

Residual efficacy of insecticides sprayed on different types of wall surfaces against *Anopheles arabiensis* in Ethiopia

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Abstract

Background: Despite the available control interventions, malaria remains a major public health problem in Ethiopia. Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are the core vector control interventions in the country. The residual efficacy of insecticides needs to be evaluated when sprayed on different wall surfaces to determine the frequency of insecticide applications. Thus, the current study aimed to evaluate EthioProxure 50% WP, Fludora Fusion, and SumiShield 50 WG insecticides at a community level in Ethiopia.

Methods: The study was conducted in five sentinel sites selected from Oromia (Wondogenet and Goro), Southern Nations, Nationalities and Peoples Region (Mirab Abaya), Afar (Melka-Werer), and Amhara (Finoteselam) regions in Ethiopia. Study villages were selected based on their history of not being covered by the IRS campaign for 12 consecutive months before the commencement of the study and households were then systematically selected from each sentinel site based on the availability of different wall types such as rough mud, smooth mud, painted, and cemented. The residual efficacy of three insecticides; EthioProxure 50% WP, Fludora Fusion, and SumiShield 50 WG on different wall surfaces was measured using a standard WHO wall cone bioassay, monthly for four consecutive months. The mean percentage mortality of mosquitoes was calculated for each site, insecticide, and wall type using SPSS Version 23.

Result: The result of the bioassay tests revealed that EthioProxure 50% WP performed above 80% mortality rate after 24-hour holding time during the four test rounds in all wall types and study sites. Moreover, Fludora Fusion and SumiShield 50 WG performed above the standard determined by WHO (>80% mortality) for all wall types except for cemented wall surface after 24 hours post-exposure up to the third month. However, when the holding time was extended to 48-hour and 72-hour, both Fludora Fusion and SumiShield 50WG exerted >80% mortality for all wall types and study sites throughout the four-round test intervals.

Conclusion: The result of the wall bioassay revealed that EthioProxure 50% WP, Fludora Fusion, and SumiShield 50 WG performed above the standard determined by WHO (>80% mortality) for all wall types from the initial test up to the fourth-month test interval.

Key words: Insecticides, EthioProxure, SumiShield, Fludora Fusion, *Anopheles arabiensis*

Introduction

Despite the available control interventions and the significant reduction in mortality and morbidity in the past two decades, malaria remains a global public health problem (Badmos et al. 2021). Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are two of the front-line malaria vector control strategies taking the lion's share in alleviating the malaria burden in sub-Saharan Africa. Despite these achievements, the application of the IRS in Africa was largely overlooked during the Global Malaria Eradication Program (GMEP) (Oxborough 2016).

The emergence and spread of insecticide resistance pose a significant and increasing threat to the control of malaria and other mosquito-borne diseases (Andriessen et al. 2015). *Anopheles arabiensis* is becoming resistant to different insecticides like DDT, permethrin, deltamethrin, lambda-cyhalothrin, and malathion in many localities of Ethiopia (Yewhalaw et

al. 2010; Balkew et al. 2010; Asale et al. 2014) threatening the sustainability of the conventional indoor vector control interventions. Moreover, PMI (2014) reported that *An. gambiae* s.l. was resistant (76% test mortality) to bendiocarb in the Asendabo town of Jimma Zone even though it was fully susceptible (100% test mortality) in Chewaka, Ilu Abba Bora Zone of Ethiopia.

Recently, in response to resistance development, new insecticides with different modes of action are being evolved. Fludora Fusion[®] and SumiShield 50 WG are the new neonicotinoid indoor residual insecticides intended for use in pyrethroid-resistant malaria vectors. These insecticides have demonstrated a promising performance against *An. arabiensis* under experimental hut (Fongnikin, 2020; Urabayala, 2018; Animut & Horstmann, 2022).

The efficacy of IRS could be influenced by different factors such as mosquito susceptibility to insecticide,

mosquito behavior (endophilic and endophagic), type of sprayable surfaces, quality of IRS, residual efficacy of an insecticide, and community acceptance (Gari and Lindtjørn 2018). Monitoring of residual efficacies of insecticides is essential to determine the periods that they remain effective in interrupting malaria transmission and schedule when to re-spray (Nigatu et al. 2019). So far community-level studies on residual efficacies of Fludora Fusion and SumiShield 50 WG are limited in Ethiopia. Thus, the current study aimed to investigate the residual efficacy of Fludora Fusion and SumiShield 50 WG besides Ethiopropoxur sprayed on different wall types under field conditions in selected sentinel sites in Ethiopia.

Materials and Methods

Study area and period: The study was conducted from January 2021 to June 2021 in five malaria sentinel sites in Ethiopia: Amhara (Finoteselam), Oromia (Wondogenet and Goro), Southern Nations, Nationalities and Peoples Region (SNNPR) (Mirab Abaya), and Afar (Melka Were) (Figure 1). The study sites are malaria endemics with different socio-demographic characteristics and are also selected as national malaria surveillance sentinel sites for monitoring entomological, parasitological, and climate factors and indices targeting malaria elimination by 2030 in different eco-epidemiological settings in the country.

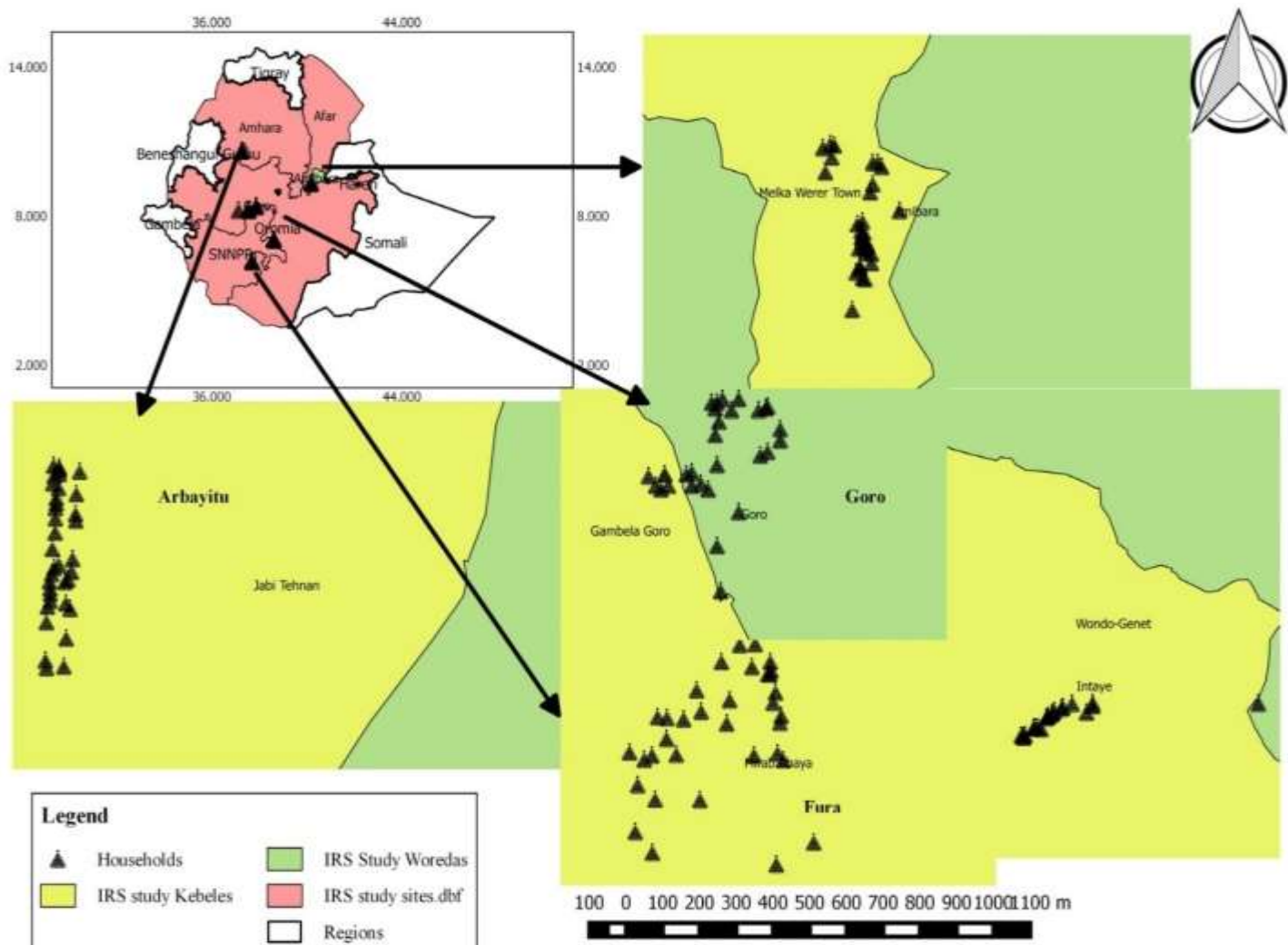


Figure 1: Map of study sites

Study design: A longitudinal study design was used to determine the residual activities of insecticides in purposively selected villages. Two villages with

relatively easy access and higher malaria transmission from each district were systematically selected. House selection was based on the availability of different

wall types such as rough mud, smooth mud, painted, and cemented. Each insecticide treatment was carried out in four houses of each wall type on the condition that all wall types were available in each study village (PMI, 2017; Nagatu et al. 2019). The global positioning system (GPS) point of each study households was taken using GPS coordinate apps installed on smart phone.

Test Insecticides

1. Ethiothopoxur-4g/m²: Ethiothopoxur 50% WP manufactured by Adami-Tulu Pesticides Processing S.Co., Ethiopia.
2. Sumi-Shield 50WG- 300 mg/m²: Sumi-Shield 50WG manufactured by Sumitomo Chemical Company, Limited, Japan.
3. Fludora Fusion- 200mg clothianidin + 25mg deltamethrin / m² manufactured by Bayer Crop Science, BCS, Nigel South Africa.

Indoor Residual Spraying operation

The indoor residual spraying operation was conducted by well-trained personnel who were doing the IRS activity within the Woreda. They were recruited from the local community and supervised by a study team from the Ethiopian Public Health Institute (EPHI) and the Ministry of Health (MOH). Preparation was undertaken in households before the spraying including the removal of movable household contents; covering of non-movable contents with plastic sheets, and removal of wall coverings and curtains. Household occupants were instructed to stay outdoors during and for at least two hours post-spraying.

Spraying of candidate insecticides was done by trained spray men in the Woreda squad using a Hudson® X-pert compression sprayer (Hudson Manufacturing Company) with a flat nozzle as recommended for IRS following the WHO operational manual for indoor residual spraying (WHO 2015). 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 swathe at the point of impact is 75 cm. Five cm overlap was maintained between the swathes to make sure that no wall surface was left without insecticide.

Wall bioassay

The decay rate of the insecticides on walls was measured using standard WHO cone tests (WHO 2006) in houses with different wall surfaces, and each surface was replicated four times for each insecticide. One unsprayed house for each surface served as control. The initial cone tests were carried out three days after spraying (Nigatu et al. 2019; Fongnikin et al. 2020). Thereafter tests of treated surfaces were performed at monthly intervals for up to four 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. Three to five days-old unfed females' susceptible colony of *An. arabiensis* found in EPHI and Adama insectary was 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, and 72-hour holding period depending on the type of insecticide, and were supplied with a 10% sugar solution. Paper cups were kept in a wooden box which was covered by a damp towel to create favorable temperature (27±2 °C) and relative humidity (70±10%).

Mortality counts were taken after 24 hours post-exposure for Ethiothopoxure and every 24 hours for 72 hours post-exposure for Fludora Fusion and Sumishield insecticides. The knockdown rate was also recorded after 30 min exposure time. 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 Abbot's formula.

Human and environmental safety: 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. 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 hours after spraying. The adult present at the time of spraying was advised to tell and control their children not to intentionally touch the sprayed walls for at least one day after spraying since the walls remained wet for about a day. For environmental safety, over-left pesticides were disposed of using standard WHO safety procedures (WHO 2006).

Ethical consideration

The study protocol was reviewed by the Internal Review Board of the Ethiopian Public Health Institute (EPHI-IRB) and received a certificate of approval (protocol number EPH-IRB-308-2020). Permission was also obtained from the Woreda Health Office and Kebele Administration, and consent from household heads before the commencement of the study.

Data analysis: Data were entered in the well-prescribed data forms and then entered into an Excel

sheet. Cone bioassay mortality was pooled for each treatment and the mean percentage mortality of mosquitoes was calculated for each site, insecticide, and substrate type using SPSS 23 software (SPSS Inc., Chicago, IL). The results were compared with the current WHO threshold value of >80% mortality (WHO 2006).

Results

Thirty minutes post-exposure Knockdown rate (kdr):

A total of 27000 susceptible female *An. arabiensis*

colonies obtained from EPHI and Adama insectary were exposed for bioassay throughout the four-month trial in the five sentinel sites. The knockdown rate was recorded for each insecticide 30 minutes post-exposure and the results are presented in Figure 2. The knockdown rate of EthioPropoxure 50% WP was above 95% for all wall types in the first-round test while the knockdown rate started to decline below 95% in the second and third months except for cemented and painted wall types.

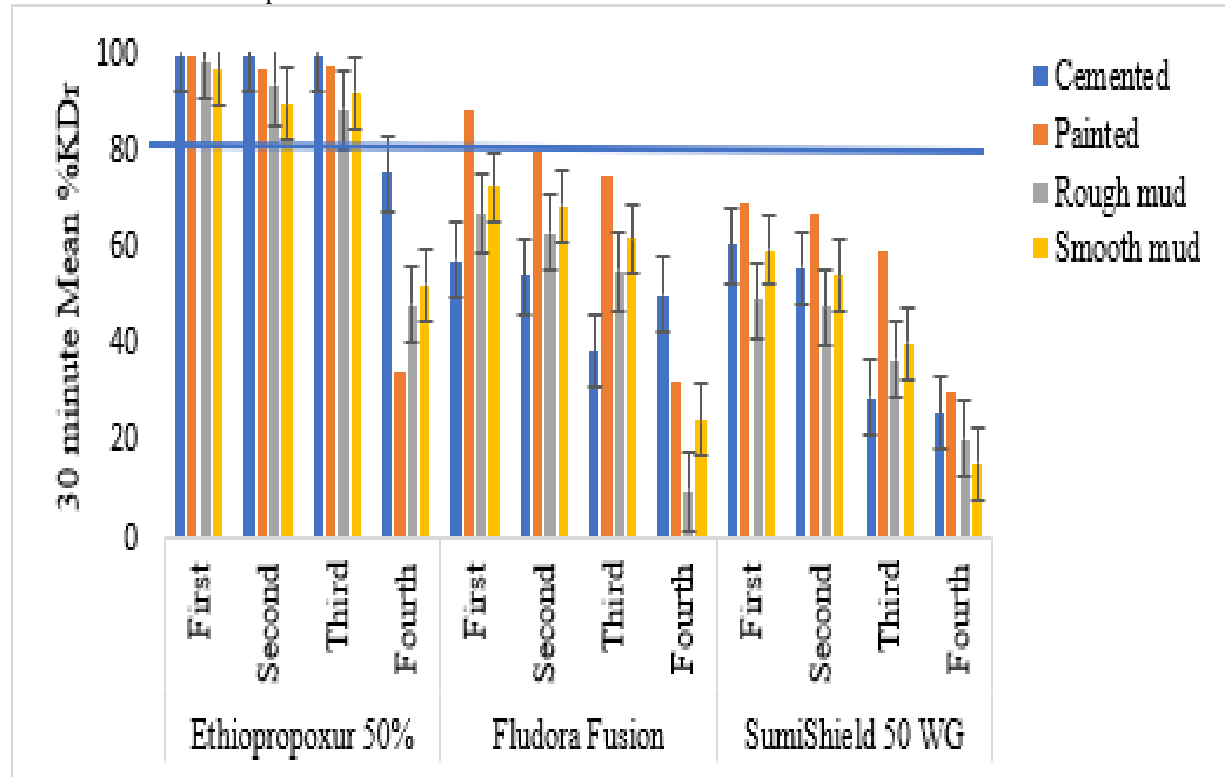


Figure 2: Knockdown effect of test insecticides against susceptible *Anopheles arabiensis* after 30 min exposure

The *kdr* of EthioPropoxure 50% WP declined below 80% during the fourth month in all wall types. Fludora Fusion performed above 80% *kdr* on the first round on painted wall surfaces but *kdr* for cemented, rough mud, and smooth mud remained below 80%. Moreover, SumiShield 50 WG demonstrated a knockdown rate below 80% (ranging between 15.5% and 69.16%) (Figure 2) across the different round tests and wall types.

Cone bioassay mortality after the 24-hour holding time: The results of the cone test for EthioPropoxure 50% WP, Fludora Fusion, and SumiShield 50 WG were recorded 24 hours post-exposure and summarized in Figure 3. EthioPropoxure 50% WP

performed above the WHO cutoff value (>80% mortality) for all wall types throughout the four-round test intervals. A similar result was also observed for Fludora Fusion and SumiShield 50 WG in the first, second, and third-round tests except for cemented wall types which demonstrated <80% mortality during the third-round test. Furthermore, during the fourth-round test, 24-hour post-exposure mortality of Fludora Fusion and SumiShield 50 WG declined below 80% on cemented, rough mud, and smooth mud (only for Fludora Fusion) wall types. While for painted wall type mortality after 24 hours during the fourth-round test remained above the WHO cut-off value (>80% mortality) for both insecticides.

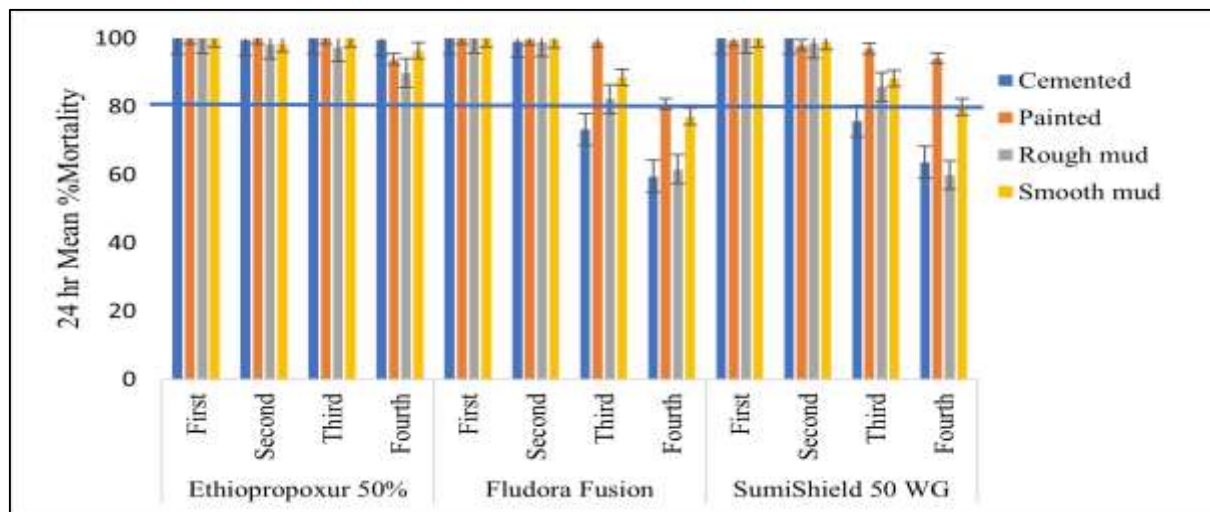


Figure 3: Mean percent mortality of susceptible *Anopheles arabiensis* after 24-hour exposure times to insecticides sprayed on different wall types

Mean percent mortalities after 24 hours of holding time across each study site was also summarized for each insecticide in all wall types across the sentinel sites in Figure 4a-c. The result indicated that Ethiopropoxure 50% WP demonstrated a mortality rate ranging between 90-100% in all sites for all the test wall types up to the third month. However, during the fourth-month bioassay, mortality declined to 82% on rough mud and 83% on painted and smooth mud in Finoteselam while mortality exceeded 90% in the rest study sites. In addition, the mortality rate for Fludora Fusion remained at 100% until the third month in Finoteselam (painted and smooth mud), Goro

(painted, smooth mud, and rough mud), Melka Werer (cemented, painted, and smooth mud). During the fourth month, the mortality rates declined to below 80% in Finoteselam (rough mud), Goro, and Mirababaya (painted). Moreover, the mortality rate of Fludora Fusion declined to below 47% on cemented, painted, and smooth wall types in Wondogenet sites during the third and fourth-month bioassay tests. Furthermore, SumiShield 50WG demonstrated an 88%-100% mortality rate in all sites and for all wall types until the third month except for Wondogenet for which the mortality rate starts to decline below 49% during the third month in all wall types.

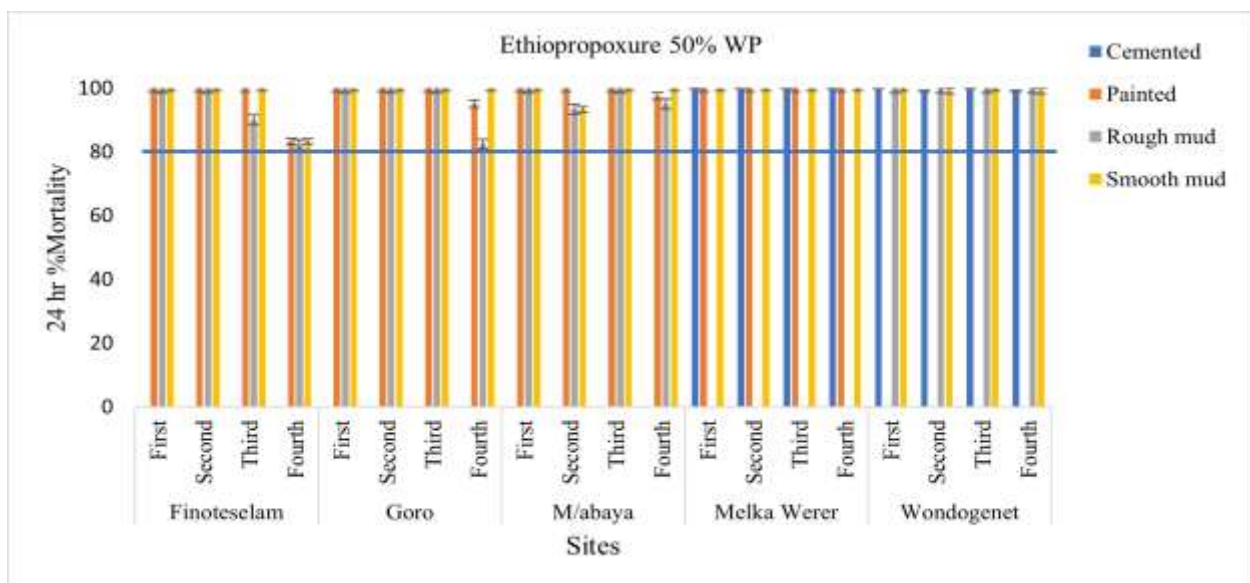


Figure 4a: Mean percent mortality of *Anopheles arabiensis* after 24-hour exposure to Ethiopropoxure 50% WP in different sentinel sites

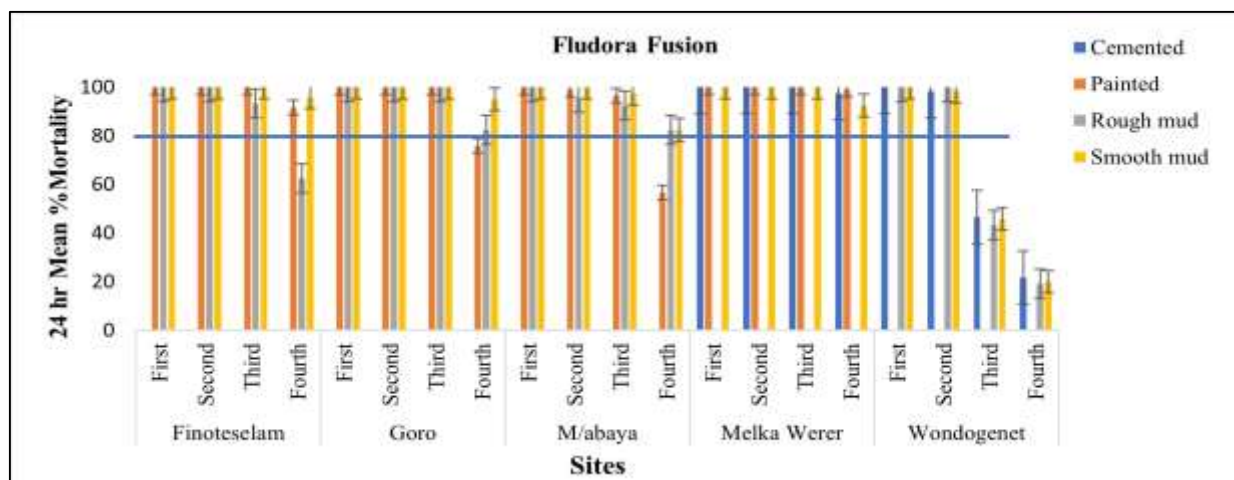


Figure 4b: Mean percent mortality of *Anopheles arabiensis* after 24-hour exposure to Fludora Fusion in different sentinel sites

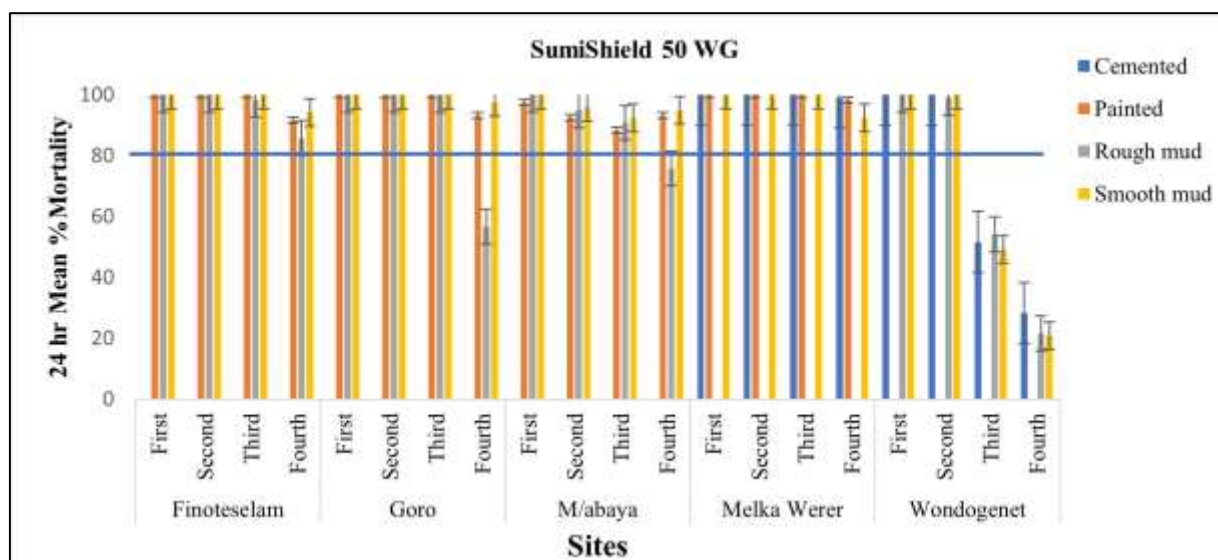


Figure 4c: Mean percent mortality of *Anopheles arabiensis* after 24-hour exposure to SumiShield 50 WG in different sentinel sites

Cone bioassay mortality after 48-hour holding time:

As the Fludora Fusion and SumiShield 50 WG insecticides claimed to have a delayed effect, mortality was recorded every 24 hours until 72 hours. Hence, at 48 hours post-exposure both Fludora Fusion and SumiShield 50 WG demonstrated a mortality rate above 80% for smooth mud, rough mud, and painted wall types in all the four-round bioassays.

However, on cemented wall type mortality declined below the WHO cut-off value (>80% mortality) during the fourth round of bioassay Figure 5. Even though, the mortality of both insecticides on cemented wall type dropdown to below 80% at 48 hours of exposure, their efficacy at 72 hours remained above the WHO cut-off value (>80% mortality).

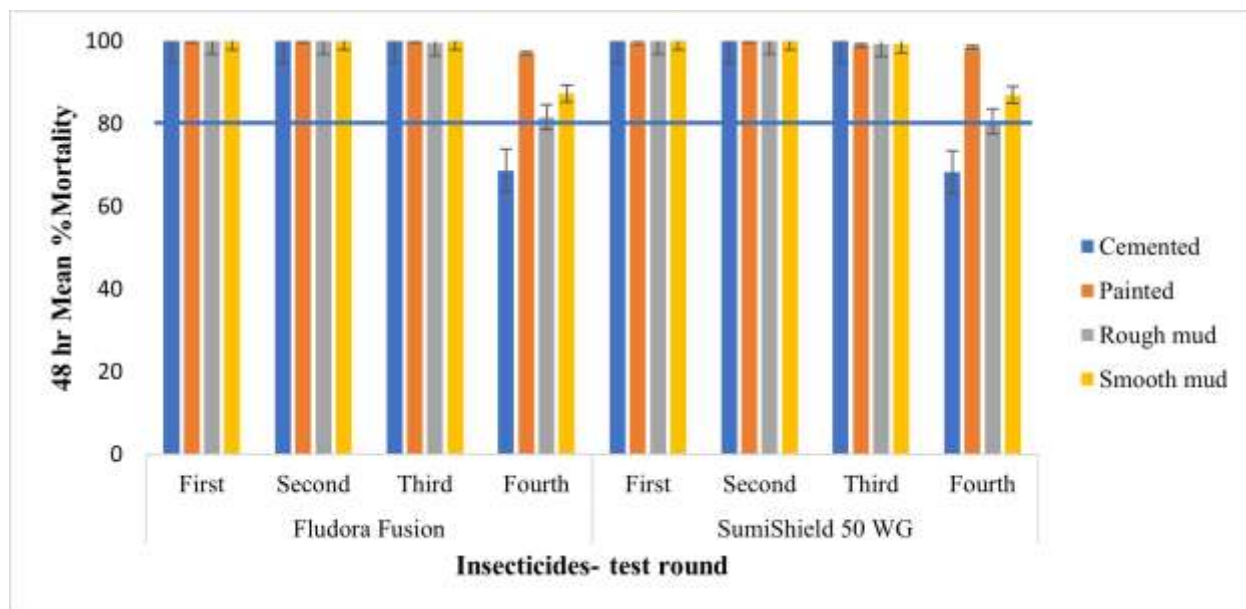


Figure 5: Mean percent mortality of *Anopheles arabiensis* after 48-hour exposure to Fludora Fusion and SumiShield 50 WG sprayed on different wall types across different time intervals

Discussion

The result of the present study revealed that the knockdown effect varied among the different insecticides, time intervals, and wall surfaces. The efficacy of indoor residual spraying could be influenced by wall types, spray quality, and insecticide formulations (Gari and Lindtjørn 2019). Ethioipropoxur 50% WP demonstrated the highest knockdown effect on cemented and painted wall types throughout the four months. A similar finding was reported by Yewhalaw et al. (2017) in Sekoru and Ziway experimental hut study. In addition, the study from southwest Ethiopia (Shellie Mella) revealed a 100% knockdown effect of propoxur on painted wall surfaces (Desalegn et al. 2018).

The knockdown effect for Fludora Fusion, and SumiShield 50 WG remained low (<80%) for all wall types and test rounds except for Fludora Fusion on painted wall type for which the knockdown rate during the first round was 88.75%. The previous study on the experimental hut indicated that the knockdown effect of Fludora Fusion on dung, smooth, and rough mud remained above 80% for about nine months while the knockdown effect of SumiShield 50WG was below 10% for all wall types (Animut and Horstmann 2022). The difference in knockdown rate with the current study could have occurred due to the difference in study settings. The current study was conducted under operational field conditions (community level) and several factors such as the culture of the community, temperature, humidity, and spray quality can influence the residual effects of the test insecticides.

The mortality rate for Ethioipropoxur 50%WP sprayed at 4g/m² diluted in 40ml water remained above the WHO cut-off value (>80%) after 24-hour holding time throughout the four test rounds in all wall types and study sites. The finding is in line with the previous studies conducted in Wondogenet and Mirab Abaya where Propoxur 50% WP induced above 95% mortality throughout the four months. However, the overall effectiveness of Fludora Fusion and SumiShield 50WG remained above 80% mortality during the first, second, and third test rounds after 24-hour holding time and up to the fourth round when the holding time was extended to 48 hours in all wall types except on cemented wall type for which mortality starts to decline below 80% starting the third month. Moreover, considering the study sites the performance of SumiShield 50WG and Fludora Fusion declined to below 80% mortality after 24 hours during the third and fourth months on cemented, rough, and smooth mud wall surfaces in the Wondogenet study site. Similarly, a previous study conducted by Marti-Soler et al. (2021) in Mozambique revealed that the performance of SumiShield 50WG after 24-hour and 48-hour holding time remained consistently below the 80% mortality rate on cemented and mud-plastered wall types. According to the finding from the PMI (2021), a decay rate study of Fludora Fusion sprayed in the Abaya and Gelana Districts of Oromia, and SumiShield 50WG sprayed in Abe Dongoro and Menge Districts revealed that the mortality of *An.arabiensis* exceeds 80% up to four months. Besides, the residual activity of SumiShield 50 WG was reported to be ≥80% at a 24-hour holding time for

about five months under operational field conditions. When the holding time was extended to 72, 96, and 120 hours, the effectiveness of both Fludora Fusion and SumiShield 50WG remained above 80% mortality (Uragayala et al. 2018). Similarly, in the current study, the performance of both Fludora Fusion and SumiShield 50WG after 72 hours was 100% for all wall types up to the fourth-round test. Furthermore, experimental hut studies from Adami Tulu Judo Kombollcha District revealed that Fludora Fusion induced over 80% mortality against the susceptible colony of *An.arabiensis* for about 12 months (Animut and Horstmann 2022).

The limitation of the current study is that bioassay follow-up was discontinued after four months due to financial constraints. Because of this, we are unable to determine the effective duration of the candidate insecticides after four months. Despite the limitations, the current finding provides evidence of the efficacy of Ethiothopoxur 50WP, SumiShield 50WG, and Fludora Fusion at the community level in different socio-demographic sentinel sites. Based on the current findings, the aforementioned insecticides can be used for indoor residual spraying against malaria vectors in Ethiopia. Further, studies are required to investigate community acceptance, spray quality, any adverse effects on spray man and the community, and the impact on vector distribution and malaria incidences.

Conclusion

The result of the wall bioassay revealed that Ethiothopoxur 50% WP, Fludora Fusion, and SumiShield 50 WG performed above the standard determined by WHO (>80% mortality) on painted, cemented, smooth, and rough mud wall types from the initial test up to the fourth-round test interval.

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Competing interest: The authors declare that they have no competing interests.

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