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Article

The residual efficacy of SumiShield™ 50WG and K-Othrine® WG250 IRS formulations applied to different building materials against *Anopheles* and *Aedes* mosquitoes

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Simple Summary: The Anopheles mosquitoes that transmit malaria are targeted by the use of indoor residual sprays (IRS), insecticides applied to the walls of homes to kill mosquitoes which rest there when coming into houses in search of a blood meal. SumiShield™ 50WG is an IRS based on the insecticide clothianidin, developed to kill mosquitoes which have become resistant to other forms of insecticide. SumiShield™ 50WG was applied to cement, wood, and mud tiles, representative of typical building materials in areas where malaria is endemic. For 18 months the ability of these treated surfaces to kill adult female mosquitoes exposed to them was measured. The IRS was highly effective against insecticide-susceptible and resistant *Anopheles gambiae* and *An. funestus*, though not *Aedes aegypti* or *Culex quinquefasciatus*. IRS was shown to be more effective and long lasting on cement and mud than on wood tiles. Overall, the results suggest SumiShield™ 50WG is well suited for malaria control.

Abstract: Insecticides with novel modes of action are required to complement the pyrethroids currently relied upon for controlling malaria vectors. One example of this is the neonicotinoid clothianidin, which is found in SumiShield™ 50WG used in indoor residual spraying (IRS). In a preliminary experiment, mortality in insecticide susceptible and resistant *An. gambiae* adults exposed to SumiShield™ 50WG-treated filter papers reached 80% by 3-days post-exposure and 100% by 6-days post-exposure. Next, cement, wood, and mud tiles were treated with SumiShield™ 50WG or K-Othrine® WG250 (deltamethrin IRS formulation) and insecticide resistant and susceptible *Anopheles* and *Aedes* were exposed to these surfaces periodically for up to 18-months. Pyrethroid resistant *Cx. quinquefasciatus* were also exposed at 9 months. Between exposures tiles were stored in heat and relative humidity conditions reflecting those found in the field. On these surfaces, SumiShield™ 50WG was effective at killing both susceptible and resistant *An. gambiae* for 18 months post-treatment, while mortality amongst the resistant strains when exposed to deltamethrin (K-Othrine® WG250) IRS was not above that of the negative control. Greater efficacy of SumiShield™ 50WG was also demonstrated against insecticide resistant strains of *An. funestus* compared to deltamethrin, though the potency was lower when compared with *An. gambiae*. In general, a higher efficacy of SumiShield™ 50WG was observed on

cement and mud compared to wood. SumiShield™ 50WG demonstrated poor residual activity against *Aedes aegypti* and *Culex quinquefasciatus*. Overall, the results suggest SumiShield™ 50WG is well suited for malaria control.

Keywords: Indoor residual spray (IRS); Vector control; *Anopheles*; *Aedes aegypti*; *Culex quinquefasciatus*; Neonicotinoids; Pyrethroid; Insecticide resistance; SumiShield; K-Othrine.

1. Introduction

Insecticide treated nets (ITNs) and Indoor residual spraying (IRS) continue to be the two primary methods used in vector control strategies against malaria [1]. Insecticides are key to the control of other mosquito-borne diseases such as arbovirus infections transmitted by *Aedes*, as well as the control of nuisance biters such as *Culex* species. IRS can greatly reduce disease transmission risk by decreasing the survival of mosquitoes as well as biting density [2]. However, the substantial progress made in the reduction of disease transmission, particularly malaria, is under threat from the increasing spread of insecticide resistance to conventional insecticides, namely pyrethroids, carbamates and organophosphates [3], [4]. Although there are other vector control tools (e.g., larval source management) and new technologies in development (e.g., transgenic mosquitoes and the use of symbionts), insecticides remain essential in the control of endophilic vectors. Therefore, there is an urgent need to develop new insecticides and formulations for IRS, effective against mosquitoes that exhibit resistance to currently approved insecticide classes. To investigate the risk of cross resistance in pyrethroid resistant populations, it is valuable to test new chemistries against well characterised strains of insecticide susceptible and resistant mosquitoes. Laboratory strains can be maintained in a controlled and consistent manner to allow comparison to be made between studies and between compounds[5].

Currently, there are five insecticide classes used in IRS products prequalified by the World Health Organization (WHO): pyrethroids, carbamates, organophosphates, and the most recently added, neonicotinoids [6]. SumiShield™ 50WG, developed by Sumitomo Chemical Ltd, is a formulation of clothianidin, a neonicotinoid, a class of insecticides which act as an agonists of nicotinic acetylcholine receptors within mosquitoes. This novel mode of action gives neonicotinoids the potential to provide control of vectors in areas of high pyrethroid resistance. SumiShield™ 50WG has already been shown to be effective in Phase I trials [7], [8], in Phase II trials in areas of high intensity insecticide resistance [9], [10], and in Phase III trials in India [11] and Tanzania [12].

The success of an IRS programme depends on several factors, including vector resting behaviour, residual efficacy of insecticides, spray coverage and the quality of spraying [13]. The typical target residual efficacy of an IRS product is 6 months, but this efficacy can vary greatly depending on the nature of the sprayed surfaces [14]–[16]. A laboratory study using deltamethrin IRS (K-Othrine WP 5%) against a susceptible *Anopheles* strain found that it retained efficacy (defined by the WHO as >80 % mortality) for 2 months on mud, 4 months on plaster and wood, and 4.5 months on cement [14]. Similarly, a study in Cameroon showed that deltamethrin IRS (K-Othrine® WG 250) sprayed on concrete walls had the longest residual efficacy (6 months), followed by mud (4.5 months), then wood (3.5 months) [16]. A study in Zanzibar determined that pirimiphos-methyl IRS (Actellic® 300CS) applied on various wall surfaces (mud, oil, water painted, lime washed walls, unplastered cement and stone blocks) remained effective for at least 8 months after spraying [17].

Some studies have investigated the residual efficacy of SumiShield™ 50WG on different surfaces, including mud and cement [11], [18] . In India, against insecticide resistant *An. culicifacies*, Sreehari et al. [18] observed SumiShield™ 50WG to have a residual life of

15 - 25 weeks on cement and mud plastered houses, depending on mosquito holding period post-exposure (24-hour mortality – 120-hour mortality). An additional 32% of mosquitoes died between 24 and 120 hours when exposed on a treated cement wall, and 40% on a mud wall, leading to clothianidin being referred to as causing delayed mortality in addition to that observed using standard protocols. Similarly, [11] observed the residual efficacy of SumiShield™ 50WG in houses against *An. culicifacies* increased from 5 to 6 months when the holding period was extended from 24 to 120 hours.

Residual efficacy experiments of IRS formulations applied to different surface types in a controlled laboratory environment can provide vital information to help make predictions about efficacy in operational use in different settings. Temperature and humidity can be controlled while the surfaces are treated and stored, to standardise conditions over the course of a long experiment and between treatments. Spray application can also be performed accurately, using a Potter Tower [19], [20]. The Potter Tower is recommended by the WHO for laboratory studies to test insecticide residual activity and is an internationally recognized method of chemical spraying [21]. Other studies have assessed the residual efficacy of SumiShield™ 50WG in a field setting, using a compression sprayer to treat huts. However, they often show high variability in spray uniformity, as illustrated by a phase III study in India, where the target dose of SumiShield™ was 300mg AI/m^2, but the mean dose applied was 516.6mg AI/m^2, with some villages having a dose ratio as high as x2.4 [11]. A similar study in Tanzania achieved a closer target dose (average 363.4 mg AI/m^2) [12], making comparison between studies difficult. Kweka *et al.* [12] and Uragayala *et al.* [11] used mud only versus mud plastered walls with lime coating, with mortality based on a 168- and 120-hour holding period of mosquito's post-exposure, respectively. The residual efficacy of IRS formulations on mud surfaces may be affected by specific physical and chemical properties of the mud.

This controlled laboratory experiment aimed to assess the residuality of SumiShield™ 50WG (hereafter referred to as 'SumiShield') in comparison to K-Othrine® 250WDG (hereafter referred to as 'K-Othrine'), a deltamethrin-based product that has been widely employed for IRS. Testing was conducted on surfaces commonly used for building houses in areas where IRS is employed (i.e., mud, cement, and plywood). First the speed of kill against a pyrethroid susceptible and a resistant strain of *Anopheles gambiae* was tested in a WHO tube assay to determine the most appropriate holding period. Then, susceptible and pyrethroid-resistant laboratory strains of *An. gambiae*, *An. funestus*, *Aedes aegypti*, and *Culex quinquefasciatus* were exposed to treated mud, cement and wood surfaces. Finally, the effect of increasing exposure time was investigated in susceptible and resistant laboratory strains of *Ae. aegypti*, and susceptible *An. gambiae*.

2. Materials and Methods

2.1. Mosquito strains

All mosquitoes were reared from colonies maintained in the Liverpool Testing Establishment (LITE) at the Liverpool School of Tropical Medicine (LSTM, UK) according to the methods described by [5]. Adult female mosquitoes, 2-5 days old, allowed to mate but not blood feed, were used for all bioassays. Seven mosquito strains were used in the residuality experiment. *An. gambiae* s.l VK7 2014 (resistant) and Kisumu (susceptible), *An. funestus* FuMoz-R (resistant) and Fang (susceptible), *Ae. aegypti* Cayman (resistant) and New Orleans (susceptible). Resistance profiles of these strains are available at Williams *et al.* [5]. A strain of *Cx. quinquefasciatus* (Muheza) was also tested. Colonised from coastal Tanzania in the early 1990s and since selected for permethrin resistance [22], this strain is highly resistant to permethrin, deltamethrin, DDT and dieldrin, and susceptible to fenitrothion and propoxur (Authors' unpublished data).

2.2. Test surfaces

Three surfaces, representative of materials which may be used to construct dwellings in areas of IRS application, were treated for efficacy testing: wood, cement, and mud.

The wood surfaces (12 cm^2 squares) were cut from untreated beech wood approximately 1 cm thick.

The cement surfaces (10 cm diameter circles, ~ 5 mm thick) were prepared by sieving sand and cement powder separately to remove any dirt and large particles then combining ~ 600 ml of sand, 600 ml of cement powder and 300 ml of purified (Millipore) water, mixing thoroughly to a thick paste. Petri dishes 10 cm in diameter were filled, pushing the surface down firmly to ensure there were no gaps and flattening until the top surfaces were smooth. Surfaces were dried at 27 °C ± 2 °C and 80% ± 10% RH) for a minimum of 30 days prior to testing.

The mud surfaces (10 cm diameter circles) were made from unfired mud bricks provided by the Institut de Recherche en Sciences de la Sante (IRSS), Burkina Faso. The mud was collected from their field station in Vallée du Kou 7, Burkina Faso (4°24'W, 11°24'N). Mud bricks were broken down into dust, reconstituted by adding small amounts of purified (Millipore) water and mixing using hands, continuing to add water until the mud was firm and smooth in consistency. This mud was used to fill metal molds which hold a cylinder of mud, approximately 1 cm deep and with a diameter of 10 cm. Mud surfaces were stored in a climate-controlled stability cabinet (27°C ± 2 °C and 80% ± 10% RH) for a minimum of 30 days to allow them to dry and produce a smooth mud surface. A mud sample was also supplied to ACS Testing Ltd (Poole, Dorset) to determine the physical characteristics and chemical properties of the mud (Supplementary Material, Table S1).

2.3. Preparation of test surfaces

SumiShield and K-Othrine formulations were diluted in purified (Millipore) water and applied to each test surface using a 'Potter Tower' (Potter Precision Laboratory Spray Tower, Burkard Scientific). The target application rate was 300 mg AI/m^2 for SumiShield and 25 mg AI/m^2 for K-Othrine, the manufacturers' recommended application rate. Negative control surfaces were sprayed with purified (Millipore) water only. Prior to use the Potter Tower was calibrated to ensure less than 10% variation in spray density across the treated surface and less than 10% variation in spray weight between applications.

Seven replicate plates of each treatment (SumiShield, K-Othrine, and control) were treated per surface type (wood, cement, mud) to produce a set of plates for bioassays (3

plates of each type and treatment) and for spare reserves (4 plates of each). This allowed each species to be exposed to a different set of plates. Two strains (resistant and susceptible) of each species were exposed to the same set of plates at each time point. The same surfaces were used for bioassays at each time point, except where surfaces were damaged and where this happened, they were replaced with reserve plates. After spraying and between bioassays the surfaces were stored in a climate-controlled stability cabinet (30°C ± 2 °C, 80% ± 10% RH), vertically and unsealed, with air circulation and in the dark to represent typical field conditions.

2.4. WHO susceptibility tube bioassay

2.4.1 Investigating additional mortality beyond 24h post-exposure to clothianidin

Because clothianidin, the active ingredient (AI) in SumiShield has been seen to have additional kill activity over time, beyond the typical 24 hour holding period, a preliminary test, using WHO susceptibility tube bioassays, was conducted to determine how long mosquitoes should be held after exposure to SumiShield to record maximum mortality. Filter paper (Whatman No. 1) was cut into 12 cm x 15 cm pieces. 264 mg of clothianidin was dissolved in 20 ml of purified (Millipore) water, and 2 ml was pipetted onto each paper to give a surface concentration of 13.2 mg AI per paper, or 733.3 mg AI/m^2. Eight replicate papers were made, alongside six negative water only control papers, and a positive control paper treated with 275 AI mg/m^2 fenitrothion. Papers were dried overnight in a fume hood before being stored in silver foil at 5°C until use. All bioassays were performed within 1 month of the papers being made. Two 0.75% w/w permethrin papers (provided by Universiti Sains Malaysia on behalf of the WHO) were also used as positive controls.

Twenty-five mosquitoes were exposed to each clothianidin, permethrin or control paper for 60-minutes, or fenitrothion for 120-minutes, in a standard WHO tube bioassay [23]. Knockdown was scored 30- and 60-minutes post-exposure, and mortality was scored 24-hours post-exposure, then daily until 7-days post-exposure.

2.4.2. Investigating the effect of varied exposure time on clothianidin efficacy

An additional study investigated how varying the time of exposure to SumiShield affected mosquito mortality. Mosquitoes were exposed to test filter papers in a WHO tube susceptibility bioassay, using the same methods for preparing papers, conducting bioassays as described above. Susceptible (New Orleans) and resistant (Cayman) *Ae. aegypti*, and susceptible *An. gambiae* (Kisumu) were exposed to SumiShield papers for a range of exposure times (15 minutes to 7 hours). Mosquitoes were exposed to negative water only controls for 60 minutes [23]. Knock down was scored 60 minutes post-exposure, and mortality was scored at 24, 48 and 72-hours post-exposure.

2.5. WHO cone bioassay

2.5.1. Residual efficacy of SumiShield over time

Each strain of mosquito was exposed to three replicate plates of each treatment and surface combination using the WHO cone bioassay [21]. Bioassays were repeated 24-hours and 1, 3, 5, 7, 9, 12 and 18-months after treatment of the surfaces, with the following exceptions: at month 9 Cayman and New Orleans strains (*Ae. aegypti*) were not available and the time point was omitted, and Muheza (*Cx. quinquefasciatus*) was only tested at a single time point (9 months) using the spare reserve plates.

Ten mosquitoes were aspirated into a plastic cone plugged with cotton wool, applied to each surface, held on a board at 45 degrees, and left for 30 minutes before being

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aspirated off into a holding cup and held in a stability cabinet at 27°C ± 2 °C and 70% ± 10% RH for 24 hours, with access to a 10% sugar solution provided on cotton wool [21]. Mosquitoes were scored for knock down or mortality at the end of exposure (30 minutes), 24 hours post-exposure, and daily for 7 days. A preliminary study (data not shown) found that ≥87% (N = 50, 5 replicate cups) of non-exposed 2–5-day old females of each strain survived for 7 days in holding cups under these holding conditions, and that in most strains' survival was ≥95%.

2.6. Data analysis

Post-exposure knockdown and daily mortality over 7-days is reported as an average of cone test results from three replicate surfaces or of three replicate WHO susceptibility tube tests, corrected for the control mortality using Abbott's formula [24]. Standard error was calculated between replicates of each strain and each treatment.

3. Results

3.1. WHO tube bioassay: Additional mortality beyond 24h post-exposure to clothianidin

Following exposure to SumiShield for 60 minutes in a WHO tube assay, >99% of susceptible Kisumu and resistant VK7 2014 were killed within 7-days, and mortality reached 80% in both strains by 3-days post-exposure (Figure 1).

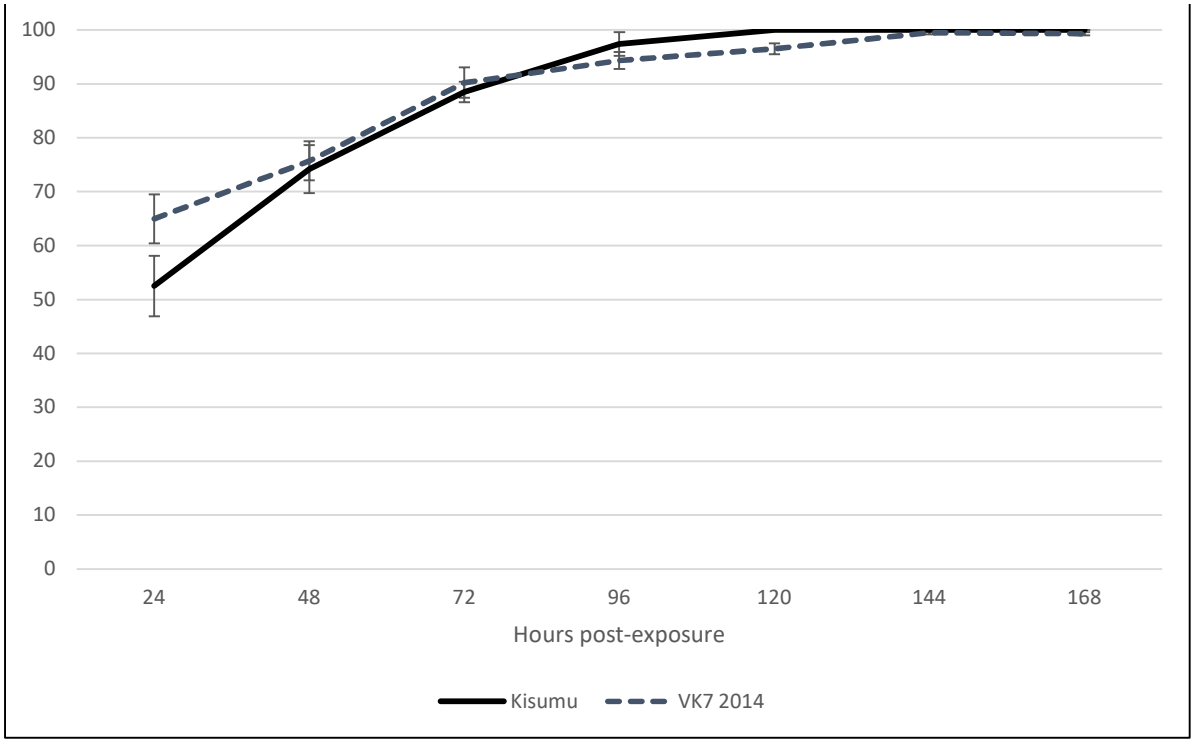

Figure 1. The average cumulative mortality of pyrethroid susceptible (Kisumu) and resistant (VK7 2014) strains of An. gambiae following exposure SumiShield in a WHO tube bioassay. Error bars represent standard error between replicate tubes of ~25 females per tube (n = 12 tubes). In each replicate of the positive control (275 mg/m2 fenitrothion) mortality was 100% by 24 hours post-exposure (n = 12 tubes) and so the data is not presented. Abbot's correction was applied where relevant.

3.2. WHO cone test: Residual efficacy of SumiShield over time

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Because mortality was seen to exceed 80% mortality and start to plateau 72-hours after exposure to SumiShield in the preliminary experiment, all results presented for the residual efficacy assay show cumulative mosquito mortality at 72-hour (3-days) post-exposure. Figures showing 24-hour and 120-hour mortality are available in Supplementary Material: Figures S1 -2). Both SumiShield and K-Othrine killed >90% of susceptible *An. gambiae* for 18 months after surfaces were treated, though there was some variability in results on mud surfaces (Figure 2). SumiShield was also very effective against resistant *An. gambiae*, which were not killed by the K-Othrine, with 100% mortality observed at 18-months (with exceptions at 3 and 9 months where mortality dropped). Mortality was less consistent over time in both resistant and susceptible *An. funestus* and varied more between surface type. However, SumiShield™ 50WG killed over 50% of exposed susceptible *An. funestus* over the 18 months period, and consistently performed better than K-Othrine against the resistant strain.

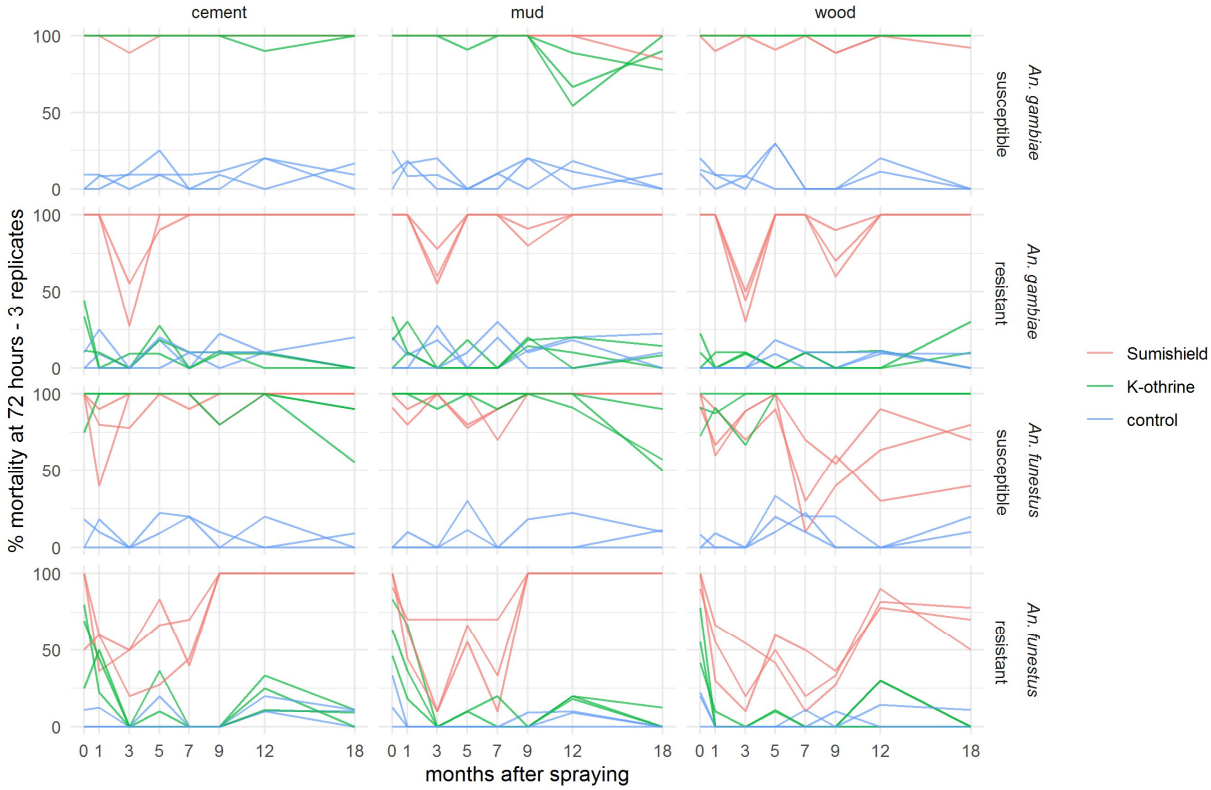

Figure 2. Residual efficacy of SumiShield and K-Othrine IRS treatments applied to different surface types against *Anopheles* mosquitoes. Mortality of resistant and susceptible strains of *An. gambiae* and *An. funestus* 72-hours after exposure to cement, mud and wood surfaces treated with SumiShield or K-Othrine is presented, in comparison to control surfaces treated with water only. Mosquitoes were exposed in a WHO cone bioassay at 24-hours, and 1, 3, 5, 7, 9, 12, and 18 months after surfaces were treated. Data from 3 replicates of each treatment and surface type are presented as separate lines.

To evaluate the additional benefit that might be achieved by a non-pyrethroid IRS used against pyrethroid resistant *Anopheles* populations, the difference in 72-hour mortality between SumiShield and K-Othrine treatments was calculated for each pair of treatments (Figure 3). In *An. gambiae* SumiShield induced very similar mortality to K-Othrine against the susceptible strain on cement and mud, and slightly worse on wood surfaces, but it killed a greater proportion of the resistant strain on all surfaces at all time points. In *An. funestus* K-Othrine killed more of the susceptible strain in most time points,

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particularly on wood, but SumiShield outperformed against the resistant strain in all but two replicate tests. There is a trend towards better relative performance of SumiShield in the later time points, suggestive of a greater residual efficacy, particularly on cement and mud surfaces.

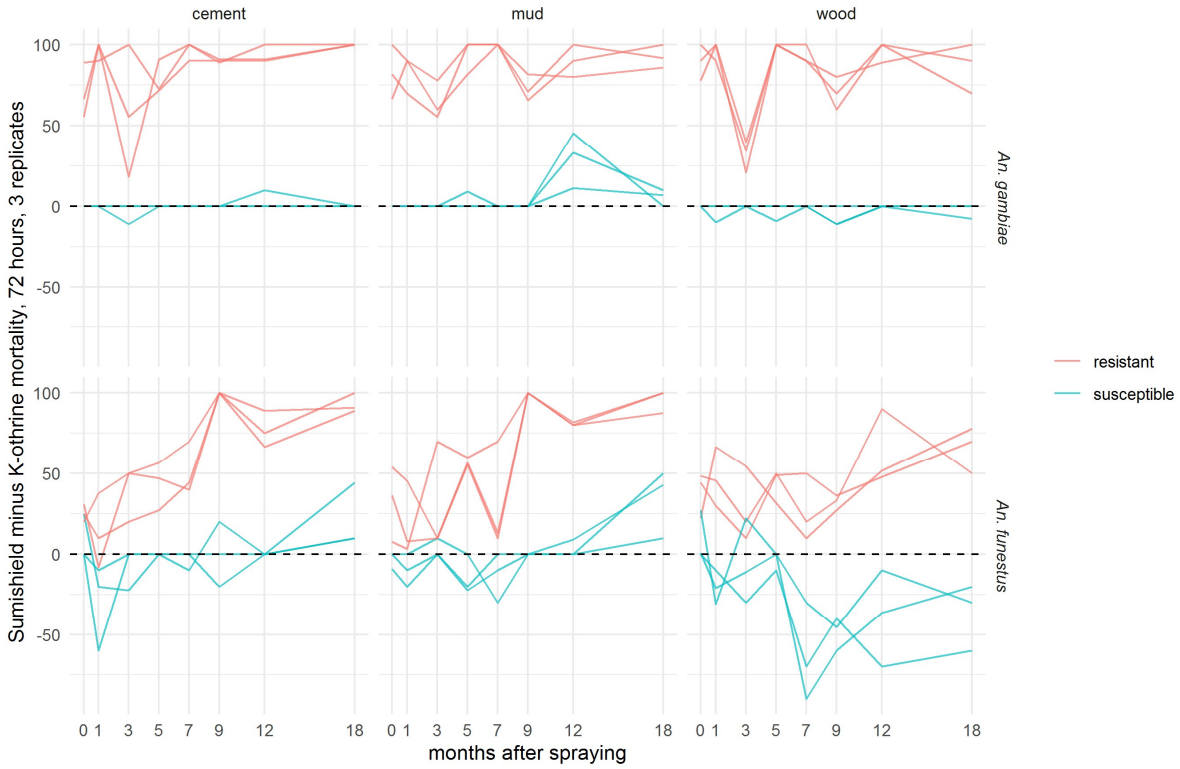

Figure 3. Efficacy of SumiShield compared directly with K-Othrine in *Anopheles* mosquitoes. Values represent the additional kill observed over time with SumiShield when testing resistant mosquitoes compared to susceptible. Here mortality of mosquitoes from insecticide-susceptible and resistant strains of *An. gambiae* and *An. funestus* observed 72-hours after exposure to cement, mud or wood surfaces treated with K-Othrine was subtracted from mortality observed after exposure to SumiShield. Mosquitoes were exposed in a WHO cone bioassay 24 hours, 1, 3, 5, 7, 9, 12, and 18 months after treatment. Data from 3 replicates of each treatment and surface type are presented separately.

Although SumiShield was specifically designed to target malaria vectors, its efficacy against *Ae. aegypti* was assessed in parallel. An opportunistic bioassay was also performed at 9 months post-treatment against the resistant Muheza strain of *Cx. quinquefasciatus*. This used backup surfaces which were made alongside the surfaces used for bioassays but previously untested. To control for any differences between these surfaces and those which had been used before, Kisumu (susceptible *An. gambiae*) were also exposed to these surfaces. The results from the backup surfaces matched those from the results of the standard Kisumu bioassays at this time point (data not shown). Mortality in susceptible New Orleans and resistant Cayman *Ae. Aegypti* strains exposed to SumiShield treated surfaces never exceeded 50%; K-Othrine also performed poorly against the resistant strain but killed 100% of the susceptible strain up to 18-months post-treatment, except on mud where mortality dropped from 5 months (Figure 4A). For *Ae. aegypti*, there was no measured advantage of SumiShield over K-Othrine in any bioassays, even against the resistant strain (Figure 4B). Results were quite variable between replicate bioassays with *Cx. quinquefasciatus* (Figure 5), but overall, where an average of 60% and 40% were killed by Deltamethrin-treated cement and wood surfaces, respectively, 5% were killed by Deltamethrin-treated mud surfaces by 72 hours post-exposure.

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SumiShield-treated wood, mud and cement killed an average of 0, 22 and 25% of exposed Muheza females, respectively. One hundred percent mortality was observed in Kisumu exposed to all K-Othrine and SumiShield treated surfaces, and in both strains exposed to control tiles mortality was <20% (data not shown).

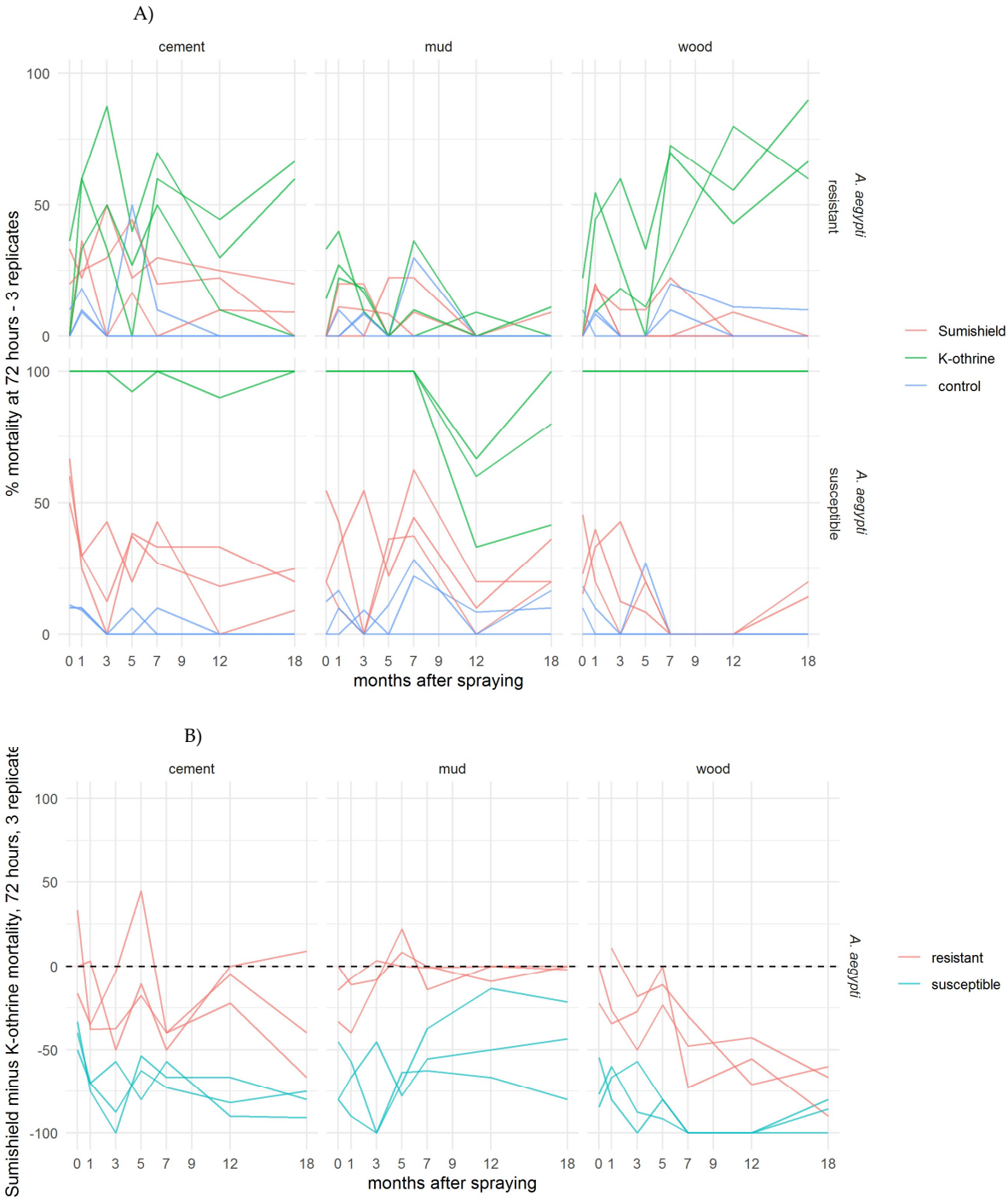

Figure 4. Residual efficacy of SumiShield and K-Othrine IRS treatments applied to different surface types against *Aedes aegypti* mosquitoes. (A) Mortality of resistant and susceptible strains 72-hours after exposure to cement, mud and wood surfaces treated with SumiShield or K-Othrine, in comparison to control water surfaces. (B) Mortality following K-Othrine exposure subtracted from mortality following SumiShield exposure. Values represent the additional kill observed over time with

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SumiShield when testing resistant mosquitoes compared to susceptible. In both assays (A & B) mosquitoes were exposed in a WHO cone bioassay 24 hours, 1, 3, 5, 7, 9, 12, and 18 months after surfaces were treated. Data from 3 replicates of each treatment and surface type are presented as separate lines.

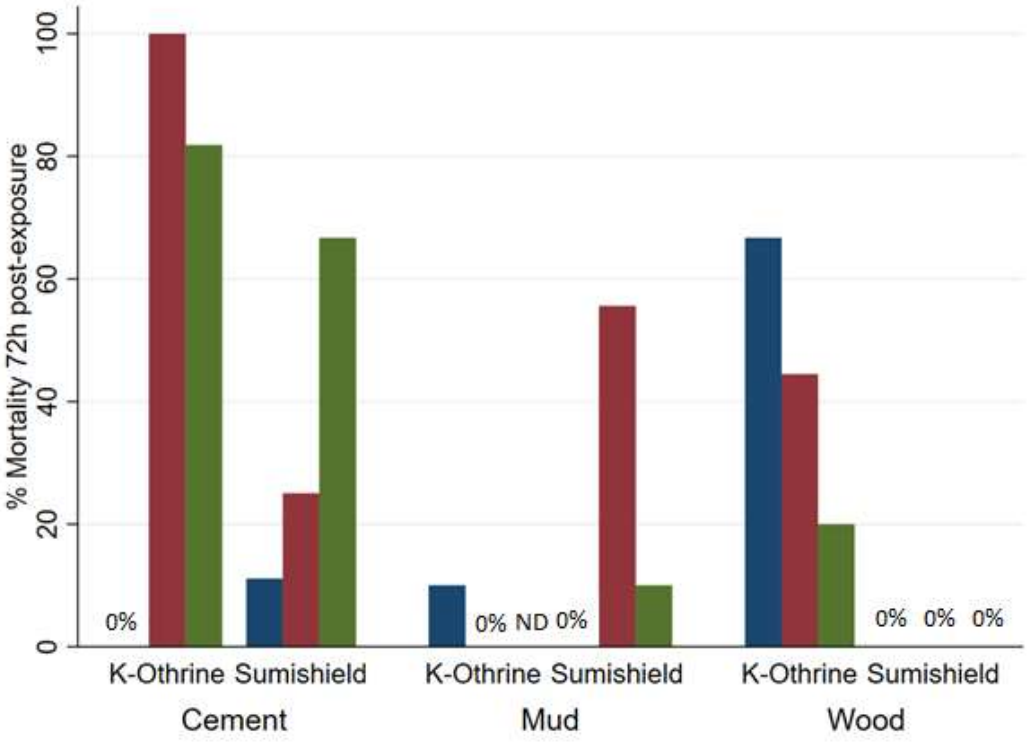

Figure 5. Mortality in *Culex quinquefasciatus* exposed to different surface types treated with K-Othrine and SumiShield. Mosquitoes were exposed for 30 minutes in a WHO cone bioassay 9 months after surfaces were treated. Results represent 3 replicates (blue, red and green bars) of each surface type, except for K-Othrine-treated mud where one tile had been used to replace a broken tile in the main experiment and results of only two replicates are shown.

To assess the duration of residual efficacy considering the speed of action of the IRS treatments, we plotted average mortality over the observation time (0-168 hours post-exposure) for each bioassay, marking the observation point at which 80% mortality was reached. Following exposure to SumiShield residual efficacy (≥80% mortality by 24-hours after exposure) lasted for the full 18 months of the experiment in Kisumu (Figure S3) and VK7 2014 (Figure S4) strains of *An. gambiae*, except for month 3 when mortality was anomalously low in VK7 2014 but recovered in months 5 onwards. Against *An. funestus* (Figure 6), mortality of the susceptible strain Fang fell below 80% at 12 months on wood and though mortality in the resistant strain FUMOZ-R dropped below 80% for several months on wood and in month 7 on mud it recovered in months 9, 12 and 18. *An. funestus* were killed more slowly by SumiShield than *An. gambiae*, particularly the resistant strain FUMOZ-R. The 80% threshold was never reached against either New Orleans (Figure S5) or Cayman (Figure S6) strains of *Ae. aegypti*. In contrast, K-Othrine exceeded the 80% threshold for the 18 months of the study against all susceptible strains (Kisumu, Fang and New Orleans, Figure S7 - S9), except for Fang and New Orleans on mud surfaces which dropped below the threshold at 12 and 18 months, though the threshold was again reached against New Orleans at 18 months post-treatment. Against the resistant strains (VK7 2014, FUMOZ-R and Cayman, Figures S10 - S12) mortality never reached 80% on any surface treated with K-Othrine. Details for control surfaces are shown in Supplementary Information, Figure S13 – S18.

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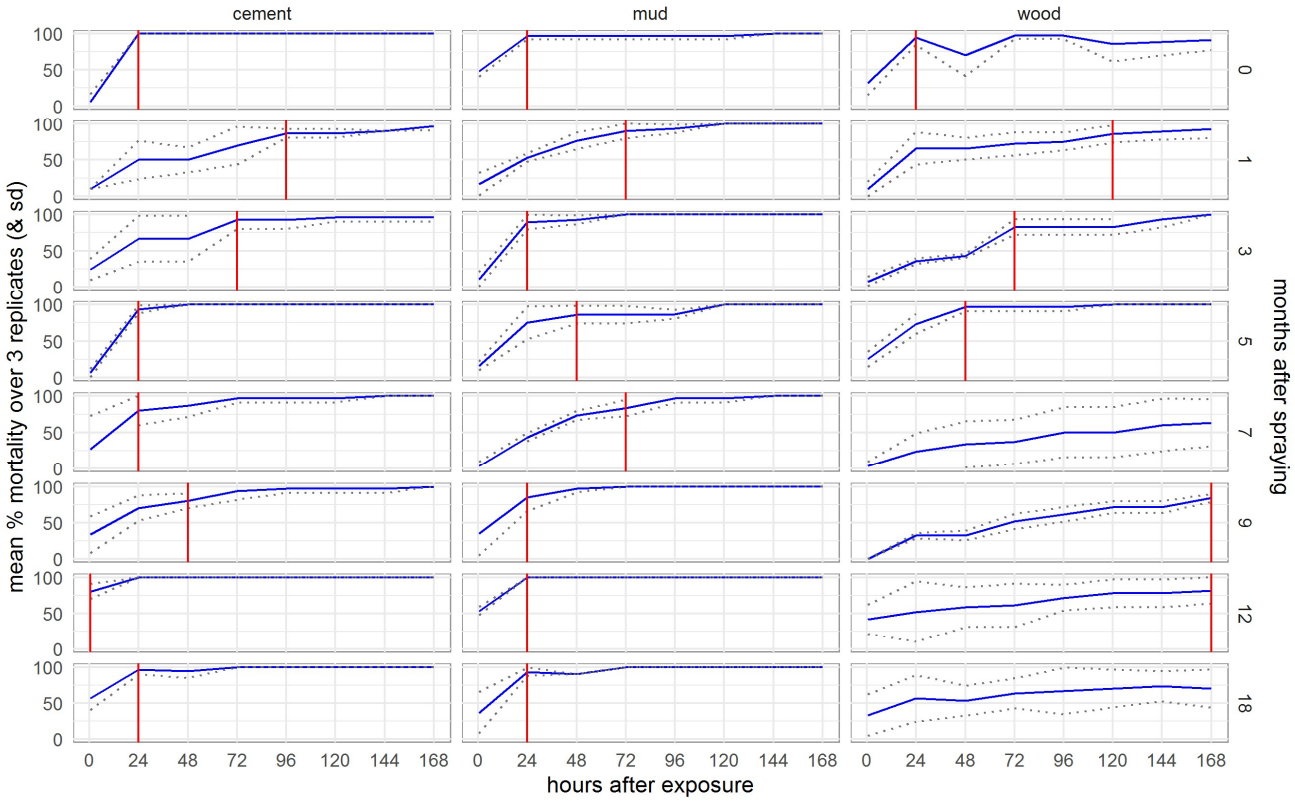

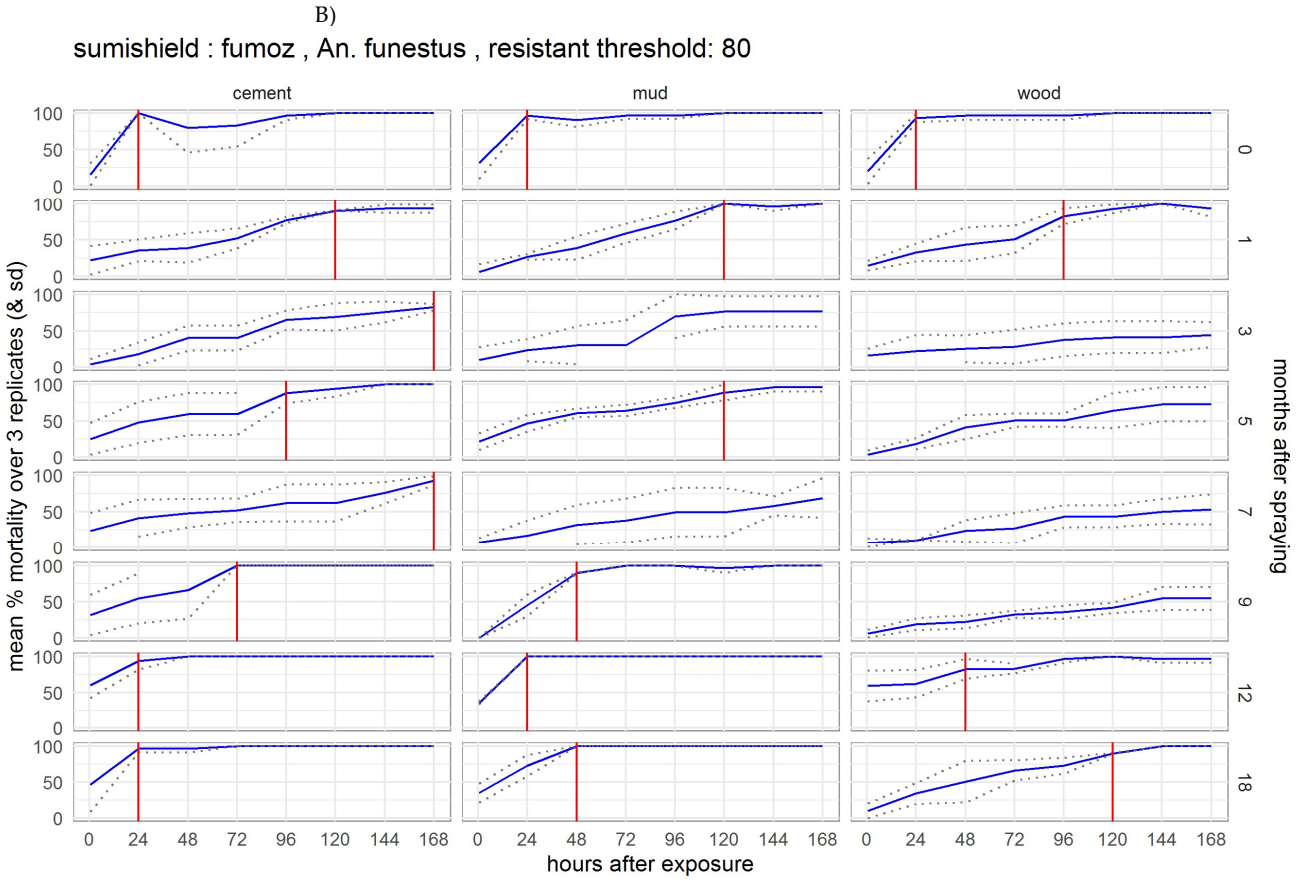

Figure 6. Speed of action of SumiShield against insecticide-susceptible (A) and -resistant (B) strains of *Anopheles funestus*. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line.

To assess the effect of application to different surfaces on the residual efficacy of SumiShield, the mortality at 72 h was aggregated for all time points by strain and surface type (Figure 7). A figure showing mortality at 120-hours post-exposure is presented in Supplementary Information: Figure S2. Across all mosquito strains tested there was poorer efficacy of SumiShield on wood surfaces, except for the resistant *Ae. aegypti* where mortality was very similar in mud and wood bioassays, and in the susceptible *An. gambiae* in which mortality was very high on all surfaces. The difference between surface types was least pronounced in *An. gambiae*. Efficacy on mud and cement was not significantly different.

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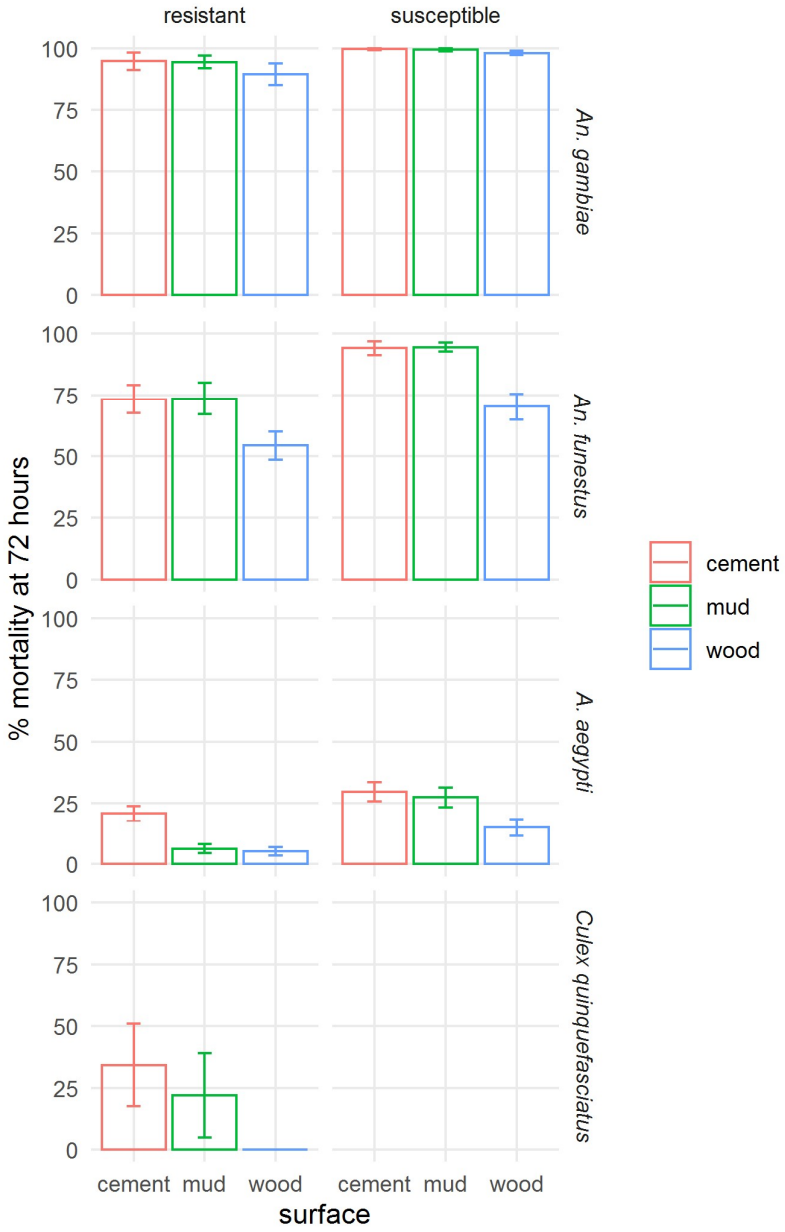

Figure 7. Effect of surface type on efficacy of SumiShield™ 50WG. Mortality of insecticide-susceptible and resistant *Anopheles gambiae*, *An. funestus* and *Aedes aegypti*, and resistant *Culex quinquefasciatus*. Average 72-hour mortality calculated across all replicate bioassays at all time points (0, 1, 3, 5, 7, 9, 12 and 18 months) for each strain and surface type is shown; error bars represent standard error across 3 replicate assays.

3.3. The effect of varied exposure time on clothianidin efficacy

SumiShield applied to a filter paper killed >80% of exposed susceptible *An. gambiae* Kisumu with an exposure time of 15 minutes by 120 h post-exposure (Figure 8). When scored at 72 h mortality was more variable but above 70% in most cases. In contrast, with an exposure time of up to 7 hours average mortality in the susceptible strain of *Ae. aegypti* (New Orleans) reached 60% only with a 7-hour exposure, and in the resistant strain (Cayman) average mortality never exceeded 20%. In all strains, 100% mortality was observed in the positive controls at all time points (data not shown).

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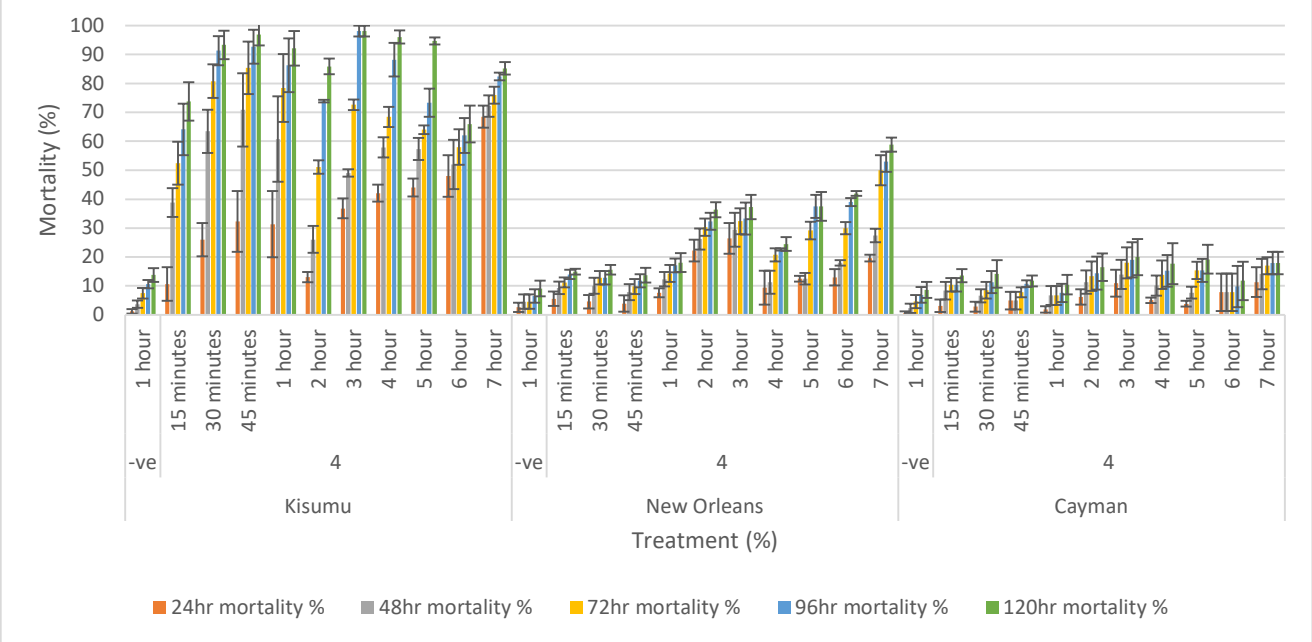

Figure 8. Effect of length of exposure to SumiShield in a WHO susceptibility tube test against susceptible (New Orleans) and resistant (Cayman) strains of *Ae. aegypti* and a susceptible strain of *An. gambiae (*Kisumu). Mosquitoes were exposed for varying lengths of time to filter papers treated with 13.2 or 733.3 mg AI/m2 of SumiShield. Error bars represent standard error between 3 replicate tubes of ~25 females per tube.

4. Discussion

Clothianidin, the active ingredient in SumiShield, is a potent insecticide which has shown promising residual efficacy against resistant malaria vectors. In a recent lab study, screening the efficacy of repurposed chemistries using CDC bottle bioassays, clothianidin was documented to have the lowest discriminating dose (8.07 µg/bottle) out of 11 AI tested [25], indicating its relatively high potency. In both Phase II hut trials and Phase III village trials SumiShield has shown good efficacy against susceptible and resistant malaria vectors. In hut trials, this efficacy has been shown to last up to 9 months against wild resistant and lab susceptible *An. gambiae* in Benin [26], [27], and in village trials up to 6 months against resistant *An. culicifacies* in India [28], [29]. These results are all based on scoring mortality after a 120-hour holding period post-exposure, rather than the standard 24-hour [30], based on observations of the delayed mortality and slower acting nature of clothianidin than pyrethroids. Similarly, this study found that mortality exceeded 80% and started to plateau at 72-hours post-exposure in the preliminary experiment, and so used 72-hour mortality as the endpoint by which to judge residual efficacy.

These studies all observed good residuality of SumiShield. However, differing methodologies, and other uncontrollable variables between these studies, precludes the assessment of efficacy across species, strain, or test surface. The completeness of our current study allows us to directly evaluate the efficacy of SumiShield against different disease vectors (*An. gambiae*, *An. funestus*, *Ae. aegypti*) and nuisance biters (*Cx. quinquefasciatus*), resistant and susceptible strains, and different surface types, which is

difficult to achieve in field conditions. The controlled nature of this lab study allows us to evaluate efficacy over time under stable conditions. This allows for direct comparisons between different groups, however it is important to acknowledge that in real life conditions are more variable, and therefore residual efficacy observed in the field may be affected by factors such as physical contact with or cleaning of walls, accumulation of dirt/dust, and fluctuating climactic conditions.

This study supports previous findings that SumiShield has potential as a potent tool to control pyrethroid resistant malaria vectors. Using a 72-hour holding period SumiShield was shown to be effective for 18 months on all surfaces tested against susceptible (Kisumu) and resistant (VK7 2014) *An. gambiae*, killing more than 80% of exposed mosquitoes in all but a few anomalous replicates with the resistant strain. This efficacy against a resistant strain is consistent with previous findings of the absence of cross resistance to clothianidin in field populations of *Anopheles* with multiple resistance mechanisms, measured using diagnostic doses of 150 µg/bottle in a CDC bottle bioassay [31] in Western Kenya [32] and 2% w/v clothianidin on filter papers in a WHO tube test [33] in sites in 16 African countries [34]. For *An. funestus* results were more variable in this residual efficacy study, and efficacy apparently varied by surface type, likely due to bioavailability and therefore uptake being affected by the nature of the surface. However, SumiShield performed consistently better than K-Othrine (deltamethrin) against the insecticide-resistant *An. funestus* strain (FUMOZ-R). Against all resistant strains tested K-Othrine only reached the 80% efficacy threshold in one species, on one surface, at one time-point (*An. funestus* FUMOZ-R strain, mud surface, 24-hour post-treatment). In all other instances, mortality remained <80% even when measured up to 7 days post-exposure. Against the susceptible strains, there was more variability, and SumiShield provided little or no increase in efficacy over K-Othrine against *An. gambiae* or *An. funestus*, particularly on wood. Nonetheless the product offers an advantage over pyrethroid products in areas of high resistance, where resistance management recommendations from the WHO would advise against use of pyrethroid-based vector control in any case [35]. The persistence of SumiShield efficacy over 6 months (indeed up to 8 months) against susceptible strains has significant impacts on its operational use and an improvement over other IRS formulations currently on the market, some of which have less than 6 months' efficacy under field conditions. There is thus no clear evidence from this study for cross-resistance to SumiShield in *An. coluzzi* collected from field sites in Burkina Faso, though there may be some in *An. funestus* collected from Mozambique. This would have to be confirmed through metabolic or molecular investigation to understand the mechanisms of any cross-resistance, most likely acting through metabolic upregulation. The potential impact of this level of cross-resistance to clothianidin on field efficacy of the product would also need to be evaluated.

Comparatively, in *Ae. aegypti* (both susceptible and resistant) the 80% efficacy threshold was never reached on any test surface treated with SumiShield. Limited efficacy was also observed in *Cx. quinquefasciatus*, even when exposure time was increased to up to 7 hours against both strains, though high variability between replicates and the single time point tested with this strain makes strong conclusions difficult to draw. Differences in

susceptibility between the two genera (*Anopheles* and *Aedes*) have been observed previously to a range of chemistries (authors' observations). This difference appears to be more than the effect of the species' size, although size can have an impact on susceptibility [36]–[38]. It is potentially related to differences in metabolism between species, though the full explanation warrants further investigation. Intrinsic activity of clothianidin, measured by topical application, was identical in susceptible *Aedes aegypti* New Orleans strain (Supplementary information, Table S2) compared to susceptible *An. gambiae* Kisumu strain [25] with almost all treated mosquitoes dying after treatment with all concentrations tested. This suggests that the reason for the lower efficacy may be due to reduced tarsal uptake, possibly related to formulation effects, or thicker tarsi in *Aedes*, rather than directly related to the innate potency of the compound. If this is the case, a longer exposure time might have revealed improved efficacy against *Aedes*, though mortality was only increased to around 60% even after 7 hours of exposure to a treated surface in the susceptible New Orleans strain. It is important to establish why efficacy varies between genera, as lack of efficacy against a subset of species may affect acceptability of the product and could result in poor uptake in the field, particularly in locations where nuisance biters predominate.

Some variation in mortality was observed in the current assay. Variation documented between experimental replicates conducted on the same day is an artefact of testing only ten mosquitoes per replicate. Variation in efficacy over time was observed, however was not linear (i.e. a reduction in 72-hour mortality in resistant *An. gambiae* (VK7) was measured at 3-months, however efficacy was restored at 5-months). This may be due to micro-variations in rearing conditions and fitness of the mosquito cohorts used at different time points, though no correlation between mosquito mortality and weight was clear between time points (Supplementary information, Figure S20). As this non-linear variation was not observed in other species at the same timepoint it is unlikely to be due to stability of SumiShield affecting bioavailability of the AI.

The WHO guidelines for evaluating IRS adulticides state that a 24-hours holding period before scoring mortality should be used in bioassays to judge product efficacy [21]. These standard testing methods were developed to assess fast acting pyrethroid insecticides and so are not suitable for AIs such as clothianidin which have a slower acting MoA. This was demonstrated by the current study, when in a preliminary test > 80% efficacy - in susceptible and resistant *An. gambiae* - was only achieved by 3-days post-exposure. Subsequently, in this current study 72-hour mortality was used to determine the products efficacy. If 24-hour mortality had been selected the residual efficacy over time would be reduced, as has been observed previously [39]. When products with a novel MoA are tested, it is vital that preliminary studies consider a wider range of outcomes than rapid knock down or kill. For example, any reductions in a mosquito lifespan, or mortality before it has time to become infectious, will have dramatic impacts on a mosquito's disease transmission potential. By only measuring immediate (within 24-hour) mortality, current tests fail to detect outcomes which could significantly relate to the effectiveness of a product under real life conditions. The additional kill more than 24h post-exposure to

clothianidin is not a limitation for an IRS application, where efficacy stems from a community effect.

Under controlled conditions SumiShield showed differential efficacy against the surface types tested. Mortality was lower on wood in comparison to cement and mud. Mud and cement are by far the most commonly used housing materials utilised across Africa, on which it is recommended IRS products be evaluated [30], so the higher efficacy on these surfaces is encouraging. To account for this differential efficacy IRS spray programmes could document house interior surfaces prior to spray treatment and could potentially factor in re-spaying at earlier time points in houses with wood interiors. This would add a level of logistical complexity, which would be best managed at a community level, though in many areas of malaria endemicity wood is not as common a building material as mud and particularly concrete. The properties of wood depends on the tree from which it originates due to factors such as coarseness of the grain. Beech was used for this study but is not common in Sub Saharan Africa, and testing residual efficacy against local woods would better predict performance of an IRS formulation.

Comparing the results from the previously untested backup surfaces, with those from the parallel surfaces - which had been treated and stored in the same way but not used for bioassays -demonstrates that using surfaces for bioassays does not diminish the efficacy of the insecticide-treated surfaces. Any loss in efficacy over time can therefore be attributed to physical or chemical changes in the surfaces and/or the applied insecticide, and not a loss of material from the surfaces during bioassays.

5. Conclusions

Clothianidin is a potent insecticide against *Anopheles* vectors of malaria, and here we show that SumiShield is an effective IRS product against *An. gambiae* and *An. funestus*, with residual efficacy up to at least 18 months on a variety of representative building materials. Long lasting killing action was demonstrated against a strain of *An. gambiae* which is resistant to a range of insecticide classes, and although results were less clear against susceptible and resistant strains of *An. funestus* SumiShield was far more effective than a deltamethrin-based IRS comparator. Results suggest that the 24h holding period used to evaluate efficacy of IRS products may not be suitable for vector control tools based on clothianidin, and a 72-hour holding period gives a more accurate measure of its efficacy. However, even with a longer holding period and a much-extended exposure period SumiShield 50WG treated surfaces were not very effective in killing *Ae. aegypti* or *Cx. quinquefasciatus*. Consideration should, however, be given to managing expectations in its performance against nuisance biters, and to the nature of the wall surfaces in the houses where it is to be sprayed. The great potential of this IRS product against mosquitoes that transmit malaria, even in areas of high pyrethroid resistance, is again demonstrated. At 18 months after treatment of surfaces, 100% efficacy was still observed on the key surfaces of mud and cement in resistant and susceptible strains of *An. gambiae* and *An. funestus.*

Supplementary Materials: The following are available online. **Figure S1.** Residual efficacy of SumiShield and K-Othrine applied to different surface types against *Anopheles* mosquitoes. Mortality of resistant and susceptible strains of *An. gambiae* and *An. funestus* at 24-hours, after exposure to cement, mud and wood surfaces treated with SumiShield or K-Othrine is presented, in comparison to control surfaces treated with water only. Mosquitoes were exposed in a WHO cone bioassay at 24-hours, and 1, 3, 5, 7, 9, 12, and 18 months after surfaces were treated. Data from 3 replicates of

each treatment and surface type are presented as separate lines; **Figure S2.** Residual efficacy of SumiShield and K-Othrine applied to different surface types against *Anopheles* mosquitoes. Mortality of resistant and susceptible strains of *An. gambiae* and *An. funestus* at 120-hours, after exposure to cement, mud and wood surfaces treated with SumiShield or K-Othrine is presented, in comparison to control surfaces treated with water only. Mosquitoes were exposed in a WHO cone bioassay at 24-hours, and 1, 3, 5, 7, 9, 12, and 18 months after surfaces were treated. Data from 3 replicates of each treatment and surface type are presented as separate lines; **Figure S3.** Speed of action of SumiShield against insecticide-susceptible *An. gambiae* Kisumu. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S4.** Speed of action of SumiShield against insecticide-resistant *An. gambiae* VK7. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S5.** Speed of action of SumiShield against insecticide-susceptible *Ae. aegypti* New Orleans. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S6.** Speed of action of SumiShield against insecticide-resistant *Ae. aegypti* Cayman. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S7.** Speed of action of K-Othrine (deltamethrin) against insecticide-susceptible *An. gambiae* Kisumu. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S8.** Speed of action of K-Othrine (deltamethrin) against insecticide-susceptible *An. funestus* Fang. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S9.** Speed of action of K-Othrine (deltamethrin) against insecticide-susceptible *Ae. aegypti* New Orleans. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S10.** Speed of action of K-Othrine (deltamethrin) against insecticide-resistant *An. gambiae* VK7 2014. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S11.** Speed of action of K-Othrine (deltamethrin) against insecticide-resistant *An. funestus* FUMOZ-R. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S12.** Speed of action of K-Othrine (deltamethrin) against insecticide-resistant *Ae. aegypti* Cayman. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S13.** Speed of action of purified water control against insecticide-susceptible *An. gambiae* Kisumu. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S14.** Speed of action of purified water control against insecticide-resistant *An. gambiae* VK7 2014. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S15.** Speed of action of purified water control against insecticide-susceptible *An. funestus* Fang. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S16.** Speed of action of purified water control against insecticide-resistant *An. funestus* FUMOZ-R. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S17.** Speed of action of purified water control against insecticide-susceptible *Ae. aegypti* New Orleans Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S18.** Speed of action of purified

water control against insecticide-resistant *Ae. aegypti* Cayman. Cumulative mortality is shown by hours post-exposure to treated cement, mud, and wood surfaces, for each month of the experiment. Time to reach the WHO-recommended 80% mortality threshold for an IRS product is marked with a red line; **Figure S19.** Effect of surface type on efficacy of SumiShield. Mortality of insecticide-susceptible and resistant *An. gambiae*, *An. funestus* and *Ae. aegypti*, and resistant *Cx. quinquefasciatus*. Average 120-hour mortality calculated across all replicate bioassays at all time points (0, 1, 3, 5, 7, 9, 12 and 18 months) for each strain and surface type is shown; error bars represent standard error across 3 replicate assays; **Figure 20.**

Table S1. The physical characteristics and chemical properties of the mud used to prepare the mud surfaces used for testing. Characterisation was conducted by ACS Testing Ltd (Poole, Dorset) to determine the physical characteristics and chemical properties of the mud.

Table S2. Intrinsic activity of clothianidin, measured as mortality after topical application in solution with acetone, in susceptible strains of *Anopheles gambiae* (Kisumu) and *Aedes aegypti* (New Orleans). Kisumu data has been previously published. Mean mortality represents an average of three cohorts of ten 2-5 day old non-blood fed female adult mosquitoes, corrected for negative control mortality using Abbott's formula.

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