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Evaluation of the Efficacy and Residual Activity of three Candidate Insecticide Formulations for Anopheles gambiae s.l. in Jimma Zone, Southwestern Ethiopia

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Abstract
One way of evaluating insecticide formulations against malaria vector is to undertake insecticide decay rate study for insecticide deposits on different Wall Surfaces using WHO cone assay. To assess the decay rate of an insecticide deposits against adult female Anopheles mosquitoes, cone bioassay test was conducted at different time intervals after the application of each candidate insecticide on different wall surfaces. Therefore, the residual life of three candidate pyrethroid insecticide formulations (deltamethrine 25%WG, lambdacyhalothrin 10%WP and lambdacyhalothrin 10%CS) was evaluated on three different wall surfaces under field conditions at two selected sites in Jimma zone. Mean knockdown and mortality rates of An. gambiae s.l. exposed to different wall surfaces sprayed with the different candidate insecticide formulations were determined from April to August 2014. The results of the study showed that at week one, the highest mortality rates of mosquitoes exposed to painted surface sprayed with labdacyaholtherin 10% WP and deltametherin 25% WG were 89.3% and 88%, respectively while the lowest mortality rates of mosquitoes exposed to non-plastered and plastered surfaces sprayed with labdacyaholtherin 10% CS were 72% and 68.6%, respectively. There was significant difference in mean knockdown and mortality rates of populations An. gambiae s.l. along time of test and insecticide formulations ($p < 0.05$.). Moreover, there was significant difference in mean knockdown rates of An. gambiae s.l. among wall surface types. There was no significant difference in mean mortality rates of An. gambiae s.l. among the three different wall surfaces ($P > 0.05$). In conclusion, populations of An. gambiae s.l. showed resistance against the three candidate insecticide formulations.
Keywords: efficacy, insecticide, knockdown, mortality, mosquitoes and residual

INTRODUCTION

Malaria spread from one person to another by female mosquitoes of the genus Anopheles. There are about 400 different species of Anopheles mosquitoes, but only 30 – 40 of these are major importance (CDC, 2006). In 2013, 123 million people were protected from malaria by IRS around the world. In Africa, 55 million people, or 7% of the population at risk, lived in households that were regularly sprayed (WHO, 2014).

To realize the full potential of IRS as a control tool, there is need to evaluate the effect of different surfaces on the availability of newer pyrethroid insecticides on sprayable surfaces in malaria vector control (Hemingway and Ranson, 2000). According to WHO (2011a) report, all recommended LLINs were treated with pyrethroids. From the points of view of both safety and effectiveness, pyrethroids are the best insecticides ever developed for public health use. The reliance of modern malaria control on pyrethroids and the increasing resistance of malaria vectors to these products put resent global efforts at risk. Determination of residual activity of insecticides is the essential information for the use of indoor spraying operation. The residual duration of pyrethroids recommended by WHO including alphacypermethrin, bifenthrin, cyfluthrin, deltamethrin, etofenprox, and lambdacyhalothrin WP, have estimated between two and six months (Najera & Zaim, 2001).

IRS carried out correctly, is a powerful intervention to rapidly reduce adult mosquito vector density and longevity and, therefore, to reduce malaria transmission. The effectiveness of IRS as a malaria control intervention arises from the fact that many important malaria vectors are endophilic (GMAP, 2008). That is, when searching for blood meals they enter human habitations or animal shelters where they rest on the walls, ceilings and other interior surfaces before and/or after feeding on the inhabitants. When a vector comes into contact with a sprayed surface, it absorbs lethal doses of insecticide, thereby reducing its lifespan. This results in a progressive decline in vector density and longevity, especially among older female mosquitoes, reduces overall vectorial capacity and contributes to a reduction in malaria transmission. IRS is most effective against indoor feeding (endophagic) and indoor resting (endophilic) vectors (WHO, 2013a). However, there are overwhelming growing evidences of insecticide resistance of vectors against pyrethroid insecticides across Africa. Given their application in LLINs and IRS (Ranson *et al*., 2011 and Coleman *et al*., 2006), the resistance to pyrethroid may compromise malaria control as LLINs may lose efficacy, although at present there are no studies linking

insecticide resistance to LLIN control failure. Thus, the objectives of this study was undertaken to evaluate the efficacy and residual effect of three candidate insecticide formulations (Pali 250WG (deltamethrin 25% WG), Revival 100WP, (lambdacyhalothrin 10% WP) and Revival 100CS, (lambdacyhalothrin 10% CS)) against field populations' of *An. gambiae* s.l. mosquitoes on different indoor wall surfaces in order to guide future interventions. And to assess the residual activity of a candidate insecticide formulations, Pali 250WG (deltamethrin 25% WG), Revival 100WP (lambdacyhalothrin 10% WP) and Revival 100CS (lambdacyhalothrin 10% CS) against field populations of *An. gambiae s.l.*

MATERIALS AND METHODS

Description of the Study Areas

Wall bioassay test of field population of *An. gambae s.l.* was assessed using WHO cone bioassay tests to deltamethrin 25%WG, lambdacyhalotherin 10%WP and lambdacyhalotherin 10%CS, in Jimma, Ethiopia from April to August 2014. Their mode of action is non-systemic insecticides with contact and stomach action. The two study sites were Kersa district (Gelo Keble) and Jimma town (Becho-Bore Keble) with an altitude of ranging from 1714m-1748m and1710m-1748m a.s.l respectively Jimma zone, Oromia Regional State, Southwestern Ethiopia (Fig 1). Other factors considered were accessibility, severe and more frequent malaria epidemics, the density and availability of the principal vector *An. gambae s.l.* and outbreaks had been reported previously. Also, the local malaria vector, *An. gambiae s.l.* is known to feed and rest indoors and therefore more susceptible to IRS control strategy that was planned to take place.

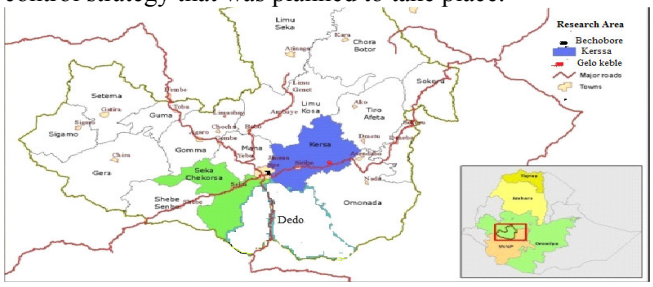

Figure 1. Map showing study sites

Study Design

The design of the study for efficacy evaluation was completely randomized while, longitudinal for residual evaluation.

Collection and rearing of field populations of *An. gambiae s.l.*

Anopheles gambiae s.l. larva and pupa were collected by dipping from a range of breeding sites (road paddies, brick pits, pools, marshes, surface water harvests mainly from Becho-Bore Kebele of Jimma town. The collection sites were determined based on: a) anticipated high vector densities to allow collection of sufficient numbers for assays, b) based on previous susceptibility assays and/or historical use of insecticides, and c) ease of access to facilitate collections.

Larvae and pupae were collected from different breeding sites using dippers and then transported to the field insectary. They were kept on larval tray for rearing and the pupae were collected using pipette and put in a beaker inside the cage to develop to adult. Larvae were provided with bakery yeast to be reared to adults under standard conditions of temperature and relative humidity. Non-blood fed adult females of 2 – 5 days old were used for bioassays. The bioassays were carried out within marked areas on the wall of selected houses to assess the persistence of the residual activity on various sprayed wall surfaces. The inhabitants were informed not to alter the marked areas by re-plastering, or painting.

Household and wall surface selection

Five houses with their wall made of mud but not plastered (up), five houses with their wall made of mud and plastered (p) and five houses with their wall made of mud, plastered and painted were randomly selected and coded from Becho-Bore Kebele. Similarly five houses with their wall made of mud but not plastered and five houses with their wall made of mud and plastered were selected and coded randomly from Gelo Kebele. Four separate plots of wall surfaces marked and labeled with the name of insecticides (WG for PALI 250WG, WP for REVIVAL 100WP, CS for REVIVAL 100CS and unsprayed to be used as control) in each house. Three WHO insecticide wall bioassay cones were fixed in each plots of wall surfaces at height of 0.5m, 1m, and 1.5 respectively from the ground.

Application of insecticide formulations on wall surfaces

Peoples living in the houses were informed to remain outside for three hours before re entering the treated

houses. Those formulations were applied using hand held compressor sprayer fitted with nozzle suitable for indoor residual application. For bio-efficacy and persistence evaluation of the candidate insecticide formulations, walls of the living room in each of the selected houses were sprayed with candidate insecticide formulations to make a homogenous residual deposit of the desired concentration. Bio-efficacy of IRS was assessed one week after treatment and then every month for the three months of the trial period.

Ten (2-5 day) aged non-blood fed female mosquitoes were introduced into conical chambers of transparent plastic for an exposure period of 30 minutes. Knockdown was counted and recorded for each respective cone after 30 minutes. After exposure mosquitoes were transferred in to 150-300ml size paper cups covered with nylon net fastened with rubber band; provided 10% sugar solution soaked in cotton wool placed on the nylon net provided and transported to the insectary room. The insectary room was maintained under standard conditions of temperature and relative humidity at (27 °C ± 2 °C and 80% ± 10% RH). Mosquitoes' mortality was recorded 24hrs post exposure for each type of wall surface and insecticides (WHO, 2006).

Concurrently similar number of An. *gambiae s.l.* was used for all three types of insecticide formulations and control cones. The efficacy and residual activity of the three candidate insecticide formulations were monitored for three months. A total of 120 mosquitos were used per house per unit time. Total of 25 houses (10 house from Gelo Kebele and 15 houses from Becho-Bore Kebele) were selected for the trial. Thus, a total of 3000 (2-5 day old) female mosquitoes (25*120) were used in each round of the trial and grand total of 12,000 female anopheles mosquitoes were used for four round (first week, month one , month two and month three) experiment.

Mean percentage knockdown and mortality were computed for each treatment. Knockdown was calculated from the percentage of mosquitoes lying on their back or side. Mortality was calculated from the percentage of mosquitoes die out (WHO, 2006).

DATA ANALYSIS

Data were analyzed using SPSS software package for windows version 20.0 and Excel MS 2007. To determine whether IRS was effective knockdown and mortality rates of populations of *An. gambiae s.l.* were calculated (WHO, 1998). Treatment was considered effective when knockdown and mortality rates of mosquitoes on exposed wall surfaces were greater than 95% and 85%, respectively. Mean knockdown and mortality rates of *An. gambiae s.l.* were compared among different wall surfaces, height of walls, and time of test and treatments using Analysis of variance (ANOVA), for significant ANOVA post hoc was checked for mean separation. $P < 0.05$ and 95% CI were considered significant during the analysis. T-test was used to compare mean knockdown and mortality rates of *An. gambiae s.l.* between the two sites. Abbott's formula was used to correct mortality rates of *An. gambiae s.l.* when mean mortality on control wall surface was between 5-20% (WHO, 2006).

Ethical Considerations

This study was reviewed and approved by the Research and Ethics Committee of College of Natural Science, Jimma University. To conduct this study, the purpose was also explained and communicated to District and Kebele officials through official letters from Jimma University and oral and written consent were obtained from head of selected households (HHs) before the study.

RESULTS

When comparing the two wall surfaces (non-plastered and plastered), the mean knockdown rates of populations of *An. gambiae s.l.* were always below 3%. The maximum mean knockdown of *An. gambiae s.l.* recorded was 2.6% for week one and month one on plastered, and also on painted-and non-plastered wall surfaces at month three (Fig. 2). For plastered wall surfaces, the maximum mean knockdown rates of populations of An. gambiae s.l. was 1.33% for week one, month one and month three in which there was no knockdown for month two. On the non-plastered wall surfaces, the maximum observed mean knockdown rate of An. gambiae s.l. was 1.67% for month three. There was no knockdown of populations of *An. gambiae s.l.* at month one and month two.

Mean knockdown rates of field populations of *An. gambiae s.l.* after exposure to sprayed wall surfaces of painted, non-plastered and plastered was presented in (Fig. 2). Different patterns of mean knockdown rates of *An. gambiae s.l.* were recorded among the three wall surfaces, by insecticide formulations, site and duration of the spray deposit (Fig. 2a). The highest mean knockdown rate of *An. gambiae s.l.* was observed on painted wall surfaces (Fig. 2b). Mean knockdown rates of *An. gambiae s.l.* exposed to painted wall surfaces sprayed with lambdacyaholtherin 100WP, deltametherin 250WG and lambdacyaholtherin 100CS after one week was 71.33%, 58% and 46.0%, respectively. The lowest mean knockdown rate of *An. gambiae s.l.* recorded for non-plastered wall surfaces sprayed with lambdacyaholtherin 100WP and deltametherin 250WG after three months was 11.3%. There was significant difference of mean knockdown rate of *An. gambiae s.l.* when the three candidate insecticide formulations (lambdacyaholtherin 10% WP, lambdacyaholtherin 10% CS and deltametherin 25% WG) sprayed on three types of wall surfaces (Table 3). Post spray mean knockdown rates of mosquitoes for

lambdacyaholtherin 10% WP after a week on painted, plastered and non plastered wall surfaces was 71.33%, 28.68% and 33.67%, respectively. Mean knockdown rates of *An. gambiae s.l.* on painted, plastered and non plastered wall surfaces after a week sprayed with deltametherin 25% WG was 58%, 26.67% and 30.67%, respectively. While mean knockdown rates of *An. gambiae s.l.* for week one on painted, plastered and non plastered wall surfaces sprayed with lambdacyaholtherin 10% CS was 46%, 23.33% and 27.67%, respectively.

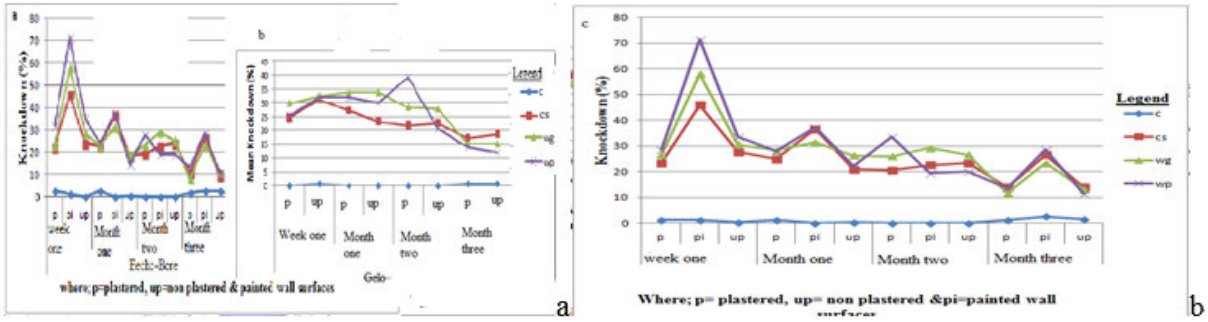

Figure 2. Mean knockdown rates (%) of field populations of *An. gambiae s.l.* exposed to different wall surfaces sprayed with candidate insecticides and on control wall surfaces

Mortality rates of mosquitoes

As showed in Figure 3, mean mortality rates of the field populations of *An. gambiae s.l.* exposed to three control types of wall surfaces (painted, non-plastered and plastered). The mean mortality rates of *An. gambiae s.l.* on non sprayed wall surfaces was recorded on the three wall surfaces, the maximum value was 9.3% on non plastered and 4.0% on plastered wall surfaces during month one and month three respectively at Becho-Bore site. The mean mortality rates of *An. gambiae s.l.* during the study time were below 5.0%. No mortality of *An. gambiae s.l.* effect was observed after exposure of mosquitoes to control wall surfaces except month one at Becho-Bore site on non plastered wall surface which was 9.3%.

The mean mortality rate of *An. gambiae s.l.* for month one, on non plastered wall surface sprayed with labdacyaholtherin 10% CS, deltametherin 25% WG & labdacyaholtherin 10% WP insecticide formulations and on control wall surface was 60%, 58%, 52% & 9.3%, respectively.The corrected mean mortality rate of *An. gambiae s.l.* for lambdacyaholtherin 10% CS, deltametherin 25% WG & lambdacyaholtherin 10% WP insecticide formulations was 55.9%, 53.75 & 47.1%, respectively.

The effects of the three candidate insecticide formulations on mean knockdown and mortality rates of *An. gamiae s.l.* exposed to sprayed wall surfaces were assessed over three months. The mean mortality rates of *An. gambiae s.l.* exposed to the sprayed wall surfaces remained low throughout the trial period (week one, month one, month two and month three) (Fig. 3b). The highest mortality rates of *An. gambiae s.l.* sprayed with lambdacyaholtherin 10% WP on painted, plastered and non plastered wall surfaces during week one was 85.33%, 83.33% and 81.0%, respectively. Mean mortality rates of *An. gambiae s.l.* on painted, non plastered and plastered wall surfaces sprayed with deltametherin 25% WG during week one was 88%, 77.67% and 76.33%, respectively during week one. The mean mortality rates of *An. gambiae s.l.* sprayed with lambdacyaholtherin 10% CS was below 85.0% irrespective of time of test and wall surface types. The highest mean mortality rates of *An. gambiae s.l.* on painted, non-plastered and plastered wall surfaces during week one was 82.0%, 74.0% &72.67%, respectively and declined from week one to month three. Mean mortality rate of *An. gambiae s.l.* on painted wall surfaces at month three for all sprayed the three candidate insecticide formulations was 48%.The lowest mean mortality rate of *An. gambiae s.l.* mosquitoes recorded was 42.66% on plastered wall surface sprayed with deltametherin 25% WG at month three. There was no significant difference observed on mean mortality rates of *An. gambiae s.l.* exposed to plastered, non plastered and painted sprayed wall surfaces ($P > 0.05$).

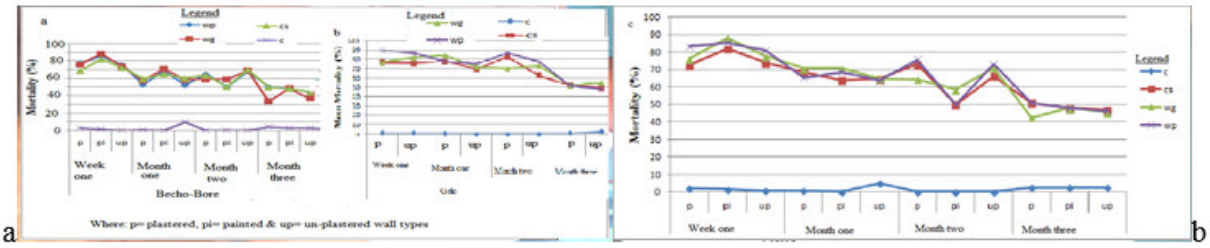

Figure 3. Mean mortality rates (%) of field population of *An. gambiae s.l.* exposed to wall surfaces sprayed with candidate insecticides and on control wall surfaces.

Analysis of variance (ANOVA) revealed that there was a significant difference in mean knockdown and mortality rates of populations of *An.gambiae s.l.* among time of test and treatments ($p < 0.05$). There was also significant difference in mean mortality rates of *An.gambiae s.l.* exposed to wall surface sprayed with lambdacyaholtherin 100CS for both lambdacyaholtherin 100WP and deltametherin 250WG. However, mean mortality rates of *An. gambiae s.l* exposed to wall surfaces sprayed with lambdacyaholtherin 100WP and deltametherin 250WG were similar ($p > 0.05$).

The residual efficacy of the candidate insecticide formulations were varied between porous (plastered and non-plastered) and non porous (painted) sprayed wall surfaces. At week one, mean mortality rates of *An. gambiae s.l.* exposed to plastered, non plastered and painted wall surfaces sprayed with deltamethrin 25%WG was 76.33%, 77.67% and 88.0%, respectively. Observed mean mortality rates of *An. gambiae s.l.* exposed to plastered, non plastered and painted wall surfaces sprayed with lambdacyaholtherin 10% WP during week one was 83.33%, 81.0% and 85.33%, respectively. While observed mean mortality rates of *An. gambiae s.l.* exposed to plastered, non plastered and painted wall surfaces sprayed with lambdacyaholtherin 10% CS during week one was 83.33%, 81.0% and 85.33%, respectively.

At month one observed mean mortality rates of *An. gambiae s.l.* exposed to plastered, non plastered and painted wall surfaces sprayed with deltamethrin 25%WG was 68.67%, 64.67% and 64.0%, respectively. Mean mortality rates of *An. gambiae s.l.* on plastered, non-plastered and painted wall surfaces sprayed with lambdacyaholtherin 10% WP during month one was 70.67%, 65.0% and 70.67%, respectively. While during this time observed mean mortality rate of populations of *An. gambiae s.l.* exposed to plastered, non-plastered and painted wall surfaces sprayed with lambdacyaholtherin 10% CS was 65.33%, 63.67% and 68.67%, respectively. Some of observed mean mortality rates of *An. gambiae s.l.* above 85% was on painted wall surfaces during week one. Mean mortality rates of *An. gambiae s.l.* exposed to painted wall surface sprayed with lambdacyaholtherin 10% WP and deltametherin 25% WG insecticide formulations was 85.33% and 88.0%, respectively at Becho-Bore and 89.3% on plastered and 86.6% on non-plastered wall surfaces sprayed with lambdacyaholtherin 10% WP insecticide formulation at Gelo site.

Mean knockdown and mean mortality rates of *An. gambiae s.l.* were compared among wall surface types, height of walls, time of test and treatments using analysis of variance (ANOVA). Those having significant difference test of $p < 0.05$, post hoc tests was done to assess the efficacy of the given candidate insecticide formulations (Table 1). ANOVA reveals that the mean knockdown and mean mortality of *An. gambiae s.l.* among those factors such as time of test and treatments there was significant difference ($P < 0.05$). For wall surface types, mean knockdown of *An. gambiae s.l.* was significantly difference ($p < 0.05$) while mean mortality rates of *An. gambiae s.l.* there was no significant difference ($p > 0.05$). For height of test there was no significant difference ($p > 0.05$). Post hoc tests of multiple comparisons among treatments based on observed means of knockdown and mortality rates of *An. gambiae s.l.* the control was significantly difference than the three candidate insecticide formulations. Lambdacyaholtherin 100CS insecticide formulation was also significantly different from both lambdacyaholtherin 100WP and deltametherin 250WG. While there was no significant different between lambdacyaholtherin 100WP and deltametherin 250WG insecticide formulations ($p > 0.05$).

Table 1 shows significant test of mean knockdown and mortality percentage rates of populations of *An. gambiae s.l.* the interaction among the independent variables. For mean knockdown rates of *An. gambiae s.l.* there was no significant difference between the two sites ($p = 0.758$). However, there was significant difference in mean mortality rates of *An. gambiae s.l.* between the two sites ($p = 0.000$).

Table 1: Summary of GLM multivariate analysis of variance of mean knockdown and mortality (%) rates of *An. gambiae s.l.*

Variables	Dependent Variable	Df	Mean Square	F test	p-value
Site	Knockdown	1	29.751	0.095	0.758
	Mortality	1	207.204	15.360	0.000*
Time	Mortality	3	26375.139	160.272	0.000*
	Knockdown	3	11458.108	82.160	0.000*
Treatments	Mortality	3	271020.556	1646.897	0.000*
	Knockdown	3	47683.941	341.918	0.000*
Wall type	Mortality	2	377.250	2.292	0.102
	Knockdown	2	9241.312	66.265	0.000*
Height	Mortality	2	250.833	1.524	0.218
	Knockdown	2	222.656	1.597	0.203
time * Treatments	Mortality	9	3501.065	21.275	0.000*
	Knockdown	9	1834.913	13.157	0.000*
time * Wall type	Mortality	6	1603.250	9.742	0.000*
	Knockdown	6	2538.368	18.201	0.000*
time * height	Mortality	6	89.306	0.543	0.776
	Knockdown	6	75.712	0.543	0.776
Treatments * Wall type	Mortality	6	383.028	2.328	0.031*
	Knockdown	6	1213.590	8.702	0.000*
Treatments * height	Mortality	6	433.056	2.632	0.015*
	Knockdown	6	79.462	0.570	0.755
Wall type * height	Mortality	4	195.250	1.186	0.315
	Knockdown	4	89.875	0.644	0.631
time * Treatments * Wall type	Mortality	18	358.806	2.180	0.003*
	Knockdown	18	554.350	3.975	0.000*
time * Treatments * height	Mortality	18	281.898	1.713	0.032*
	Knockdown	18	61.684	0.442	0.979

* Significant at $p < 0.05$

Table 2 indicates mean separation of knockdown and mortality of populations of *An. gambiae s.l.* (post hoc tests) among treatments. Based on mean separation of mortality rates of *An. gambiae s.l.*, there was no significant difference between deltamethrin 250WG and lambdacyhalotherin 100WP and deltamethrin 250WG and lambdacyhalotherin 100CS. While there was significant difference between lambdacyhalotherin 100WP and lambdacyhalotherin 100CS. The mean mortality of *An. gambiae s.l.* exposed to wall surfaces sprayed with deltamethrin 250WG, lambdacyhalotherin 100WP and with lambdacyhalotherin 100CS insecticide formulations out of the exposed ten mosquitoes was 6.45, 6.65 and 6.36, respectively. Based on mean separation of knockdown of *An. gambiae s.l.*, there was no significant difference between deltamethrin 250WG and lambdacyhalotherin 100WP. While there was significant difference between lambdacyhalotherin100CS and deltamethrin 250WG and lambdacyhalotherin 100CS and lambdacyhalotherin 100WP insecticide formulations. The mean knockdown of *An. gambiae s.l.* exposed to wall surfaces sprayed with deltamethrin 250WG, lambdacyhalotherin 100WP and lambdacyhalotherin 100CS out of the exposed ten mosquitoes was 2.6, 2.7 and 2.4, respectively.

Table 2: Post hoc test result for multiple comparison of mean of knockdown and mortality rates of populations of *An. gambiae s.l.* by treatments (LSD).

Dependent Variable	Treatments	Mean	Mean ± SE	95% CI
Knockdown	Lambdacyhalotherin 100WP	27.0^{a}	27.0 ± 2.0	(25.0, 29.0)
	Deltamethrin 250WG	26.1^{a}	26.1 ± 1.9	(24.1, 28.0)
	Lambdacyhalotherin 100CS	23.5^{b}	23.5 ± 1.6	(21.9, 25.1)
	Control (C)	0.8^{c}	0.8 ± 2.3	(-1.4, 3.1)
Mortality	Lambdacyhalotherin 100WP	64.7^{a}	64.7 ± 2.7	(62.0, 67.4)
	Deltamethrin 250WG	64.6^{ab}	64.6 ± 2.2	(62.4, 66.8)
	Lambdacyhalotherin 100CS	62.0^{b}	62.0 ± 2.3	(59.7.0, 64.3)
	Control (C)	1.5^{c}	1.5 ± 2.5	(-0.1, 4.0)

Means with the same letter(s) in the same column are not significantly different from each other at $P < 0.05$

Table 3 indicates mean separation of knockdown and mortality of populations of *An.gambiae s.l.* (post hoc tests) among residual time. Except between month one and month two, there is significant difference among

the residual times ($p < 0.05$).

Table 3: Post hoc test result for multiple comparisons of mean of knockdown (%) and mortality (%) rates of populations of *An. gambiae s.l.* by time of test (LCD).

Dependent Variable	Time of tests	Mean	Mean ± SE	95% CI
Knockdown	Week one	26.07[a]	26.07 ± 2.25	(23.82, 28.32)
	Month one	20.53[b]	20.53 ± 2.25	(18.28, 22.78)
	Month two	18.60[b]	18.60 ± 2.25	(16.35, 20.85)
	Month three	12.17[c]	12.17 ± 2.25	(9.92, 14.42)
Mortality	Week one	59.60[a]	59.60 ± 2.46	(57.12, 62.08)
	Month one	50.50[b]	50.50 ± 2.48	(48.02, 52.98)
	Month two	50.27[b]	50.27 ± 2.48	(47.79, 52.75)
	Month three	36.23[c]	36.23 ± 2.48	(33.75, 38.71)

Means with the same letter(s) in the same column are not significantly different from each other at $P < 0.05$.

Mean separation of knockdown of population of *An. gambiae s.l.* (post hoc tests) among wall surface types, there was no significant difference between plastered and non plastered wall surfaces ($p > 0.05$). However, there was significant difference between absorbent and non absorbent wall surfaces ($p < 0.05$). The mean knockdown rates of population of *An. gambiae s.l.* exposed to wall surfaces sprayed with all the three candidate insecticide formulations starting from week one to third month were below 95% and the mean mortality rates of population of *An. gambiae s.l.* was below 85% except week one on pained wall surface sprayed with deltametherin 25% WG and lambdacyhalothrin 10% WP insecticide formulations. Both mean knockdown and mean mortality rates of population of *An. gambiae s.l.* were decaling from the first test time to third month.

DISCUSSION

The World Health Organization (WHO) recommends 12 insecticides in four classes (organochlorines, organophosphates, carbamates and pyrethroids) for indoor residual spraying (IRS) at specific doses (Najera & Zaim, 2002). These however differ in their residual life when sprayed on different wall surfaces. The effectiveness of insecticide depends on a complex set of factors. These include intrinsic toxicity, mode of action and stability and its effect on the vector (Najera *et al*., 1998).

The findings of this study revealed that there was possibility of resistant of populations of *An. gambiae s.l.* during week one exposed to all the three wall surfaces sprayed with labdacyaholtherin 10% WP insecticide formulation and on painted wall surface sprayed with deltametherin 25% WG and labdacyaholtherin 10% CS insecticide formulations having mean mortality rates of *An. gambiae s.l.* between 80% – 97% % (WHO, 2006). And for the rest residual time the mean mortality rates of *An. gambiae s.l.* exposed to wall surfaces sprayed with the three candidate insecticide formulations (deltametherin 25% WG, labdacyaholtherin 10% WP and labdacyaholtherin 10% CS) was below 80%. This shows the resistance occurrence of field population of *An. gambiae s.l.* to the corresponding insecticide formulations in the study sites; mean mortality rates of *An. gambiae s.l.* 25.7% for lambdacyhalothrin and 8% for deltametherin have been reported at Omo Nada (Asendabo) district of Jimma zone (PMI-AIRS, 2013).

The residual lifespan of IRS insecticide formulations is of key importance. Based on the mean separation of both knockdown and mortality rates of *An. gambiae s.l.* among residual time there was significance difference except between month one and month two. The mean mortality rates of *An. gambiae s.l.* for week one was 5.96 (95% CI, 5.712, 6.208); month one was 5.05 (95% CI, 4.802, 5.298); month two was 5.03 (95% CI, 4.779, 5.275) and month three was 3.62 (95% CI, 3.375, 3.871). Similar study by Okumu *et al*. (2012) showed that activity of the IRS declined significantly within two months.

Based on observed mean mortality rates of *An. gambiae s.l.* there was no significant when the insecticide formulations were sprayed on plastered, non-plastered and painted wall surfaces this clearly showed that the residual efficacy of the three candidate insecticide formulations was similar. This could be attributed to the strong resistance of the local *An. gambiae s.l.* population against pyrethroids. The mortality rates on the different sprayed wall surfaces remain ineffective in killing field populations of *An. gambiae s.l.* in week one, month one, month two and month three (Table 5). The mortality rates of *An. gambiae s.l.* on the different types of sprayed wall surfaces were low on painted, non-plastered and plastered (Fig. 3). This finding is consistent with the findings of a study by Yewhalaw *et al*. (2011) who reported the existence of multiple insecticide resistance in populations of *An. gambiae s.l.* in the study sites. The resistance levels of population of *An. gambiae s.l.* to the pyrethroids varied greatly across candidate insecticide formulations and time of test. The resistance levels to the pyrethroids varied greatly from susceptibility to resistance across treatments and time of test. Pyrethroid used in Africa for IRS and LLINs has increased greatly between 2002 - 2009 (Berg *et al*., 2012) and has probably accelerated the development and spread of pyrethroid resistance (Ranson *et al*., 2011and Czeher *et al*., 2008). Concurrent use of pyrethroids for indoor residual spraying and LLINs could increase the

pressure for resistance development in vector populations (WHO, 2011b). There are concerns that increasing pyrethroid (deltamethrin) resistance will reduce effectiveness of both IRS and LLINs (PMI, 2014a). In 2009, 19 countries in the African region reported using pyrethroids for indoor residual spraying against malaria. These countries included Ethiopia, Kenya, Liberia, Madagascar, Mali, Mozambique, Nigeria, Rwanda, Senegal, Tanzania, and Uganda, all of which have high coverage rates of LLINs for malaria control (WHO, 2010).

Insecticide resistance is a major impediment in malaria vector control. There was rapidly spread of pyrethroid resistance in the past decade throughout Sub-Saharan Africa (PMI, 2014b). Anopheles mosquito resistance to insecticides has been detected in 64 countries with on-going malaria transmission, affecting all major vector species and all classes of insecticides (GPIRM, 2012). Current vector control tools remain effective; however, if left unchecked, insecticide resistance could lead to a substantial increase in malaria incidence and mortality. The global malaria community needs to take coordinated action to prevent insecticide resistance from emerging at new sites, and to urgently address it at the sites where it has been identified (WHO, 2013b). *An. gambiae s.l.* was resistant to deltamethrin in Jimma and other project sites of African indoor residual spraying project (AIRS) in Ethiopia (PMI-AIRS, 2013). Moreover, populations of *An. arabiensis* developed resistance to permethrin, deltamethrin and lambdacyhalothrin (Yewhalaw *et al*., 2014). Another study conducted by Massebo *et al*. (2013) around southern Ethiopia also showed that populations of *An. arabiensis* were resistant to lambdacyhalothrin, cyfluthrin, alphacypermethrin and deltamethrin. A similar study conducted by Abate & Hadis (2011) in northern, northwestern, central and southern Ethiopia confirmed the development of high level pyrethroid resistance in populations of *An. gambiae s.l.*

There are increasing reports of malaria vectors that have developed resistance to the pyrethroids commonly used in LLINs and pyrethroid resistance is now firmly established throughout Africa (Ranson *et al*., 2011 and Coleman *et al*., 2006). This resistance to pyrethroids may compromise malaria control as LLINs may lose efficacy, although at present there are no studies linking insecticide resistance to LLIN control failure. *An. arabiensis* is the primary malaria vector species in the southwest of Ethiopia, and is the only vector species of the *An. gambiae* complex found in Jimma, Tiro-Afeta, Omo-Nada and Kerssa districts. Studies done within these areas indicate that populations of *An. Arabiensis* were resistant to DDT, permethrin, deltamethrin, malathion (Yewhalaw *et al*., 2011 and 2010).

Generally the findings of this study revealed that there was resistance of *An. gambiae s.l.* populations to the three candidate insecticide formulations (deltametherin 25% WG, labdacyaholtherin 10% WP and labdacyaholtherin 10% CS) with percentage mean mortality rate of below 80%. Recently, global malaria-control efforts rely heavily on a single class of insecticide the pyrethroids both for IRS and to treat bed nets. This class of insecticide is used in most IRS programmes, and it is the only insecticide used in WHO-recommended LLINs. However, increasing resistance of malaria vectors to pyrethroids and to other insecticides may jeopardize global malaria control efforts (WHO, 2013b). Recognizing the threat posed by insecticide resistance, WHO released the Global Plan for Insecticide Resistance Management in malaria vectors (GPIRM, 2012). The residual life span and efficacy of most insecticides are affected by the chemical nature of the sprayed surface (Ansari *et al*., 1997). Therefore, the residual efficacy and the persistence of insecticide may vary on different types of surface. Currently insecticide resistance is the most critical challenge facing global malaria vector control efforts, and is central to the planning and implementation of an effective IRS programme. As outlined in the GPIRM, the insecticide resistance status of the local vectors must be determined before selecting the insecticides to be used in the IRS programme (WHO, 2013c). Pates and Curtis (2005), suggest that IRS is effective if the mosquito species concerned is endophilic and rests on the insecticide-treated surfaces for a sufficient time to pick up a lethal dose.

Conclusion and Recommendations

In view of the results, the evaluation of residual effects of the three candidate insecticide formulations (lambdacyhalothrin 10% WP, deltametherin 25% WG and lambdacyhalothrin 10% CS) on different wall surfaces (painted, plastered and non-plastered wall surfaces) had established a baseline set of data that can be used to show the occurrence of resistance of populations of *An. gambae s.l.* against those insecticide formulations before using by the national malaria control program for IRS in the study area. And to establish the efficacy of insecticide formulations at the selected application rates against the target vector species, before applying to all or most households in the community. Knowing how long a residual insecticide will last is important information for vector control, since it indicates the minimum interval between spraying to maintain the resistance of the insecticide. Any insecticide formulations to be used for IRS should be tested in real use conditions at community level so that the results would guide the decision makers on the spray cycles. In the presence of the resistant *An. gambiae s.l.* populations in the study areas alternative new vector control tools should be used and an insecticide resistance management strategy plan should be developed and implemented. The IRS program to be effective against malaria control it is better to determine the dosage by studying at the application areas rather than somewhere else studied.

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