

Susceptibility of *Anopheles gambiae* s.l. and Residual Efficacy of Klypson 500WG and 2GARD-WP Under Operational IRS Conditions in Ethiopia

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Abstract

Background

Insecticide susceptibility and residual activity determine the effectiveness of indoor residual spraying (IRS) for malaria control. Clothianidin-based insecticides have shown prolonged efficacy in experimental huts and limited field trials, but evidence on Klypson 500WG and 2GARD-WP is lacking. In this study, we evaluated the susceptibility of *Anopheles gambiae* s.l. to deltamethrin, pirimiphos-methyl, and clothianidin, and assessed the residual efficacy of Klypson 500WG and 2GARD-WP alongside SumiShield 50WG in Ethiopia.

Methods

The susceptibility of wild *Anopheles gambiae* s.l. was tested against clothianidin, pirimiphos-methyl, and deltamethrin following WHO protocols. The residual efficacy of Klypson 500WG, 2GARD-WP, and SumiShield 50WG was assessed monthly on mud and painted surfaces using cone bioassays against *An. arabiensis*, with mortality recorded between 24 to 120 hours after exposure. Results were interpreted based on WHO thresholds. Logistic regression models determined the effective residual lifespan of the insecticides, while mixed-effect logistic regression identified predictors of IRS performance.

Results

Anopheles gambiae s.l. populations were fully susceptible to clothianidin in most sites, though slightly reduced mortality in Dilla (93.2%) and Werkamba (97.3%) suggests the need for continued vigilance. Resistance to deltamethrin was evident in Mekhoni (77.3%) and Werkamba (64.3%), while pirimiphos-methyl achieved complete mortality in both sites. Klypson 500WG and 2GARD-WP maintained $\geq 80\%$ mortality for 7–10 months on mud walls and up to 10–11 months on painted walls at 120-hour holding time, but only for 2–6 months at shorter holding times (24–72 hours). Regression analysis indicated significantly lower mortality odds for Klypson 500WG and 2GARD-WP compared to SumiShield 50WG, though differences were moderate.

Conclusion

Clothianidin remains effective for *An. gambiae* s.l. control across Ethiopian sites, with reduced mortality in Dilla and Werkamba, suggesting possible early resistance and highlighting the need for continuous monitoring to preserve its effectiveness. Retained susceptibility to pirimiphos-methyl highlights that organophosphates may remain viable alternatives, while deltamethrin resistance highlights potential limitations for ITNs' performance. Klypson 500WG and 2GARD-WP demonstrated prolonged residual efficacy under field conditions, with performance broadly comparable to SumiShield 50WG. These

findings provide operational evidence on Klypson 500WG and 2GARD-WP and underscore the importance of continued monitoring to guide IRS policy and resistance management.

Background

Malaria remains a significant public health concern despite decades of control efforts. In 2023, approximately 249 million malaria cases were reported worldwide, remaining nearly the same as in 2022, with Africa representing the majority [1]. In Ethiopia, malaria transmission continues at high levels, with over 7.3 million cases documented between January and October 2024 [2]. This ongoing burden highlights the importance of implementing effective and long-lasting vector control interventions.

Anopheles arabiensis, a member of the *An. gambiae* complex is the primary malaria vector in Ethiopia, with *An. funestus*, *An. pharoensis*, and *An. nili* serving as secondary vectors [3]. More recently, the invasive vector, *An. stephensi* has been detected across multiple regions [4–7], raising concerns about its contribution to increased transmission. The coexistence of diverse vector species highlights the importance of monitoring insecticide resistance and residual efficacy to sustain malaria control.

Vector control remains central to malaria prevention, with insecticide-treated nets (ITNs) and IRS recognized as the most effective strategies in sub-Saharan Africa [8, 9]. Both ITNs and IRS interventions have averted nearly 78% of malaria cases in the region since 2001 [10]. In Ethiopia, IRS was introduced with DDT in the mid-1960s but discontinued in 2009 due to widespread resistance, leading to a shift to deltamethrin [11]. Subsequent resistance promoted a switch to bendiocarb in 2012 [12]. With increasing resistance to pyrethroids, carbamates, and organophosphates, WHO has pre-qualified clothianidin-based IRS products with a novel mode of action [13]. These insecticides are neonicotinoids: clothianidin (SumiShield 50WG, Sumitomo Chemical Pvt. and Klypson 500WG, Tagros Chemical India PLC) and a mixture of clothianidin and deltamethrin (2GARD-WP, Tagros Chemical India PLC, Fludora Fusion, BAYER) [13–16].

Clothianidin-based IRS products have demonstrated prolonged residual efficacy [17, 18]. In Ethiopia, SumiShield 50WG and Fludora Fusion maintained high mortality for several months in experimental huts [19, 20], and under Ethiopian field conditions, they remained effective for more than four months [21, 22], although evaluations were discontinued before efficacy decline could be determined. Most assessments have therefore been hut-based, with limited field data. Importantly, the residual efficacy of Klypson 500WG and 2GARD-WP remains unknown, despite their active ingredients and dosages being equivalent to SumiShield 50WG and Fludora Fusion, respectively. As formulation characteristics may vary between manufacturers, empirical evaluation is required to confirm their performance under Ethiopian settings.

In addition to residual efficacy, susceptibility monitoring of local vector populations is essential. The widespread emergence of insecticide resistance to pyrethroids, carbamates, and organophosphates in Ethiopia [23, 24] has already necessitated shifts in IRS policy, and continued resistance evolution threatens the durability of current interventions. Regular monitoring of insecticide susceptibility and

residual efficacy is therefore critical to guide resistance management and ensure IRS remains effective to halt transmission.

Therefore, this study evaluates the residual activity of Klypson 500WG and 2GARD-WP alongside Sumishied 50WG, while also assessing the susceptibility of the primary vector to these IRS insecticides. By generating locally relevant evidence, this work aims to strengthen national malaria control strategies and support effective resistance management.

Methods

Study area

The study was conducted from February 2023 to 2024 across six sites in Ethiopia: Batu (Oromia), Dilla (Southern Ethiopia), Harbu (Amhara), Melka-Werer (Afar), Mekhoni, and Werkamba (Tigray) (Fig. 1). Batu is situated in central Ethiopia at 7° 56' N and 38° 42' E, at an elevation of 1,657 meters above sea level (m.a.s.l.), with an annual temperature range of 15–29°C and annual rainfall between 700 and 800 mm [25]. Dilla is located at 6° 25' N, 38° 18' E, at approximately 1,476 m.a.s.l., with an average annual rainfall of 1464 mm [26]. Precipitation here is bimodal, peaking during April-May and September-October [27]. Melka-Werer, at 9° 33' N and 40° 23' E, with an elevation of 723 m.a.s.l., has a semi-arid climate with annual rainfall between 200 and 500 mm [28]. Harbu, situated at 10° 55' N and 39° 47' E, at 1,502 m.a.s.l., is located 355 km northeast of Addis Ababa [29]. Mekhoni, which is 652 km north of Addis Ababa, receives around 724 mm of annual rainfall and experiences lowland temperatures ranging from 19.6 to 23.4°C (below 1500 m.a.s.l.). Werkamba, located 900 km north of Addis Ababa, has an annual rainfall of 500–800 mm [30].

All sites exhibited bimodal malaria transmission, with a major peak in September-November and a minor peak in April-May. *Plasmodium falciparum* is prevalent, followed by *P. vivax* [25, 31, 32]. Insecticide-treated bed nets and occasional indoor residual spraying are the core vector control interventions implemented in the study sites.

Study site selection and design

Districts were purposely selected based on their eligibility for the IRS campaign, malaria transmission intensity, and logistical suitability for conducting bioassays. For the IRS component, three districts: Dilla, Batu, and Melka-Werer were chosen because they meet all inclusion criteria and are suitable for wall bioassays, including safe transportation of live mosquitoes. Within each district, two villages with relatively higher malaria transmission and better accessibility were systematically selected, with the additional criterion that they had not received IRS in the previous 12 months to ensure no residual insecticides. Households were then chosen by lottery, with a preference for different types (mud and painted). Each insecticide treatment was replicated in four houses of each wall type, with one unsprayed house of each type included as a control [33]. For susceptibility assays, we included additional districts:

Harbu, Raya-Azebo, and Werkamba to assess the resistance status of malaria vectors across a broader geographic area, as susceptibility testing is a one-time, less resource-intensive activity compared to monthly IRS bioassays. While several districts met the broader eligibility criteria, our evaluation of IRS was limited to three sites.

Study procedures

Insecticide susceptibility testing

Susceptibility of *An. gambiae* s.l. was assessed following WHO guidelines [34]. Larvae were collected from different breeding sites of the study villages, transported to the local insectary, and reared to adults. During rearing, larvae were provided with Tetramin fish food. When pupated, they were transferred to cages (30 × 30 cm bug dorm). Emerged adults were provided with a 10% sugar solution soaked in clean cotton wool. Non-blood-fed, 2-5-day-old female mosquitoes (n = 25 per replicate) were exposed to deltamethrin and pirimiphos-methyl using WHO tube tests and to clothianidin (4 µL/mg) using WHO bottle bioassays.

Indoor residual spraying (IRS) and decay rate assessment

Treatment insecticides

SumiShield 50WG: Water-dispersible granule with active ingredient clothianidin (50% w/w) sprayed at 300 mg a.i./m² (Sumitomo Chemical Co. Ltd., Japan).

Klypson 500WG: Water-dispersible granule with active ingredient clothianidin (500 g/kg), sprayed at 300 mg/m² (Tagrose Chemicals India Pvt. Ltd., India).

2GARD-WP: Wettable powder with active ingredients 50% clothianidin and 6.25% deltamethrin sprayed at 200 g/m² and 25 mg/m² (Tagrose Chemicals India Pvt. Ltd., India).

IRS application procedures

Before conducting the IRS, spray operators were trained in WHO-recommended procedures [35]. Informed consent was obtained from household heads, who were advised that insecticides would be sprayed on the interior walls of their house. All movable items must be removed outdoors, non-movable items must be covered with plastic sheets, and no re-entry is allowed for at least 2 hours post-spray. Indoor residual spraying (IRS) was conducted by locally recruited, trained spray operators under the supervision of the Ethiopian Public Health Institute (EPHI) and the Ministry of Health (MoH). Hudson® X-pert compression sprayers with a flat nozzle fitted with a constant flow valve (CFV) were calibrated with water before insecticide spray mixing [35]. SumiShield 50WG and Klypson 500WG were sprayed at 300

mg clothianidin active ingredient per square meter, while 2GARD-WP was sprayed at 200 mg clothianidin active ingredient and 25 mg deltamethrin active ingredient per square meter. A distance of 45 cm from the nozzle tip to the surface to be sprayed was maintained during spraying. At this distance, the width of the swath at the point of impact is 75 cm. A 5 cm overlap was maintained between the swaths to make sure that no wall surface was left without insecticide.

Human and environmental safety considerations

Spray operators were provided with personal protection equipment (PPE) and clothing, including gloves, hats, goggles, or clear plastic visors (to protect their faces and eyes when spraying overhead), washable cotton overalls, and field boots. Two sets of protective clothing with different colors were provided for the spray man. Spray operators were also checked for any side effects, including complaints of transitory skin burning or eye watering.

At the time of obtaining informed consent from the participating households, they were educated to take safety precautions to avoid any possible risk during and after the spraying of their houses. On the day of spraying, all family members were again advised by a spray team supervisor to remain out of the rooms during the spray and up to two to three hours after spraying. The adult householders present at the time of spraying were advised to tell and control children and were advised not to allow their children to intentionally touch the sprayed walls for at least one day after spraying, as the walls remained wet for approximately 24 hours. For environmental safety, all empty insecticide sachets collected at the end of each spraying day were punctured to prevent reuse and returned to the central facility for safe disposal in accordance with WHO safety procedures [36].

Residual activity assessment

Bio-efficacy and decay rate of the insecticides on walls were measured using standard WHO cone tests [36] in houses with different wall surfaces, and each surface was replicated four times for each chemical. One unsprayed house for each surface served as a control. The initial cone test was carried out three days after spraying [17, 33]. Thereafter, tests of treated surfaces were performed at monthly intervals up to 11 months. In a single house, three cones were fixed using fine steel pins on the walls at the lower, middle, and upper parts. Cones were not fixed on areas 60 cm above the ground and 60 cm below the roof. Two- to five-day-old unfed susceptible female colonies of *An. arabiensis* from the Ethiopian Public Health Institute (EPHI) and Adama Public Health Research and Referral Laboratory Center were used. Ten mosquitoes were gently transferred into each cone and exposed for 30 minutes. At the end of exposure time, the mosquitoes were transferred into insecticide-free holding paper cups for a 24, 48, 72, 96, and 120-hour holding period and were supplied with a 10% sugar solution. Paper cups were kept in a wooden box covered by a damp towel to create a favorable temperature (27 ± 2) and humidity (70 ± 10) [37]. Mortality counts were taken 24 hours post-exposure, and every 24 hours for 120 hours for each insecticide. Mosquitoes were classified as dead if they were immobile or unable to stand

or fly in a coordinated way. When the average mortality of the controls was between 5% and 20%, test mortality was corrected using Abbott's formula.

Data management and statistical analysis

Data was entered into the standardized forms and subsequently compiled in Excel. The susceptibility test results were analyzed and interpreted based on the WHO susceptibility analysis and interpretation criteria [34]. Cone bioassay mortality was pooled by treatment, and the mean percentage mortality of mosquitoes was calculated for each site, insecticide, and substrate across holding time and evaluation months.

We first conducted descriptive analyses of cone bioassay outcomes to summarize distributions across sites, insecticides, wall types, months post-spraying, bioassay holding times, and cone positions. To assess predictors of IRS performance, we fitted mixed-effect logistic regression models (generalized linear mixed models, GLMMs) with a logit link and binomial distribution. The binary outcome was defined as effective ($\geq 80\%$ mortality) and not effective ($< 80\%$ mortality). Fixed effects included insecticide, wall type, months post-spraying, holding time, and cone positions, while site was specified as a random intercept to account for repeated measures and site-level heterogeneity. Odds ratios (ORs) and 95% confidence intervals (CIs) were derived by exponentiating the log-odds estimates, and effects were considered statistically significant if the 95% CI did not include 1. In addition, logistic regression predictions were used to estimate the number of consecutive months during which the IRS maintained $\geq 80\%$ mortality for each insecticide, wall type, and bioassay holding time. All analyses were conducted in R (version 4.4.3) using RStudio (version 2025.09.2–418).

Results

Susceptibility of *Anopheles gambiae* s.l. to clothianidin, deltamethrin, and pirimiphos-methyl

The susceptibility status of *An. gambiae* s.l. field populations and laboratory colonies of *An. arabiensis* was assessed against diagnostic doses of clothianidin using the WHO bottle assay [38] and deltamethrin and pirimiphos-methyl using the WHO tube bioassays [34]. Results are summarized in Fig. 2. After a 24-hour holding time, exposure to clothianidin 4 $\mu\text{g/mL}$ resulted in 100% mortality of *An. gambiae* s.l. in Batu, Harbu, and Melka-Werer. In contrast, mortality rates in Dilla and Werkamba were slightly lower at 93.15% and 97.33%, respectively. Similarly, deltamethrin (0.05%) induced complete mortality (100%) of *An. gambiae* s.l. in Harbu, Batu, and Dilla. However, reduced mortality was recorded in Mekhoni (77.33%) and Werkamba (64.28%). Pirimiphos-methyl was evaluated only in Mekhoni and Werkamba, where it achieved 100% mortality of *An. gambiae* s.l. Moreover, the Ethiopian Public Health Institute (EPHI) colony of *An. arabiensis* exhibited full susceptibility to both clothianidin and deltamethrin insecticides with 100% mortality at 24-hour post-exposure.

Residual activity of clothianidin-based indoor residual spraying insecticides

Mean monthly mortality rate

Cone bioassays were conducted monthly (0–11 months) post-application on mud and painted walls to evaluate the residual efficacy of Klypson 500WG, 2GARD-WP, and SumiShield 50WG at Dilla, Batu, and Melka-Werer sites. Mortality of *An. arabiensis* was recorded every 24 hours post-exposure up to 120 hours. All the results were Abbott corrected when necessary. The mortality trends for each insecticide and wall type are presented in Fig. 3. All three IRS insecticides demonstrated strong initial efficacy, with mortality consistently exceeding 95% during the first two to three months (M0-M2) across sites and wall types.

Klypson 500WG (clothianidin) showed high mortality (> 95%) during the first three months (M0-M3) across all sites and wall types. Painted walls consistently maintained higher efficacy, with mortality at 96–120 hours remaining close to 100% throughout the mid-study period. On mud walls, however, early mortality (24-hour) declined more rapidly after M4-M5, falling below 40% by M7-M9, and to < 30% by M10-11. Despite a decline in rapid kill, delayed mortality (96–120 hours) remained relatively high (> 70–80%), even in later months, consistent with the slow-acting nature of clothianidin.

2GARD-WP (clothianidin + deltamethrin) similarly achieved near complete mortality (> 95%) during the early months, with painted walls maintaining higher efficacy than mud walls. By M9-M11, mortality at 24 hours had declined to < 50% on mud walls, while delayed mortality (96–120 hours) remained 70–85%, underscoring the persistence of clothianidin activity.

SumiShield 50WG (clothianidin) also achieved consistently high mortality (> 95%) in the first three months (M0-M3), particularly on painted walls. On the mud wall, early mortality declined more rapidly after M4-M5, dropping below 40% by M8-M11. Nonetheless, delayed mortality at 96–120 hours remained above 75–85% across sites, indicating the slow-acting but residual effect of clothianidin.

Across all treatment insecticides, the painted wall consistently sustained higher efficacy than the mud walls, and delayed mortality (96–120 hours) remained high even when early mortality (24–48) declined. These findings revealed the strong initial performance of clothianidin-based IRS formulations and their gradual reduction in achieving rapid mortality over time, while demonstrating sustained residual activity.

Determinants of residual efficacy

We used a mixed-effect logistic regression model to assess the effect of insecticide type, wall type, holding time, bioassay months, and cone position on mosquito mortality, with site included as a random intercept. The model included 17040 observations across the three study sites, and the results are presented in Table 1. The intercept indicated a baseline odds of mortality of 11.47 (95% CI: 6.06–21.73, $p < 0.00$). Compared to SumiShield 50WG, both 2GARD-WP (OR = 0.85, 95% CI: 0.75–0.95, $p = 0.006$) and Klypson 500WG (OR = 0.78, 95% CI: 0.69–0.88, $p < 0.001$) demonstrated significantly reduced odds of *An. arabiensis* mortality. Mud walls showed substantially lower efficacy (OR = 0.38, 95% CI: 0.34–0.42, $p < 0.001$) compared to painted walls. Month effects showed a progressive decline in efficacy over time.

Mortality odds were reduced by M2 (OR = 0.52, 95% CI: 0.37–0.72, $p < 0.001$). By M3 (OR = 0.26, 95% CI: 0.19–0.35, $p < 0.001$) and M4 (OR = 0.10, 95% CI: 0.08 – 0.14, $p < 0.001$), efficacy had dropped sharply with odds ratios approaching zero from M5 onwards ($OR \leq 0.02$, all $p < 0.001$), indicating near complete loss of residual efficacy by M11. In contrast, mortality increased exponentially with post-exposure time, reflecting the delayed effect of neonicotinoid insecticides. Compared to 24 hour, the odds ratio rose sharply at 48 hour (OR = 4.03, 95% CI: 3.44–4.71, $p < 0.001$), 72 hour (OR = 13.73, 95% CI: 11.60–16.26, $p < 0.001$), 96 hr (OR = 90.06, 95% CI: 74.34–109.10, $p < 0.001$), and peaked at 120 hour (OR = 293.97, 95% CI: 236.19–365.89, $p < 0.001$). Furthermore, cone positions did not influence mosquito mortality. Neither the middle (OR = 1.05, 95% CI: 0.93–1.19, $p = 0.397$) nor the upper (OR = 0.92, 95% CI: 0.82–1.04, $p = 0.185$) positions differ from the lower reference.

Table 1

Predictors of IRS effectiveness. Odds ratios (OR) with 95% confidence intervals (CI) and p-values are derived from mixed-effect logistic regression models with site included as a random effect. Odds ratios < 1 indicate reduced odds of IRS effectiveness relative to the reference category, while odds ratios > 1 indicate increased odds of effectiveness. P-values reported as 0.000 are presented as < 0.001.

Predictor	Odds Ratio	95% CI	p-value
(Intercept)	11.47	6.06–21.73	< 0.001
Insecticide2GARD-WP	0.85	0.75–0.95	0.006
InsecticideKlypson_500WG	0.78	0.69–0.88	< 0.001
Wall-type Mud Wall	0.38	0.34–0.42	< 0.001
Month_M1	0.73	0.52–1.02	0.065
Month_M2	0.52	0.37–0.72	< 0.001
Month_M3	0.26	0.19–0.35	< 0.001
Month_M4	0.10	0.08–0.14	< 0.001
Month_M5	0.02	0.01–0.02	< 0.001
Month_M6	0.01	0.01–0.01	< 0.001
Month_M7	0.01	0.00–0.01	< 0.001
Month_M8	0.00	0.00–0.01	< 0.001
Month_M9	0.00	0.00–0.00	< 0.001
Month_M10	0.00	0.00–0.00	< 0.001
Month_M11	0.00	0.00–0.00	< 0.001
Time_48hr	4.03	3.44–4.71	< 0.001
Time_72hr	13.73	11.60–16.26	< 0.001
Time_96hr	90.06	74.34–109.10	< 0.001
Time_120hr	293.97	236.19–365.89	< 0.001
Cone_position Middle	1.05	0.93–1.19	0.397
Cone_positionUpper	0.92	0.82–1.04	0.185

Logistic regression prediction showed that the effective duration of IRS ($\geq 80\%$ mortality) varied by insecticide treatment, wall type, and holding time (Table 2). Painted walls persistently retained longer efficacy than mud walls. At 120-hour post-exposure, all three insecticides (SumiShield 50WG, Klypson 500WG, and 2GARD-WP) maintained effectiveness for up to 9–11 months on painted walls, while mud

walls showed shorter durations of 7–10 months. At shorter holding times (24–72 hours), effective durations were limited to 2–6 months, with SumiShield 50WG generally outperforming Klypson 500WG and 2GARD-WP across sites.

Table 2

Predicted duration of IRS effectiveness ($\geq 80\%$ mortality) for each site, insecticide, wall type, and post-exposure holding time. Values represent the number of consecutive months post-spraying for which the predicted mortality remained $\geq 80\%$. Confidence intervals (95% CI) are shown in parentheses. Predictions were obtained from a mixed-effect logistic regression model with site as a random effect.

Site	Insecticide	Wall type	24hr	48hr	72hr	96hr	120hr
Melka-Werer	SumiShield_50WG	Painted Wall	3 (2–4)	5 (4–5)	5 (5–6)	8 (7–10)	11 (10–11)
		Mud Wall	0 (0–2)	4 (3–4)	5 (4–5)	6 (5–8)	9 (7–10)
	2GARD-WP	Painted Wall	3 (1–4)	4 (4–5)	5 (5–5)	8 (7–9)	10 (9–11)
		Mud Wall	0 (0–2)	3 (2–4)	5 (4–5)	6 (5–7)	9 (7–10)
	Klypson_500WG	Painted Wall	3 (1–3)	4 (4–5)	5 (5–5)	8 (7–9)	10 (9–11)
		Mud Wall	0 (0–1)	3 (2–4)	5 (4–5)	6 (5–7)	8 (7–10)
Batu	SumiShield_50WG	Painted Wall	4 (3–4)	5 (5–5)	6 (5–7)	10 (9–11)	11 (11–12)
		Mud Wall	3 (1–3)	4 (4–5)	5 (5–5)	8 (7–9)	10 (9–11)
	2GARD-WP	Painted Wall	4 (3–4)	5 (5–5)	6 (5–7)	10 (8–11)	11 (11–12)
		Mud Wall	2 (0–3)	4 (4–5)	5 (5–5)	7 (6–9)	10 (9–11)
	Klypson_500WG	Painted Wall	4 (3–4)	5 (4–5)	6 (5–7)	10 (8–10)	11 (10–12)
		Mud Wall	2 (0–3)	4 (3–5)	5 (5–5)	7 (6–9)	10 (9–11)
Dilla	SumiShield_50WG	Painted Wall	2 (0–3)	4 (3–5)	5 (5–5)	7 (6–9)	10 (9–11)
		Mud Wall	0 (0–1)	3 (1–4)	4 (4–5)	5 (5–6)	8 (6–9)
	2GARD-WP	Painted Wall	1 (0–3)	4 (3–5)	5 (5–5)	7 (6–8)	10 (8–10)
		Mud Wall	0 (0–0)	3 (1–3)	4 (3–5)	5 (5–6)	7 (6–9)
	Klypson_500WG	Painted Wall	1 (0–3)	4 (3–4)	5 (4–5)	7 (6–8)	9 (8–10)

Site	Insecticide	Wall type	24hr	48hr	72hr	96hr	120hr
		Mud Wall	0 (0–0)	2 (1–3)	4 (3–5)	5 (5–6)	7 (6–9)

Discussion

The present study provides important insights into the susceptibility status of *Anopheles gambiae* s.l. populations and the residual performance of clothianidin-based indoor residual spraying (IRS) formulations across different sites and wall types. Susceptibility assays revealed complete susceptibility of *An. gambiae* s.l. to deltamethrin within the Great Rift Valley of Ethiopia (Batu and Dilla) and the Harbu sites. High resistance was detected in the northern sites of Raya-Azebo and Werkamba, indicating a clear spatial heterogeneity in pyrethroid susceptibility. This pattern suggests that while pyrethroids may retain operational values in certain areas, their effectiveness is severely compromised in others, such as northern Ethiopia, reflecting localized selection pressure. Notably, the detection of complete susceptibility to deltamethrin contrasts with earlier Ethiopian reports, where *An. gambiae* s.l. was consistently resistant to pyrethroids [23, 24, 39]. Our findings are corroborated by recent genomic studies showing that pyrethroid resistance-conferring mutations, such as L995F and copy number variations in *Cyp450* detoxification genes, occur at higher frequencies in Northern Ethiopia (Werkamba and Raya-Azebo), but lower in the Great Rift Valley, Ethiopia [40]. This genetic evidence aligns with phenotypic resistance observed in Raya-Azebo and Werkamba, while the lower frequency in the Rift Valley explains the unexpected susceptibility. However, whether our current finding of susceptible *An. gambiae* s.l. across the Rift Valley of Ethiopia represents a genuine restoration of susceptibility or temporal fluctuation remains uncertain. This highlights the need for longitudinal monitoring to determine if deltamethrin susceptibility is being re-established in these populations.

In contrast to the variability observed in deltamethrin, both pirimiphos-methyl and clothianidin demonstrated strong efficacy against *An. gambiae* s.l. populations. Pirimiphos-methyl induced full susceptibility in Raya-Azebo and Werkamba, areas where pyrethroid resistance was high, underscoring its continued value as an IRS alternative in Ethiopia. Clothianidin also induced full susceptibility in most sites, with only a slight reduction in mortality observed in Dilla and Werkamba sites. These findings are consistent with previous evaluations from Ethiopia, where pirimiphos-methyl has repeatedly shown high effectiveness against pyrethroid-resistant populations [23, 24, 39], and clothianidin has been reported to maintain sustained susceptibility [39, 41, 42]. The alignment of our findings with these studies highlights the sustained efficacy of organophosphates and neonicotinoids as critical alternatives for resistance management. Importantly, the slight reduction in clothianidin-induced mortality of *An. gambiae* s.l. in Dilla and Werkamba suggests that vigilance is required, as early shifts in susceptibility could signal emerging tolerance. In previous studies, a similar alarming finding has been reported from Cameroon [43]. Continuous monitoring will therefore be essential to extend the effective life span of these insecticides in Ethiopia.

Beyond susceptibility findings, the residual efficacy evaluation of clothianidin-based insecticides offers valuable evidence on how long these insecticides remain effective when sprayed on different wall types under operational field conditions. Cone bioassays revealed that residual activity was strongly influenced by insecticide types, wall type, holding time, and follow-up months. Across sites, SumiShield 50WG maintained efficacy for up to 11 months, while Klypson 500WG and 2GARD-WP sustained $\geq 80\%$ mortality for up to 10 months, and in some cases up to 11 months on painted walls (e.g., Batu) under extended holding times. Although SumiShield 50WG demonstrated marginally longer persistence, the differences were modest, and all clothianidin-based IRS products tested provided extended protection, consistent with previous findings [19, 20, 44–49]. This prolonged efficacy across formulations underscores the value of clothianidin-based IRS for malaria vector control, with slight variation observed across sites and wall types.

Painted walls consistently maintained higher and sustained mortality than mud walls. This difference is potentially due to the smoother, less porous surface of painted substrates, which reduces insecticide absorption and allows for more uniform retention of active ingredients. In contrast, mud walls are highly porous and alkaline, leading to rapid sorption and degradation of insecticides [50, 51], thereby shortening the effective duration. Previous hut-based evaluations of clothianidin-based IRS insecticides in Ethiopia, including SumiShield 50WG [20] and Fludora Fusion[19], demonstrated similar trends: high initial efficacy across wall types, persistence of $\geq 80\%$ mortality for up to 10–12 months, and diminishing difference between mud and painted surfaces at longer holding times. These findings highlight the capacity of clothianidin-based IRS insecticides to provide prolonged protection under controlled hut conditions. In contrast, our operational household study revealed consistently stronger persistence on painted walls, suggesting that under real community conditions, wall porosity and environmental variability are likely to accelerate insecticide decay on mud walls. This is also further supported by the findings of Agossa et al. 2018 [44], where they showed painted walls outperform mud walls in retaining IRS insecticides.

Mortality increased substantially with longer post-exposure holding times, reflecting the delayed lethal action characteristics of neonicotinoids [52]. At 24 hours, mortality often declined below 50% after 6–8 months, but at 72–120 hours, mortality remained $\geq 80\%$ through 9–11 months across most treatments. This cumulative effect has been emphasized in WHO cone-bioassay guidelines and WHO PQT/VCP Implementation Guidance, which recommend extended observation periods when assessing clothianidin efficacy. Our findings, therefore highlight the importance of considering holding time in IRS evaluations, as short-term mortality may underestimate the true protective effect of clothianidin insecticides.

Collectively, our findings showed that *An. gambiae* s.l. populations in Ethiopia remain susceptible to clothianidin and pirimiphos-methyl, while deltamethrin resistance is more pronounced in northern areas but less evident in the Rift Valley. Clothianidin-based IRS (SumiShield 50 WG, Klypson 500 WG, and 2GARD-WP) insecticides provided residual efficacy lasting 7–11 months, with painted walls consistently outperforming mud surfaces. Importantly, the reduced mortality observed with clothianidin in Dilla and Werkamba sites suggests possible early resistance, highlighting that continuous monitoring of these insecticides is imperative to preserve their effective lifespan. In our study, we were unable to perform

chemical analysis on the Whatman filter paper, which was fixed on the sprayable walls during spray operation, due to financial and supply constraints. This limited us to determining the exact dosage sprayed on the walls. However, to ensure quality, spraymen were trained, and sprayers were carefully calibrated to maintain consistent and recommended dosages. As our scope was restricted to susceptibility and residual efficacy, further studies are required to evaluate the entomological and epidemiological impact of long-lasting IRS insecticides, thereby verifying their role in sustaining malaria vector control under diverse ecological conditions.

Conclusion

Our study revealed that *An. gambiae* s.l. populations in Ethiopia remain susceptible to clothianidin and pirimiphos-methyl, while deltamethrin resistance shows marked geographic variation. Importantly, reduced mortality of *An. gambiae* s.l. to clothianidin in Dilla and Werkamba suggests possible early resistance, emphasizing the need for continuous monitoring to extend the lifespan of these insecticides. Clothianidin-based IRS: SumiShield 50 WG, Klypson 500 WG, and 2GARD-WP provided longer residual efficacy, lasting for 7–10 months on mud walls and up to 11 months on painted surfaces, suggesting their potential for sustained vector control. While the study shows promising residual effects, further investigations are needed to confirm the insecticides' impact on epidemiological and entomological indicators of transmission to inform decision-making for vector management.

Abbreviations

WHO

World Health Organization

ITNs

Insecticide-Treated Nets

LLINs

Long-lasting Insecticide-Treated Nets

IRS

Indoor Residual Spraying

EPHI-IRB

Ethiopian Public Health Institute Institutional Review Board

Declarations

Ethical approval and consent to participate

The study was reviewed by the Ethiopian Public Health Institute-Institutional Review Board (EPHI-IRB) and has received the certificate of approval with approval No. EPHI-IRB-483-2022. We also received IRB approval for the insecticide resistance from the Natural and Computational Sciences-Institutional Review Board (CNS-IRB) of Addis Ababa University.

Consent for publication

Not applicable

Availability of data and materials

The data analysed during the current study are available from the corresponding author on reasonable request

Competing interest

The authors declare that they have no competing interests.

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Authors' contributions

AE and AW conceptualised and designed the study, analyzed data, interpreted the findings and wrote the manuscript. MT, SK, and KL contributed to data analysis and collection. YW, FG, TK, BA, EK, WM, DH, GG, AH, FT conducted data collection and processing. GA, HS, MH, GT, GT and BGB facilitated data collection and processing. LG, HT interpreted the findings. All authors revised the manuscript.

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Figures

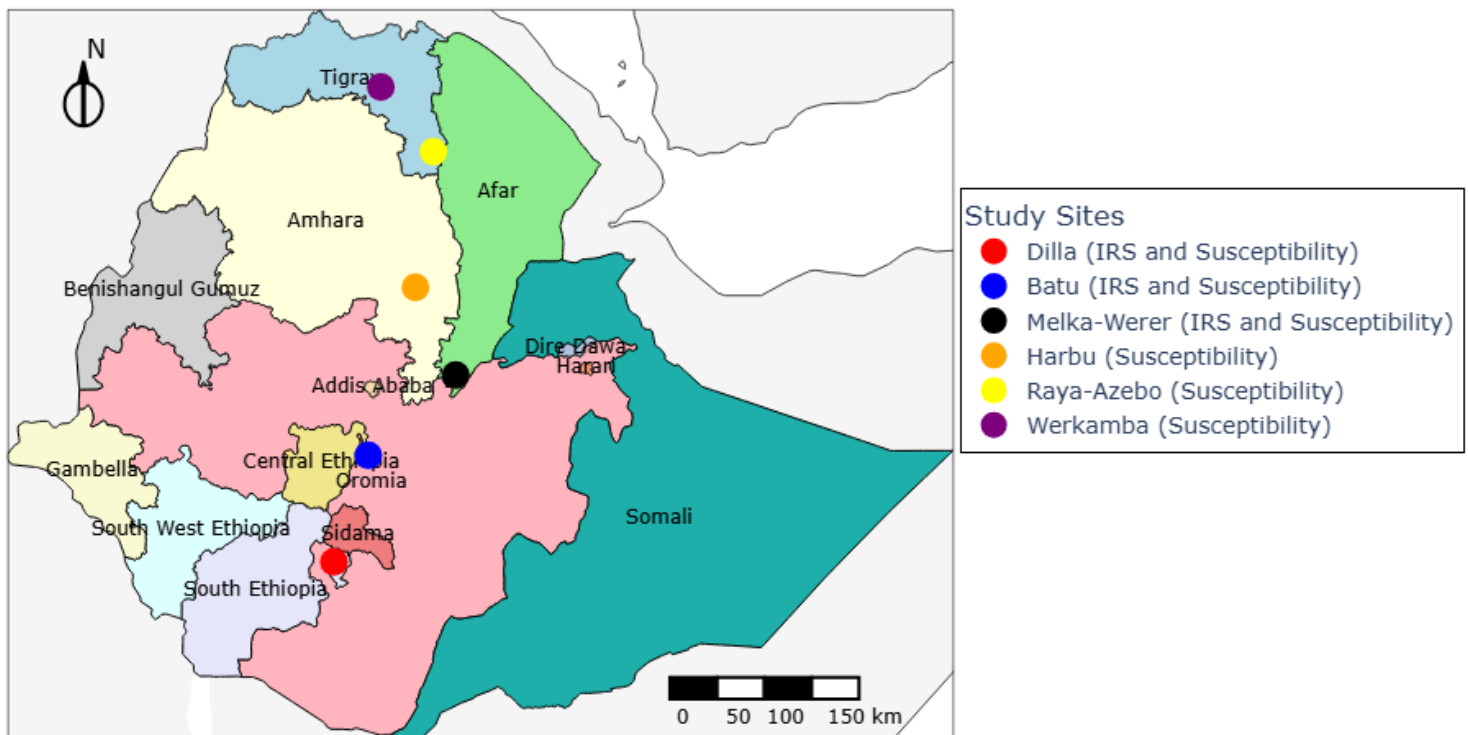


Figure 1

Locations of study sites for indoor residual spraying effectiveness and susceptibility testing

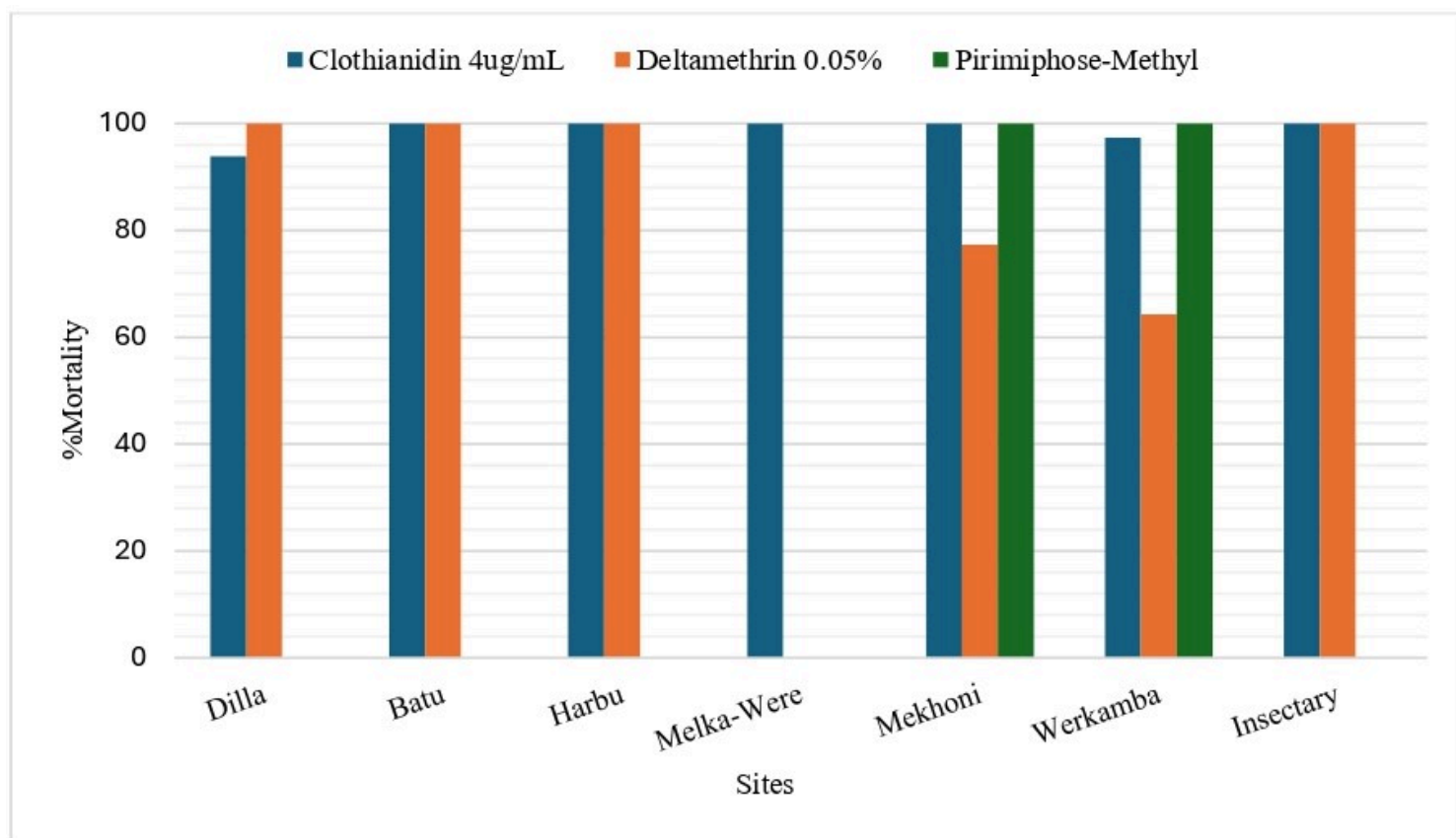


Figure 2

Mean percent mortality of *Anopheles gambiae*.I. and susceptible insectary *Anopheles arabiensis* colony against clothianidin, deltamethrin and pirimiphos-methyl.



Figure 3

Monthly mean mortality of IRS-treated surfaces by site, wall type, insecticide, and bioassay holding time. Lines represent mean % mortality across months (M0-M11), stratified by insecticide. Rows correspond to site and wall type combinations, while columns show bioassay holding times (24-120 hours). The dashed horizontal line indicates the 80% mortality threshold used to define IRS effectiveness.