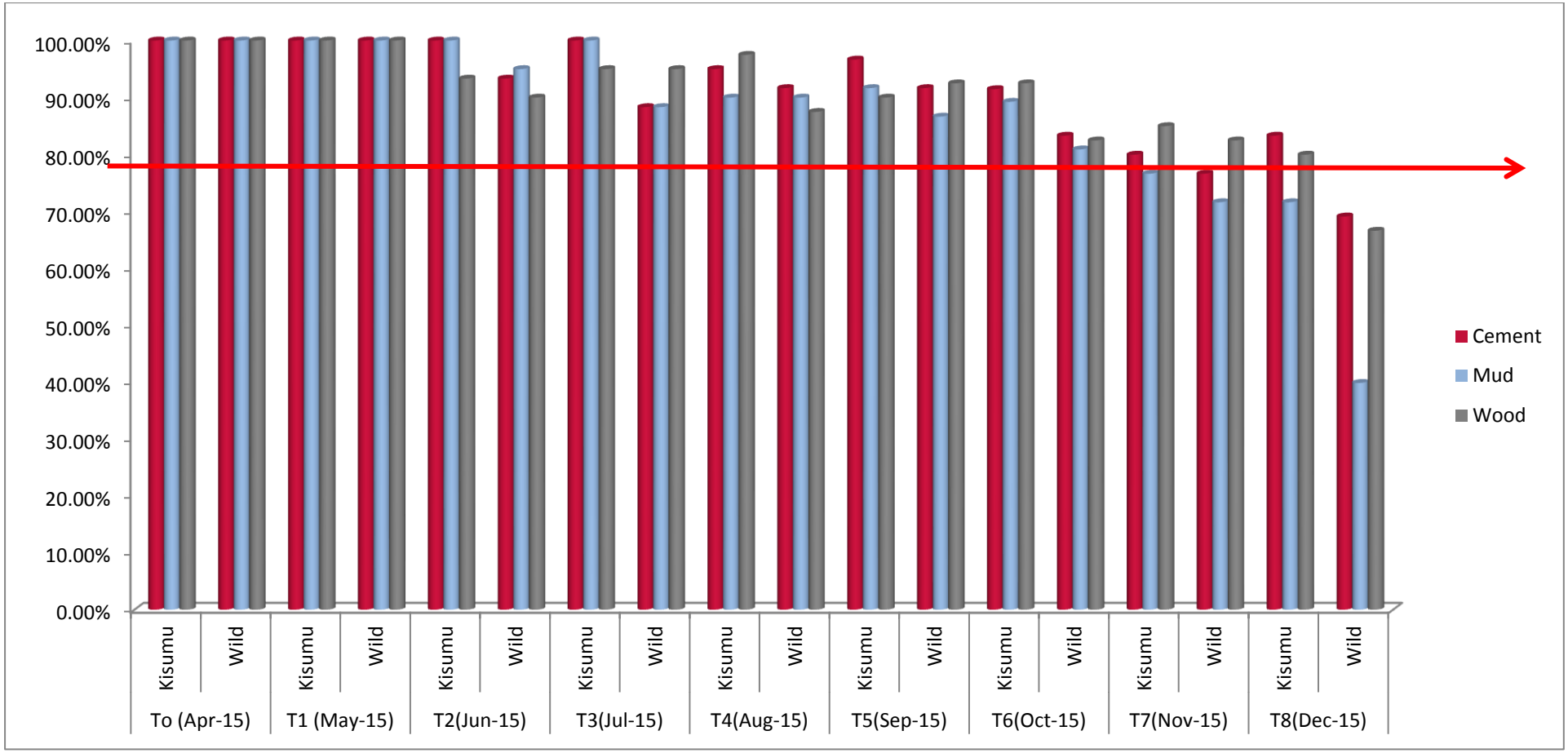


FIGURE 27: PIRIMIPHOS-METHYL DECAY RATE OF *AN. GAMBIAE* 'KISUMU' STRAIN, WALL SURFACES, GUABULIGA, WMD

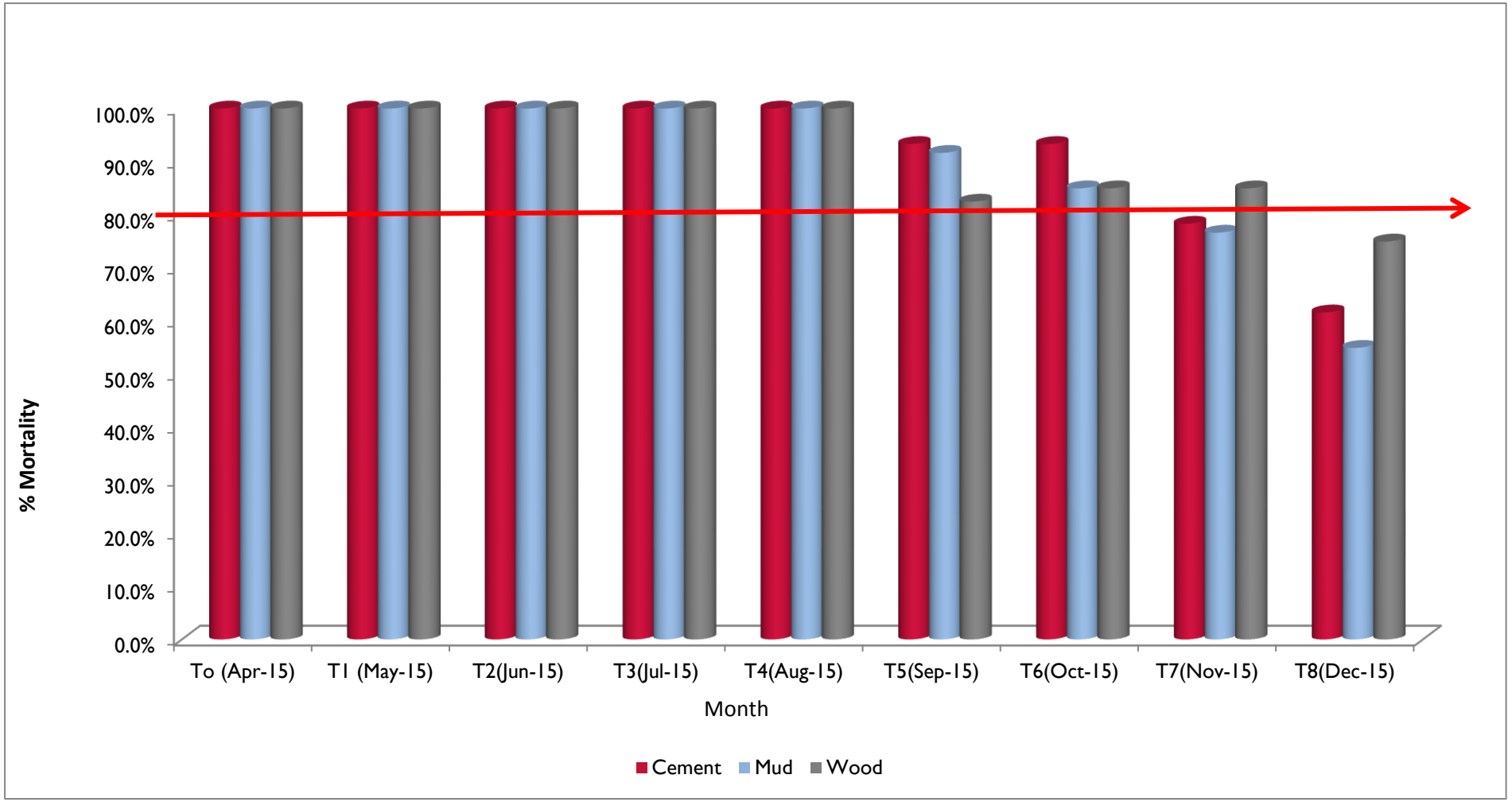


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FIGURE 28: PIRIMIPHOS-METHYL DECAY RATE OF *AN. GAMBIAE* 'KISUMU' STRAIN, WALL SURFACES, NAA NORI, EMD

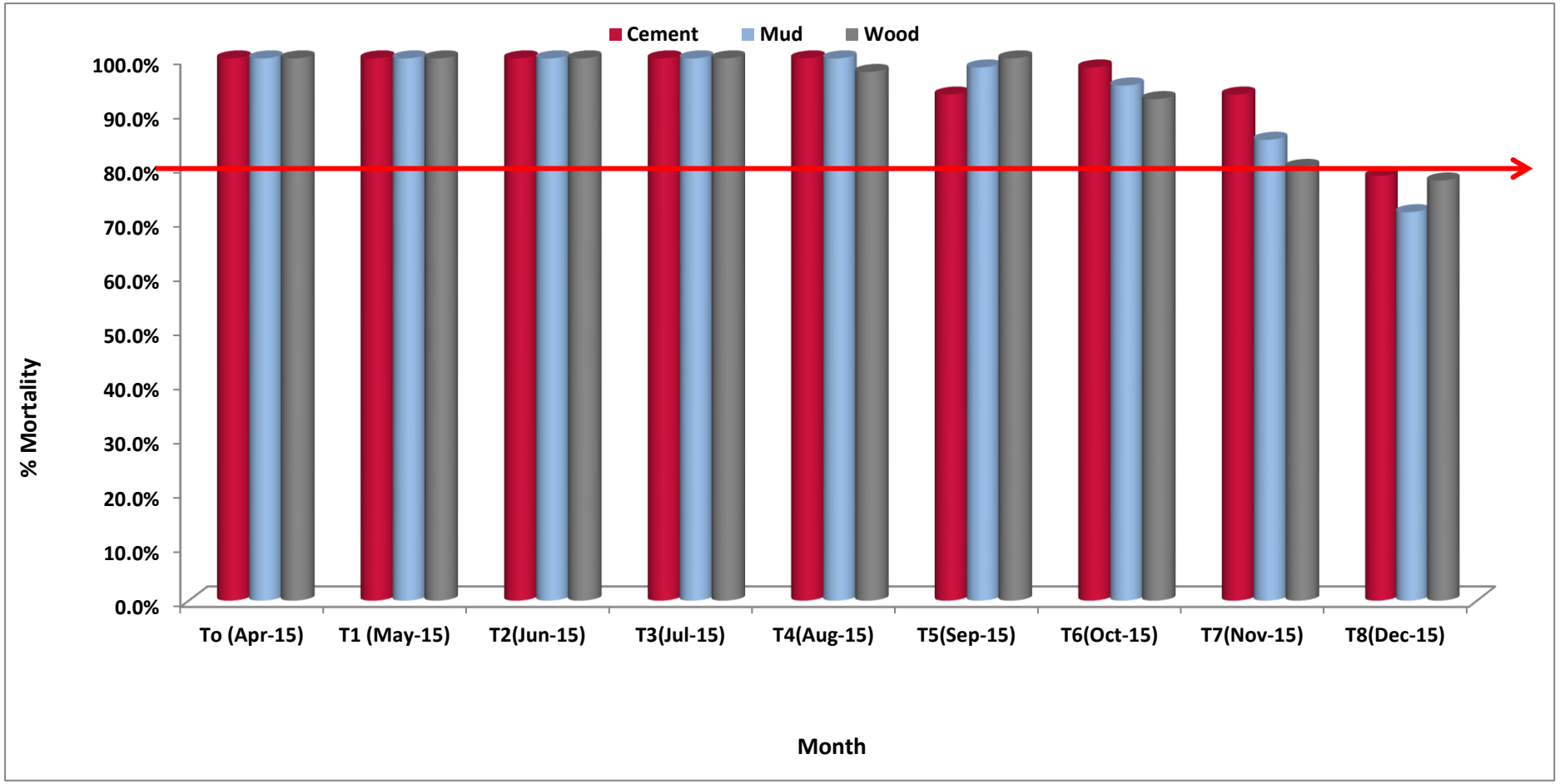

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FIGURE 29: PIRIMIPHOS-METHYL DECAY RATE OF *AN. GAMBIAE* 'KISUMU' STRAIN, WALL SURFACES, NANPONTI BAUK, BYD

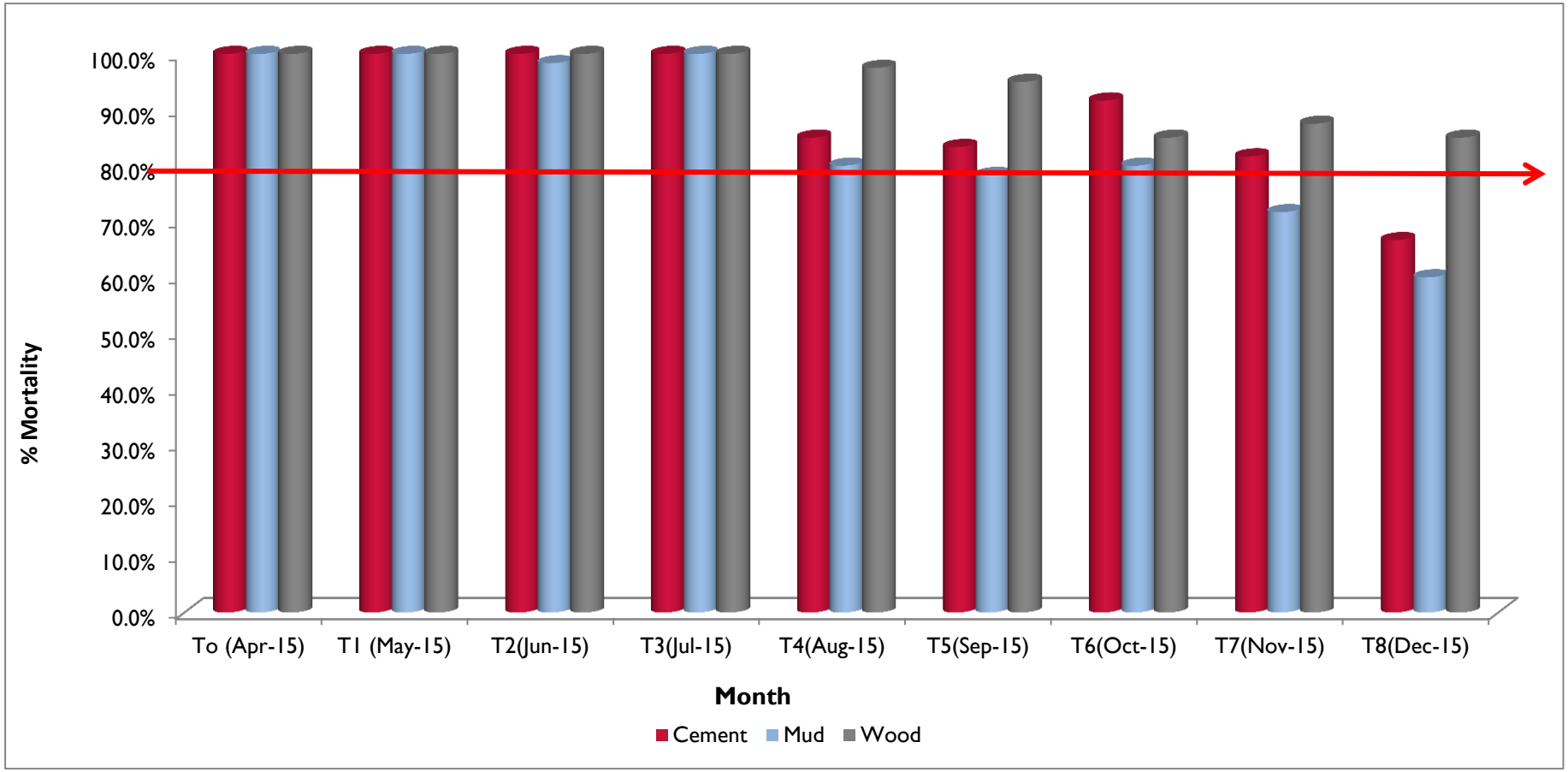

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FIGURE 30: PIRIMIPHOS-METHYL DECAY RATE OF *AN. GAMBIAE* 'KISUMU' STRAIN, WALL SURFACES, BUNBUNA, BYD

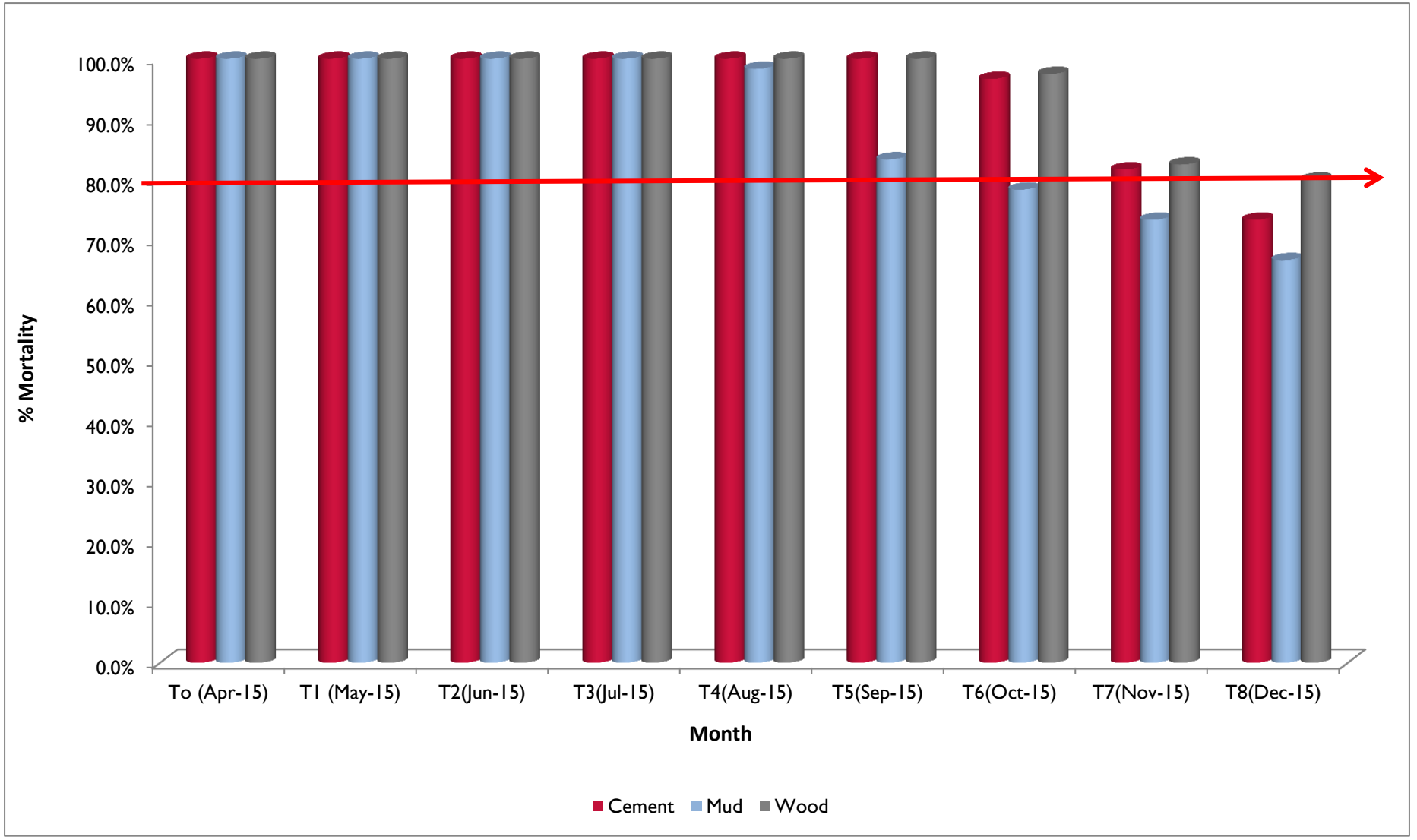

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FIGURE 31: PIRIMIPHOS-METHYL DECAY RATE OF *AN. GAMBIAE* 'KISUMU' STRAIN, WALL SURFACES, YUNYOO, BYD

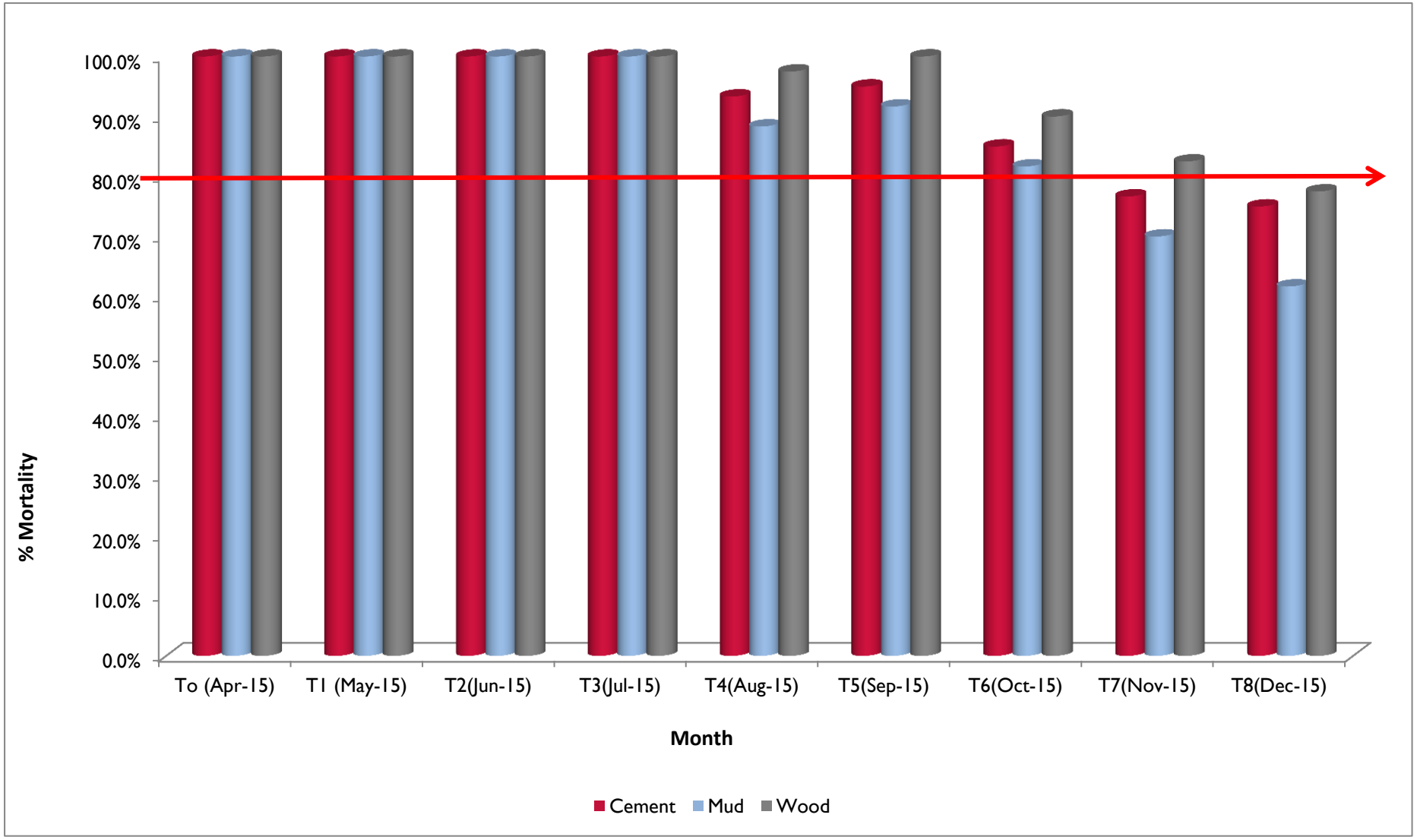
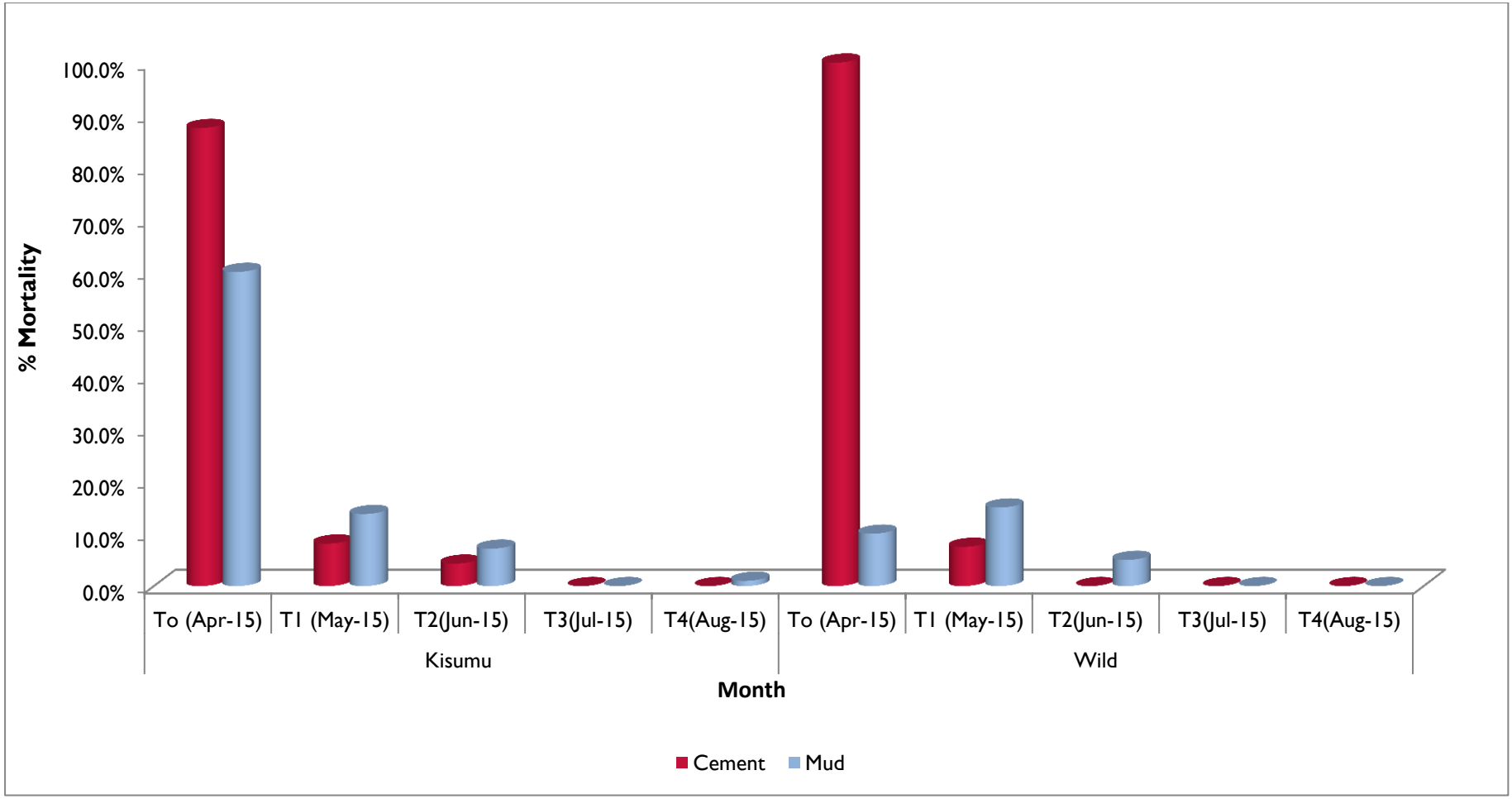


FIGURE 32: AIRBORNE EFFECT OF ACTELLIC 300CS ON KISUMU STRAIN OF *ANOPHELES GAMBIAE* S.S. AND WILD *AN. GAMBIAE* S.L. IN SPRAYED ROOMS



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4.7 Sporozoite Rates of Vectors Collected from Sentinel Sites

The NMIMR team conducted ELISA assays on a total of 3,287 *Anopheles* to determine the presence of sporozoites in their salivary glands. *An. gambiae* s.l constituted about 97.0% of the total number (3,287) of samples examined. Other species examined included *An. funestus* (20) *An. pharoensis* (25) and *An. nili* (74).

The overall sporozoite rate for both *An. gambiae* s.l. and *An. funestus* was 1.00% (402) in TD, 1.11% (361) in Savelugu, 1.25% (320) in KD, 0.46% (649) in Bunkpurugu and 1.10% (1456) in TML districts. In total, the team detected 16 (55.2%) of the sporozoites positive mosquitoes from Tamale, the control district (Table 8). In KD, the team collected all the sporozoite positive mosquitoes (4) from Gbullung. Gbullung is different from IRS and IRS withdrawn areas in that the community is surrounded by irrigation farms that sustain mosquito breeding sites for most part of the year. The team did not find any of the other *Anopheles* species examined (*An. pharoensis* and *An. nili)*, to be positive for sporozoites.

A comparison of the indoor and outdoor sporozoite rates showed relatively higher outdoor sporozoite rate than indoor in all the IRS and Non-IRS districts with the exception of Tamale (Table 9). The indoor sporozoite rates were 0.67% (150), 0.9% (223) 0.38% (263), 1.98% (101) and 1.14% (877) for SND, KD, BYD, TD and Tamale respectively whereas that for outdoor were 0.66% (301), 1.42% (211), 2.1% (97), 0.52% (386), and 1.0% (579) for TD, SND, KD, BYD and Tamale respectively (Table 9). The team found the differences in indoor and outdoor sporozoite rates to be non-significant for all the sites (Table 9).

TABLE 9: SPOROZOITE INFECTIONS IN *AN. GAMBIAE AND AN. FUNESTUS* SAMPLED FROM ALL SENTINEL SITES, JANUARY - DECEMBER 2015

Sentinel Site	# Tested by ELISA	CS +ve	Sporozoite rate
IRS			
BYD	649	3	0.46%
KD	320	4	1.25%
Non-IRS			
SND (IRS withdrawn)	361	4	1.11%
TD (IRS withdrawn)	402	4	1.00%
TML (Non-IRS)	1456	16	1.10%

TABLE 10: DISTRIBUTION OF INDOOR AND OUTDOOR TRANSMISSION OF *AN. GAMBIAE AND AN. FUNESTUS* SAMPLED FROM ALL SENTINEL SITES, JANUARY - DECEMBER 2015

Study Site	Indoor			Outdoor			Z test for difference in proportions			
	# Tested by ELISA	**CS +ve**	**Sporozoite rate**	**# Tested by ELISA**	**CS +ve**	**Sporozoite rate**	**Pooled sample proportion:**	**Standard error:**	**Z test statistic**	**p value**
IRS										
BYD	263	1	0.38%	386	2	0.52%	0.005	0.005	-0.254	0.799
KD	223	2	0.90%	97	2	2.06%	0.013	0.014	-0.862	0.389
Non-IRS										
SND (IRS withdrawn)	150	1	0.67%	211	3	1.42%	0.011	0.011	-0.675	0.499
TD (IRS withdrawn)	101	2	1.98%	301	2	0.66%	0.010	0.011	1.153	0.249
TML (Non-IRS)	877	10	1.14%	579	6	1.04%	0.011	0.006	0.186	0.852

4.8 ESTIMATION OF ENTOMOLOGICAL INOCULATION RATES OF VECTORS

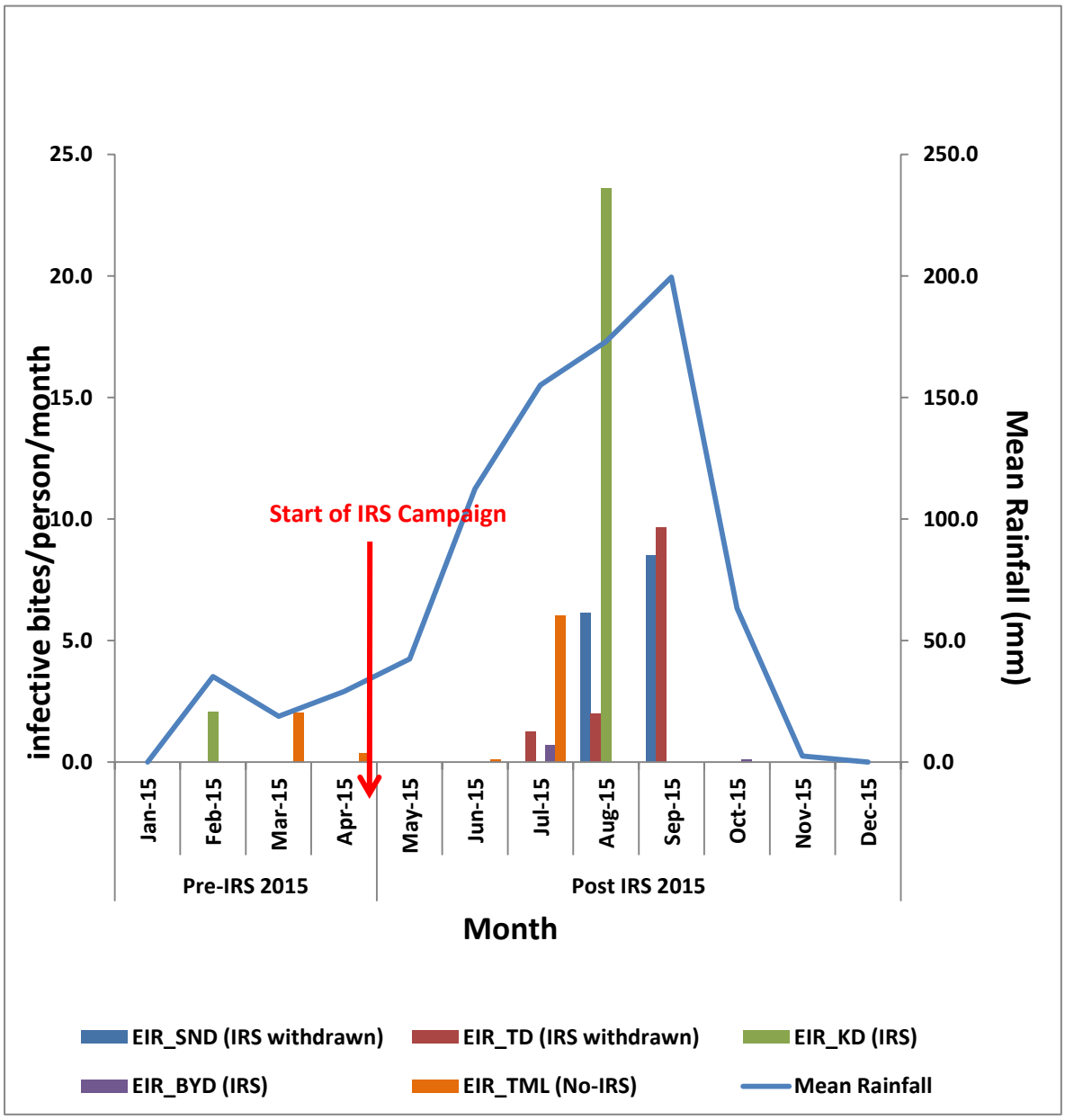
The team estimated the EIR, which measures the risk of exposure to malaria, to be 0.01 infective bites/person/night (ib/p/n) for BYD, 0.12 ib/p/n for KD, 0.04 ib/p/n for SND, 0.06 ib/p/n for TD, and 0.312 ib/m/n for TML. This translates to 0.83 infective bites/man/year (ib/m/yr), 26.21ib/m/yr, 14.65 ib/m/yr, 12.99 ib/m/yr and 8.51ib/m/yr, respectively (Table 10). Monthly trends of transmission showed that transmission was highly seasonal in both IRS and Non-IRS districts (Figure 32).

TABLE 11: ENTOMOLOGICAL PARAMETERS OF MALARIA TRANSMISSION, *ANOPHELES GAMBIAE* AND *AN. FUNESTUS,* ALL SENTINEL SITES, JANUARY - DECEMBER 2015.

Study Site	# Tested by ELISA	CS +ve	Sporozoite Rate	Mean MBR (b/p/n)	EIR (ib/p/n)	[7]Estimated[8] Annual EIR (ib/p/yr)
IRS						
BYD	649	3	0.46%	2.23	0.01	0.83
KD	320	4	1.25%	9.48	0.12	26.21
Non-IRS						
SND (IRS withdrawn)	361	4	1.11%	3.45	0.04	14.65
TD (IRS withdrawn)	402	4	1.00%	5.97	0.06	12.99
TML (Non-IRS)	1456	16	1.10%	12.20	0.13	8.51

[8] Annual EIR estimation based on sum of monthly EIRs

FIGURE 33: MONTHLY TRENDS IN EIR FOR *AN. GAMBIAE* AND *AN. FUNESTUS*, IN BYD, KD, SND, TD AND TML, 2015



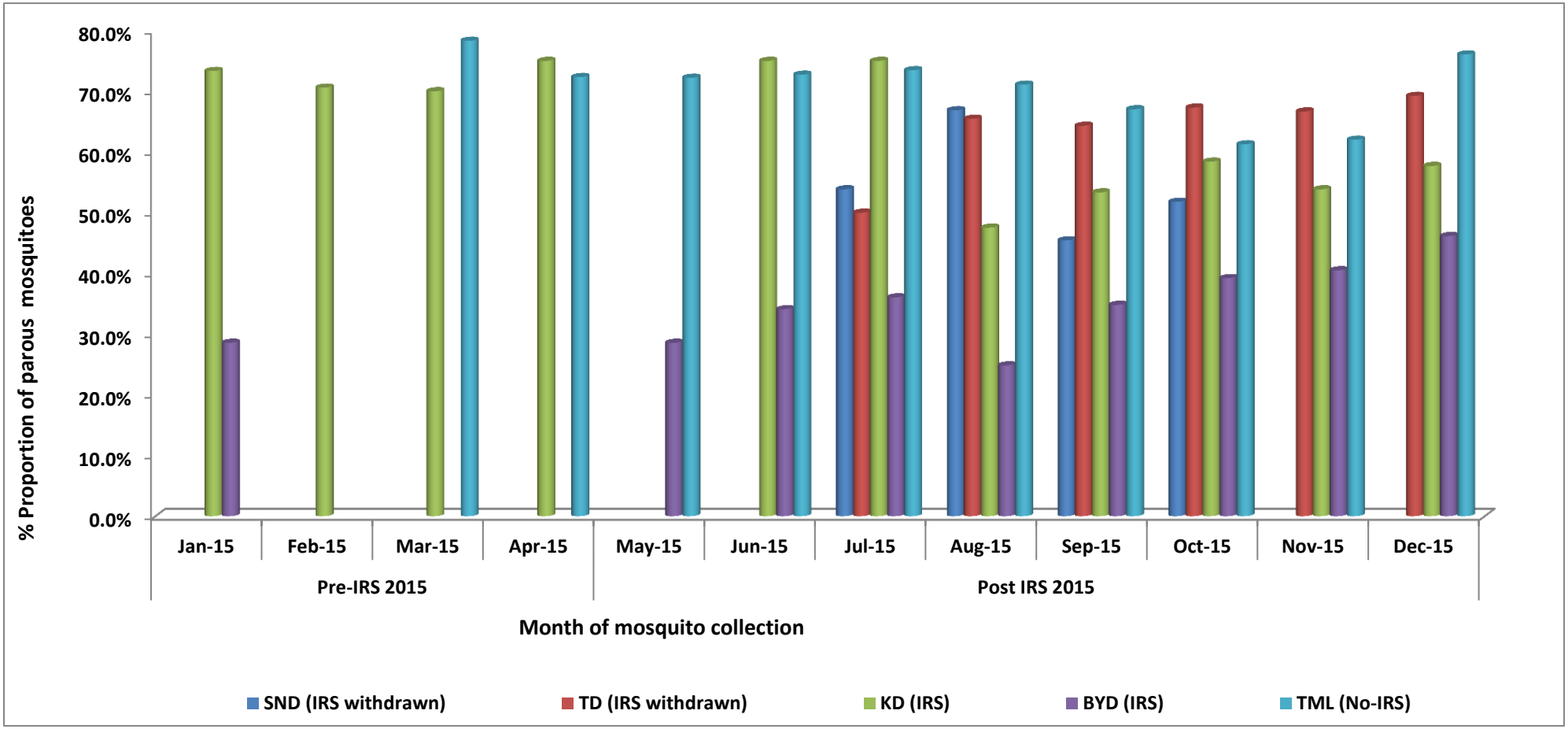
4.9 PARITY RATES

Dissections of *An. gambiae* s.l. mosquitoes collected from the study sites between January and December 2015 revealed a higher proportion of older *Anopheles* populations in the TD, SND, and TML (unsprayed districts) and in KD (IRS district). BYD recorded a mean parity rate of 30.6 percent, which was significantly lower ($p<0.05$) than the mean parity rates recorded for KD (53.1%), SND (51.2%), TD (65.1%) and TML (68.3%) (Table 11) (BYD & KD: $F_{(1,18)} = 44.41$, $p<0.0001$; BYD & SND: $F_{(1,11))}=27.93$, $p<0.0001$; BYD & TD, $F_{(1,13)} = 105.952$ $p<0.0001$; BYD & TML : $F_{(1,17)}=121.349$, $p< 0.0001$). Similarly, the mean parity rate for KD was significantly lower than the mean parity rates for TD ($p=0.009$) and Tamale ($p=0.001$), but was not significantly different from parity rates recorded in SND where the team withdrew IRS in 2015 ($p=0.836$). The team found parity rates in SND to be significantly lower than parity rates for TD ($p=0.028$) and TML ($p=0.008$). There was no significant difference between parity rates recorded for TML and TD ($p=0.553$). Figure 33 shows the monthly trends in parity rates for all sites. A comparison of 2014 and 2015 parity data in the sprayed districts showed that parity rates of *An. gambiae* s.l. in BYD increased significantly from 24.3% in 2014 to 30.6% in 2015 (representing 21% increase from the 2014) ($F_{(1,16)}=4.568$, $p = 0.048$). However, parity rates in KD decreased significantly from 66.8% in 2014 to 53.1% in 2015. The unsprayed districts, TD and TML, recorded 8.1 and 5.7 percentage decrease in parity rates respectively, compared to 2014 parity rates. However, these reductions were not significant (TD: $p=0.395$; TML: $p= 0.189$). Parity rates in SND increased significantly from 28.1% recorded in 2014 to 51.2% in 2015 ($F_{(1,8)}= 33.449$, $p<0.0001$).

TABLE 12: TOTAL NUMBER OF PAROUS FEMALE *AN. GAMBIAE* S.L., HLC, ALL SENTINEL SITES

	Dissected	Parous	%Parity	95% confidence interval	
				Lower Bound	upper Bound
IRS					
BYD (IRS)	1678	514	30.6%	28.4%	32.8%
KD (IRS)	2535	1346	53.1%	51.2%	55.0%
Non-IRS					
SND (IRS withdrawn)	1377	705	51.2%	48.6%	53.8%
TD (IRS withdrawn)	1550	1008	65.0%	62.7%	67.4%
TML (Non-IRS)	4453	3041	68.3%	66.9%	69.7%

FIGURE 34[9]: PROPORTION OF PAROUS *ANOPHELES GAMBIAE* S.L., HLC, ALL SENTINEL SITES



[9] As a result of low mosquito numbers collected and/or dried samples no dissections were done for; SND: January, February, March, April, June, July, November and December; TD: January, February, March, April, May and June; KD: May BYD: February, March, April; TML: January, February and March.

5. Discussion, Conclusion and Recommendations

5.1 Discussion

The results indicate that *An. gambiae* s.l. was the most abundant species in all the study sites, making up more than 96.6% of the total *Anopheles* species collected. Results found that the M (*An. coluzzii*) and *An. gambiae* Giles of *An. gambiae* s.s. were present in sympatry at all five sites in varying proportions. The team did not detect any *An. arabiensis* in the samples analyzed.

The monthly monitoring found that distribution of *An. coluzzii* and *An. gambiae* to be dependent on ecological and geographical factors. The S forms prefer breeding sites that are temporary and rainfall dependent with relatively low temperatures (Diabate *et. al*., 2003), while *An. coluzzii* prefer to breed in permanent environment and habitats created by human activities such as irrigation fields and regions with high temperatures (Wondji et al., 2002). Reduction in mean annual rainfall for the study area between 2010 and 2015 (from 121.9mm in 2010 to 78. 2 in 2014 and then to 69.3mm in 2015) could partly explain the general increase in *An. coluzzii* across all sites compared to previous years. It is possible that temporary rainfall dependent larval habitat that could support breeding of the S forms might have also declined over the period with the reduction in amount of rainfall. The relatively high densities of *An. coluzzii* in KD, TD, and TML (Kulaa and Tugu) could have resulted from the abundance of irrigation farmlands and other permanent and semi-permanent dams/ponds found in the area (Figure 1) that support rice farming and irrigations all year round.

The strong positive correlation observed between *An. gambiae* s.l. abundance (biting rates and indoor resting densities) and the mean rainfall, suggests that the risk of malaria transmission is highly dependent on rainfall patterns as well. IRS in the intervention areas will therefore be most beneficial if the spray campaign is timed to be completed before *Anopheles* biting rates and densities peak. The increase in biting rates and densities of mosquitoes just after IRS is a result of the rains which peaked at the end of the spray campaign.

The exophagic (outdoor feeding) behavior observed in most sites could be a result of multiple interventions in the areas (IRS in the sprayed areas and use of long-lasting insecticide-treated bed nets (LLINs) in the unsprayed areas).

The results from the insecticide susceptibility tests indicate that *An. gambiae* s.l. in the tested sites are resistant to the pyrethroids alpha-cypermethrin and deltamethrin. This could partly be due to the continuous use of alpha-cypermethrin and deltamethrin impregnated LLINS (*Interceptor*® and PermaNet® 2.0) from previous hang up campaigns and mass distributions. The occurrence of most active anopheline breeding sites around irrigated farmlands where the larvae could have had prior exposure to insecticides at the developmental stages, could also account for resistance to the pyrethroids and possible resistance to some organophosphates and carbamates as shown in the results. However, the vector is fully susceptible to pirimiphos-methyl in all IRS communities.

Determination of the presence/absence of *Kdr-w* and *Ace-1* alleles is important to understand the scope of local resistance and determine factors driving observed phenotypic resistance in areas of operation. During the previous years, the project team has detected relatively higher numbers of resistant genes from the IRS areas than non-IRS areas. The *kdr* alleles increased from 33.5% in 2010 to 94% in 2012 and near 100% in 2013 for all the study areas. However, the general observation is that the *kdr* frequency in An. *gambiae s.s* in most IRS sites has reduced in 2015 – three years after the change from pyrethroids to organophosphate. The apparent increase in *kdr-w*

homozygote susceptible alleles in the population of *An. gambiae s.s.* may be due to the change or withdrawal of pyrethroids in the IRS areas. However, the project will monitor this trend continuously in subsequent years so as to understand the dynamics of this insecticide resistance trait in malaria vectors under different insecticide pressures. The team also found high frequency of susceptible homozygous *Ace-1* allele in the M (*An. coluzzii*) and S forms analyzed from the operational areas. This could account for the high susceptibility of vector species to pirimiphos-methyl and fenitrothion observed in most areas. However, the presence of heterozygote resistant alleles in samples from BYD and TD could be an indication that resistance may be developing in the local vector species and needs to be monitored.

Results from the synergist assays suggest a role of mono-oxygenases in the resistance of *An. gambiae* s.l. from Tarikpaa to the pyrethroids tested. However, resistance to the pyrethroids was only partially abolished, and hence it is not the only mechanism involved in insecticide resistance in the area. The project is conducting further biochemical assays for confirmation of these observations.

The high EIRs recorded in KD could be as result of a high gametocyte pool already present in people in the district resulting from the IRS withdrawal in 2013. It may be too early to see the direct impact of IRS on EIRs in KD. However, follow-up IRS campaigns may further reduce the intensity of transmission as observed in BYD, which has benefitted from five years of IRS operations. In BYD, intensity was maintained at a significantly low level. The general reduction in malaria transmission also observed across most sites including TML, could also be partly due to the sustained LLIN distribution by the National Malaria Control Program through the antenatal clinics, child welfare clinics, and schools from late 2014 through 2015, especially in the non-IRS areas, as well as continuous malaria prevention awareness campaigns. These might have contributed to reducing human vector contact indoors where most transmission used to occur as observed in the previous years. The effect of IRS withdrawal at SND is evidenced in the immediate increase in parity rates, indoor resting densities, as well as EIRs, in comparison with 2014 when the project did not detect any sporozoite infections. The high pyrethroid resistance intensity observed in Tarikpaa in SND could be a reflection of the level of resistance intensity to pyrethroid in the district, meaning pyrethroid impregnated LLINs may be less effective in reducing transmission in the district.

The relatively high outdoor malaria transmission observed in 2015 could be a result of the change in feeding behavior observed in most sites. This needs further observation and investigation.

The occurrence of significantly lower parous females in BYD in comparison with other sites could be a result of the continuous IRS operations in the district since 2011. The re-introduction of IRS in KD could account for the significant reduction in proportion of parous (older) females as compared to proportion collected in 2014. Despite the high densities of *An. gambiae* s.l. recorded in KD, the proportion of older females in the district was significantly lower than for TD and TML.

5.2 CONCLUSIONS

- IRS has significantly maintained transmission at low levels in BYD. The re-introduction of IRS in KD seems to have contributed to the significant reduction in parity rates in the district as compared to TML and TD. This effect could be attributed to the impact of pirimiphos-methyl in killing high proportions of the older females *An. gambiae* and *An. coluzzii* mosquitoes that rest in the rooms. This also confirms that the local vector species in the area are still highly susceptible (98-100 percent) to pirimiphos-methyl used for the 2015 IRS operations. The project will monitor this trend in 2016.
- There was an increase in malaria transmission intensity in SND probably as a result of the withdrawal of IRS.

5.3 RECOMMENDATIONS

- The occurrence of increased malaria transmission risk during the rainy season suggests that it will be prudent to complete the spraying campaign before transmission starts in June.
- *An. gambiae* and *An. coluzzii* from the sprayed areas remain susceptible to organophosphates (pirimiphos-methyl and fenitrothion). Considering the high susceptibility of the local vector species to pirimiphos-methyl and the residual life of six to eight months documented for pirimiphos-methyl CS, we recommend that it can still be used for IRS in all the areas in 2016. However, the project needs to monitor the trends and intensity in the level of insecticide resistance, especially to organophosphates, within the malaria vector populations across the IRS areas that have sprayed pirimiphos-methyl since 2012.

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