

Residual Efficacy of Fludora® Fusion Against Anopheles Arabiensis in Simple Huts in Ethiopia

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Abstract

Emergence and spread of malaria vectors resistant to the available insecticides required a new and efficacious insecticide. Residual efficacy of Fludora® Fusion was evaluated against insecticide susceptible *Anopheles arabiensis* in ten circular huts similar to the residential huts. Fludora® Fusion WP-SB 562.5, FICAM WP80 and Clothianidin WG70 were sprayed, by experienced technician, on interior wall surfaces: paint, dung, smooth mud, and rough mud. WHO cone bioassays were carried out a month after spraying and thereafter on monthly intervals for 12 months. Knockdown was recorded at 60 minutes and mortality at 24 hours, 48 hours and 72 hours holding time post-exposure. Fludora Fusion induced 100% *An. arabiensis* mortality during the first four months post-treated on all surface types at 24 hours holding time post-exposure. Its activity remained over 80% from the fifth to the twelfth month post-treated on the surfaces with the exception of two assessment points, at seventh month and eleventh month, on paint and smooth mud surfaces. FICAM induced 100% mortality rate during the first 4 months and 92% mortality during the fifth month post-treatment on painted surfaces. Its activity was over 96% mortality 1-month post-treatment on smooth mud and rough mud surfaces and 92% mortality 2-month post-treatment on dung surfaces. Clothianidin caused 89% and 86% mortality 1-month post-treatment on smooth mud and rough mud surfaces. Fludora Fusion can be used as alternative indoor residual insecticide spraying against *An. arabiensis* in Ethiopia. However, the insecticide shall also be tested in residential houses prior to employing at a larger scale.

Introduction

Malaria is among the top diseases in Sub-Saharan Africa that cause severe sickness, death and huge economic loss every year [1, 2]. World Health Organization reported 219 million cases and 435, 000 deaths of malaria globally among which 92% of the cases and 93% of the deaths were from the WHO African Region in 2017. *Plasmodium falciparum* is the most prevalent parasite that accounted for 99.7% of the cases and the majority of malaria related deaths in the continent. Children aged less than 5 years old contributed to about 60% of the deaths in the world. On top of this, about 79% of malaria infected children had anemia. According to the report, Ethiopia had over 240,000 lower cases in 2017 compared to the cases in the 2016. However, the difference was not significant [2] which entails that malaria is still a public health problem in the country.

Vector control use to be a major malaria prevention strategy as it provides personal protection from mosquito bites and hence reduces disease transmission. Universal coverage of core vector interventions namely long lasting insecticide treated mosquito nets (LLINs) and indoor residual insecticide spraying (IRS) is being implemented in populations at risk of malaria [3]. However, the major malaria vectors have developed resistance to most of the available insecticides of choice from the four insecticide classes (pyrethroids, organochlorines, carbamates, organophosphates) commonly used in the core vector intervention strategies[4]. Resistance to pyrethroids, the only insecticide class currently used in LLINs, is reported to be highest in Africa. This together with the continued emergence of antimalarial drug resistant *Plasmodium* parasites remains an impediment to the control and elimination of malaria [2].

In Ethiopia, malaria vector control primarily depends on LLINs followed by IRS. The widespread deployment of LLINs and IRS contributed to the reduction of malaria transmission in the country in particular and in the WHO African Region in general since 2010[2, 3, 5]. Ethiopia aimed near zero malaria deaths, reduced malaria cases by 75% from baseline of 2013, and malaria eliminated in selected areas by the year 2020 and beyond [6, 7]. However,

the increasing occurrence of malaria vectors resistant to insecticides that are being used in IRS and LLINs put the current control efforts at risk [8-10].

In order to maintain the declining trend of malaria and ultimately control/eliminate the disease, having new and efficacious insecticides remains a top priority. In line with this, Bayer developed a new insecticide formulation, Fludora® Fusion, the first two-way indoor residual spray solution that combines the neonicotinoid clothianidin with a second insecticide with an unrelated mode of action- the pyrethroid deltamethrin. The residual insecticidal activity of Fludora® Fusion against insecticide susceptible and laboratory reared *An. Arabiensis* was evaluated in simple huts in central Ethiopia. *An. arabiensis* has been the most important malaria vector in the country and hence the target for control using IRS and LLINs.

Methods

3.1. Study area and hut construction

Ten circular huts were constructed in Edo Gojolla Kebele (the lowest political administrative population unit in Ethiopia), Adami Tulu Judo Kombollcha District, East Showa Zone, Oromia Regional State, Ethiopia, located at about 160 Kms from Addis Ababa, along the road to Hawassa. The huts, each having a sprayable surface of 12.56 m² (2π X 2 m x 2 m), were constructed on land owned by local farmers interested to construct the huts (cost covered by the project) and later on to own the huts at the end of the trial. The huts were made of mud bricks and thatched roofs similar to the residential huts in the area. The interior of each hut wall was divided to four equal surfaces and each surface was plastered to have one of four surface types: rough (one layer of mud), smooth (two layers of mud), cow dung (cow dung over two layers of mud) and painted (a layer of paint over a layer of lime, applied to 2nd smooth layer of mud). After construction was completed; the huts were locked and left to dry under natural environmental conditions.

3.2. Insecticide products evaluated

1. Fludora® Fusion WP-SB56.25 is a new insecticide formulation for Indoor Residual Spraying. The formulation is a Wettable Powder in water soluble bags (WP-SB) available in 100g sachets. The product contains two active ingredients: 500 g/kg clothianidin + 62.5 g/kg deltamethrin. The application rate of the product is 200 mg clothianidin/sqm and 25 mg deltamethrin/sqm.
2. Clothianidin WG70: containing 700g/kg clothianidin - applied at 200 mg/sqm
3. FICAM WP80: containing 800 g/kg FICAM - applied at a rate of 400 mg/sqm.

3.3. Insecticide spraying

Nine huts were randomized into three experimental groups (each group having three huts) and the remaining tenth hut was left as an untreated control. The walls were checked for absence of insecticidal activity prior to spraying of the insecticides. A spraying operator having long time experience undertook the spraying on December 5, 2017 following Standard Operating Procedures and WHO manual [5, 11, 12]. One group of the experimental huts was sprayed with Fludora® Fusion WP-SB56.25, the second with FICAM WP80 and the third with Clothianidin WG70 following the instructions of the manufacturer (Bayer). The control hut was sprayed with equal volume of water. The walls were sprayed to attain dosages as per the manufacturer's recommendation using the standard Hudson Xpert pump with 8002E nozzles with a volume of 10 L. One sachet containing a formulation of an insecticide was

placed in the pump containing 10 L of water and was allowed to dissolve, followed by agitation of the pump to ensure adequate mixing. The pressure inside the pump was adjusted to 55 psi after which the products were applied via indoor residual spraying (IRS) with the maximum application rate amounting to 40 mL spray/m². Calibration of the pumps was done prior to spraying to obtain uniform and good quality spraying for the target dose. Protective clothing, goggles and gloves were provided to the spray man for general safety. Sachets and waste water were disposed according to WHO guidelines [5, 12]. The huts were kept free of animal and human contact until the end of the trial.

3.4. Bioassays

An insectary colony of *An. arabiensis* DebreZeit strain, susceptible to all insecticides and maintained at the Aklilu Lemma Institute of Pathobiology, Addis Ababa University was used for evaluation of the residual efficacy of Fludora® Fusion. WHO cone bioassays [13] were carried out a month after spraying and thereafter on monthly intervals for 12 months. On the day of the bioassays, the floor of the huts was wetted with water to create favorable room temperature and relative humidity conditions for the trial. Three cones were attached on each surface type at different heights by using small nails. Ten unfed, 2-4 days old, female mosquitoes were transferred to each cone using mouth aspirator (a separate aspirator was used for each insecticide/treatment) and exposed for 30 minutes. After 30 minutes of exposure, the mosquitoes were transferred to clean holding cages and then supplied with sugar solution by moistening a pad of cotton. Cages were kept in polythene bags with damp cotton inside to create favorable temperature (+ or – 25°C) and humidity (above 60%) for the survival of mosquitoes and placed in an insecticide free room. Knockdown was recorded at 60 minutes and mortality at 24 hours, 48 hours and 72 hours holding time post-exposure in order to cover delayed mortality effects as well.

Data analysis

Data was entered into Microsoft Excel spread sheets from which percentages of knockdown and mortality were determined and results interpreted according to WHO [13]. As mortality in the control groups was above 5% no Abbott's formula [14] based mortality correction was made. The Excel data was transferred to IBM SPSS Statistics 20 and fitted to generalized linear models statistics to compare mortality effects for *Anopheles arabiensis* mosquitoes 24 hours-post exposure to the insecticides, each insecticide on four different types and heights, and time since insecticides sprayed.

Ethical consideration

This study was reviewed and approved by the Institutional Review Board (IRB) of Aklilu Lemma Institute of Pathobiology, Addis Ababa University. Permission to conduct the study was obtained from local government officials in Adami Tulu Judo Kombollcha district. Huts were constructed on lands owned by local farmers after obtaining their consent. Consent was obtained from the farmers, owning the lands, after they had been clearly informed about the study objectives, methodology, anticipated benefits, and discomforts.

Results

Residual knockdown effect of insecticides

A total of 12,600 insecticide susceptible female *Anopheles arabiensis* DebreZeit strain were exposed to wall surfaces among which 11,160 were in experimental huts and 1,440 in the control. FICAM induced 100%

knockdown (KD) against *An. arabiensis* until 4 months post-treatment on painted surfaces (Figure 1). Its KD effect, on painted surfaces, reduced starting from the fifth month. Its KD effect on dung, smooth mud and rough mud surfaces was generally low and continued to decline until the seventh month. The residual KD effect of Fludora Fusion was consistently high (>80%) on dung surfaces for 12 months and on smooth mud and rough mud surfaces for 9 months post-treatment. Clothianidin had the lowest residual KD effect (10% or below) on the surfaces compared to Fludora Fusion and FICAM through the 12 months assessment points.

Mortality rate of *An. arabiensis* at 24 hours holding time post-exposure to treated surfaces

Fludora Fusion induced over 80% *An. arabiensis* mortality during the 12 months post-treatment period on painted surfaces with the exception of two assessment points at seventh month (48%) and eleventh month (69%) (Figure 2). It caused 100% mortality for 6 months and above 80% mortality during the seventh to the twelfth month post-treatment on dung surfaces. Its activity on smooth mud surfaces was above the 80% mortality threshold for the 12 month duration of the trial. It also revealed 100% mortality on rough mud surfaces up to 4 months and 90% (on average) mortality from the fifth month to the twelfth month on rough mud surfaces. Fludora caused the most significant ($p < 0.001$) *An. arabiensis* mortality up to the seventh month post treatment and significantly ($p < 0.001$) higher mortality compared to Clothianidin up to the 12th month post-treatment. However, the difference among the number of dead mosquitoes after exposure to the four surface types was not significant ($p > 0.05$).

FICAM induced 100% mortality rate during the first 4 months and 92% mortality during the fifth month post-treatment on painted surfaces. Its activity reduced to below 80%, on these surfaces, during the sixth and seventh months. Its activity was over 96% mortality 1-month post-treatment on smooth mud and rough mud surfaces. In addition, it induced 92% mortality 2-month post-treatment on dung surfaces. Clothianidin caused 89% and 86% mortality 1-month post-treatment on smooth mud and rough mud surfaces.

Mortality rate of *An. arabiensis* at 48 hours holding time post-exposure to treated surfaces

Given that evaluations prior to 6 months post-treatment generally revealed mortality levels above the 80% WHO threshold, there was no need to additionally run delayed mortality assessments. These results therefore start from the sixth month post-treatment. At 48 hours holding time post-exposure, Fludora Fusion caused over 80% mortality except during the sixth month (69%) and the eleventh month (71%) post-treatment on painted surfaces (Figure 3). Its activity on dung surfaces ranged from 86% to 100%. Its residual activity on smooth mud and rough mud surfaces also remained above the 80% threshold from the sixth month to the twelfth month post-treatment. The residual activities of FICAM WP80 and Clothianidin WG70 were below 80%. Fludora Fusion, FICAM and Clothianidin induced relatively higher insecticidal activity with the increase in holding time of exposed mosquitoes from 24 hours to 48 hours.

Mortality rate of *An. arabiensis* at 72 hours holding time post-exposure to treated surfaces

Residual efficacy of Fludora Fusion and Clothianidin against *An. arabiensis* increased with the extension in the holding time of the mosquitoes from 24 hours to 72 hours post exposure to treated surfaces. The residual activity of Fludora Fusion on painted, dung, smooth mud and rough mud surfaces remained above 80% efficacy from the eighth month to the twelfth month post-treatment (Figure 4). The activity of Clothianidin on all surface types increased as well with the increase in the holding time of the mosquitoes exposed to treated surfaces from 24 hours to 72 hours. Its delayed activity was relatively higher on smooth mud and rough mud surfaces.

Discussion

Fludora® Fusion, a new indoor residual spraying formulated from clothianidin (a neonicotinoid) and deltamethrin (a pyrethroid), caused over the WHO threshold (80%) residual insecticidal efficacy against susceptible *Anopheles arabiensis* for a period of 12 months post-treatment on painted, dung, smooth mud and rough mud surfaces of simple huts, similar to the huts of the local inhabitants, in Ziway area, central Ethiopia. The residual efficacy of Fludora Fusion against the mosquitoes increased with the extension in holding time of the exposed mosquitoes from 24 hours to 72 hours. This indicates a longer residual persistence and insecticidal activity of Fludora Fusion against insecticide susceptible *An. arabiensis*.

In Benin, Fludora Fusion was observed to induce over 80% efficacy against wild insecticide resistant *An. gambiae* s.s for over a 9-month period on cement walls and for a 6-months period on smooth mud walls. Its efficacy was observed to be above the threshold for over a 10-month and 3-month period on cement and smooth mud surfaces respectively against laboratory susceptible "Kisumu" *Anopheles gambiae* [15]. In another study in the country, Fludora Fusion was found to induce overall mortality rates of >80% on mud and cement walls for over 8 months against wild pyrethroid resistant *An. gambiae* s.l. [16]. In Equatorial Guinea, it caused over 80% residual efficacy, on wooden surfaces, against malaria transmitting wild pyrethroid resistant *Anopheles* mosquitoes for a period of 8 months [17]. The very high and prolonged knockdown and efficacy of Fludora Fusion against the laboratory reared *An. arabiensis* warrant its potential use in IRS programs in Ethiopia. In addition, its residual insecticidal effect against susceptible and resistant malaria vectors [15-17] strengthens Fludora Fusion as the insecticide of choice for IRS programs in view of the widespread insecticide resistance.

The strong residual efficacy of Fludora Fusion on pyrethroid susceptible and resistant malaria vectors might be attributed to their complementary mode of action. As a mixture product it assures contact by the mosquito to two active ingredients at the same time [16]. Therefore, the mosquito needs to detoxify both regardless if they are pyrethroid resistant or not. Clothianidin is a molecule that belongs to the chemical class of the neonicotinoids, so it mimics the effect of acetylcholine or even nicotine and binds at the nicotinic acetylcholine receptor. That triggers a cellular influx of sodium ions at the presynaptic membrane and the opening of additional sodium channels. These open state channels represent the major binding target for the second active ingredient of the Fludora Fusion mixture which is the type II pyrethroid deltamethrin.

FICAM caused over 80% mortality for five months on painted surfaces, for two months on dung surfaces and for one month on both smooth mud and rough mud surfaces. In a previous study in the district, the mortality rate of *An. arabiensis* exposed to FICAM treated painted surfaces was observed to be high up to four months [18]. In another experimental hut trial, 5 km away from Nazareth Town (95km southeast of Addis Ababa), FICAM mixed with high pH water (pH 8.0) and sprayed on dung walls killed more than 80% of the exposed susceptible *An. arabiensis* (Adama strain) up to three months and on mud surfaces killed more than 80% only for one month [19]. In Cameroon, mortality rates of Kisumu susceptible strain of *Anopheles gambiae* s.s exposed to FICAM treated mud surfaces was observed to be over 80% on mud surfaces for three months [20]. In Madagascar, FICAM treated on cement, wood, tin, mud and vegetal materials surfaces caused a mortality rate of 89-100% against *An. arabiensis* during 3 months post treatment but varying from the 4th month [21].

Clothianidin caused over the WHO threshold (80%) *An. arabiensis* mortality 1 month after sprayed on smooth mud and rough mud surfaces. Then after, its activity remained low during the study period. The residual activity of clothianidin was generally observed to be higher on smooth mud and rough mud surfaces compared to the

painted and dug surfaces. Even so, its residual activity increased with the increase in the mosquito holding time post exposure from 24 hours to 72 hours. In Benin, at 72 hours post-exposure, the residual efficacy above the WHO threshold (80%) against susceptible *An. gambiae* s.s on smooth mud surfaces lasted only 4 months[15].

Limitation Of The Study

we were unable to carry out efficacy assessment following a fast decline in the mortality of mosquitoes during the seventh and eleventh assessment points due to the very low number of mosquitoes to repeat the experiment.

Conclusion And Recommendation

Fludora Fusion revealed a 12-month residual efficacy against insecticide susceptible laboratory breed *Anopheles arabiensis* on paint, dung, smooth mud and rough mud surfaces of simple experimental huts in Ziway area, central Ethiopia. Thus, the product can be considered as an alternative indoor residual spraying insecticide in the country. However, we recommend further evaluation of the product in human habitation houses for use at wider scale.

Declarations

Acknowledgments

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Authors' contributions

AA designed the study, collected the data, analyzed the data and interpreted the results, and prepared the draft manuscript. AA and SH reviewed the manuscript. Both authors read and approved the final version of the manuscript.

Conflict of Interest

AA declares no financial or non-financial competing interests. SH is employee of Bayer AG and is working in the product development group for vector control products. As employee it is part of his duty and responsibility to take care and monitor developing resistance in target organisms in malaria endemic countries. In his role as field trial coordinator he contributed to this publication, but his salary is independent from this publication and he will not receive any bonus.

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Figures

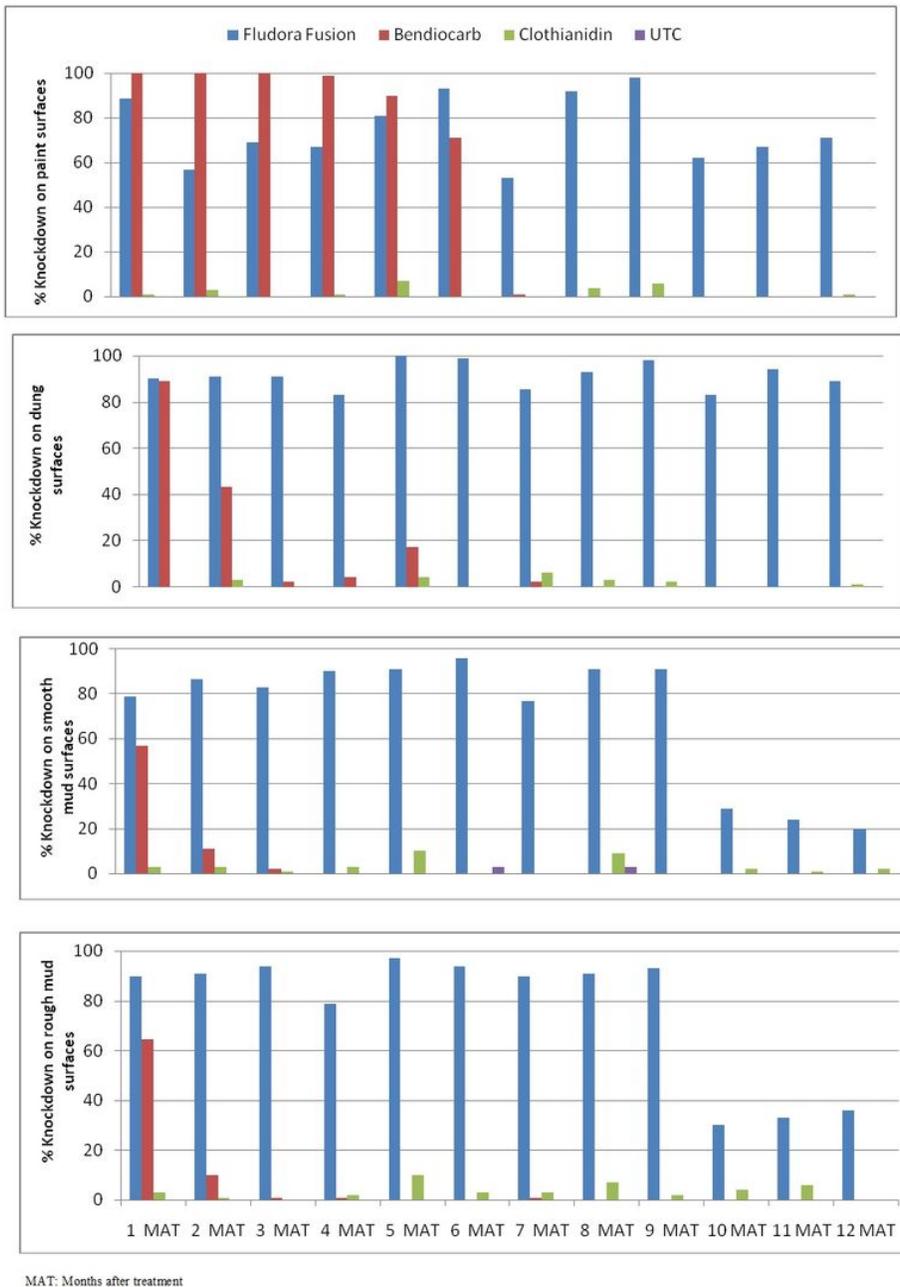


Figure 1

Residual knockdown activity of insecticides, against susceptible *Anopheles arabiensis* on four surface types of simple huts wall cone bioassays at 60 minutes holding time post-exposure, Ethiopia

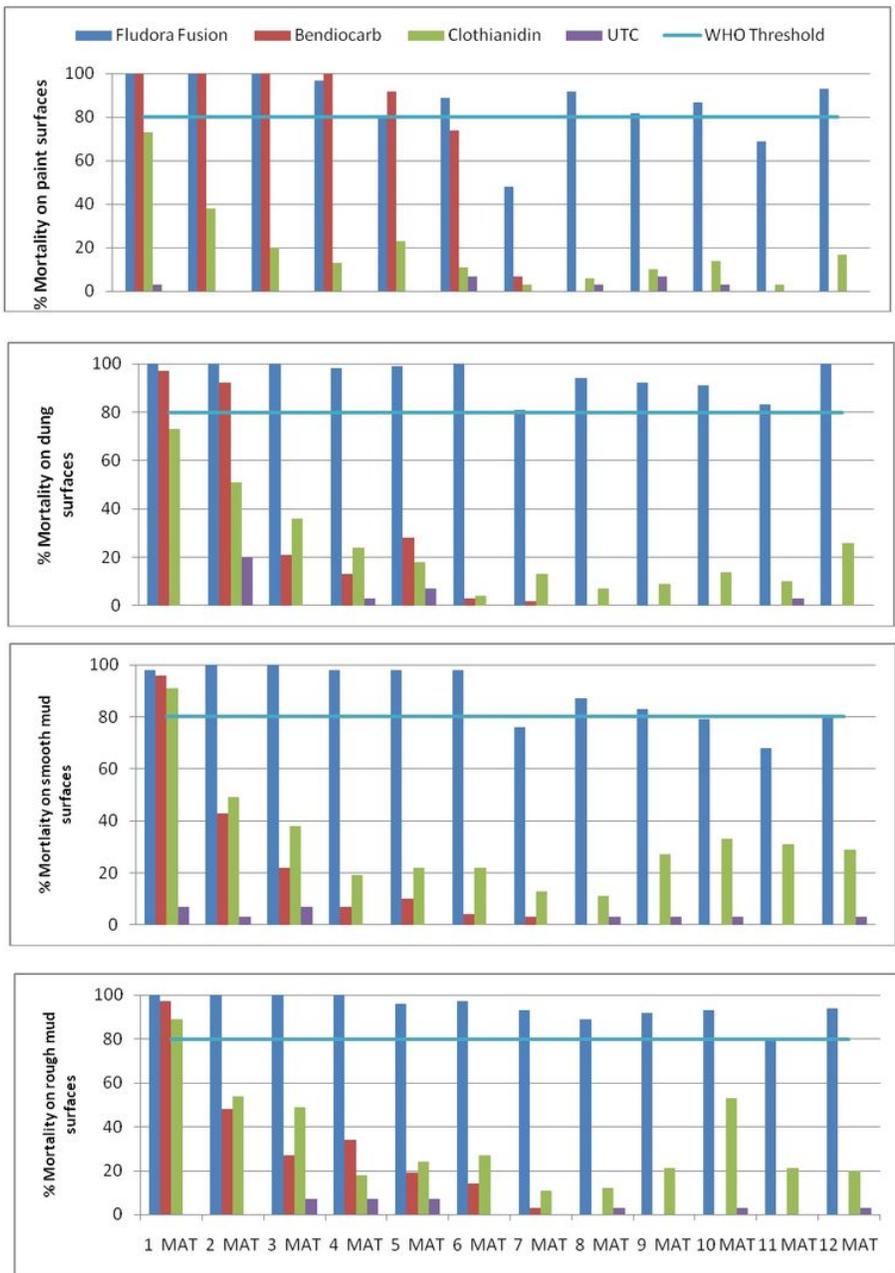


Figure 2

Residual activity of insecticides against susceptible *An. arabiensis* on four surface types of simple huts wall cone bioassays at 24 hours holding time post-exposure, Ethiopia

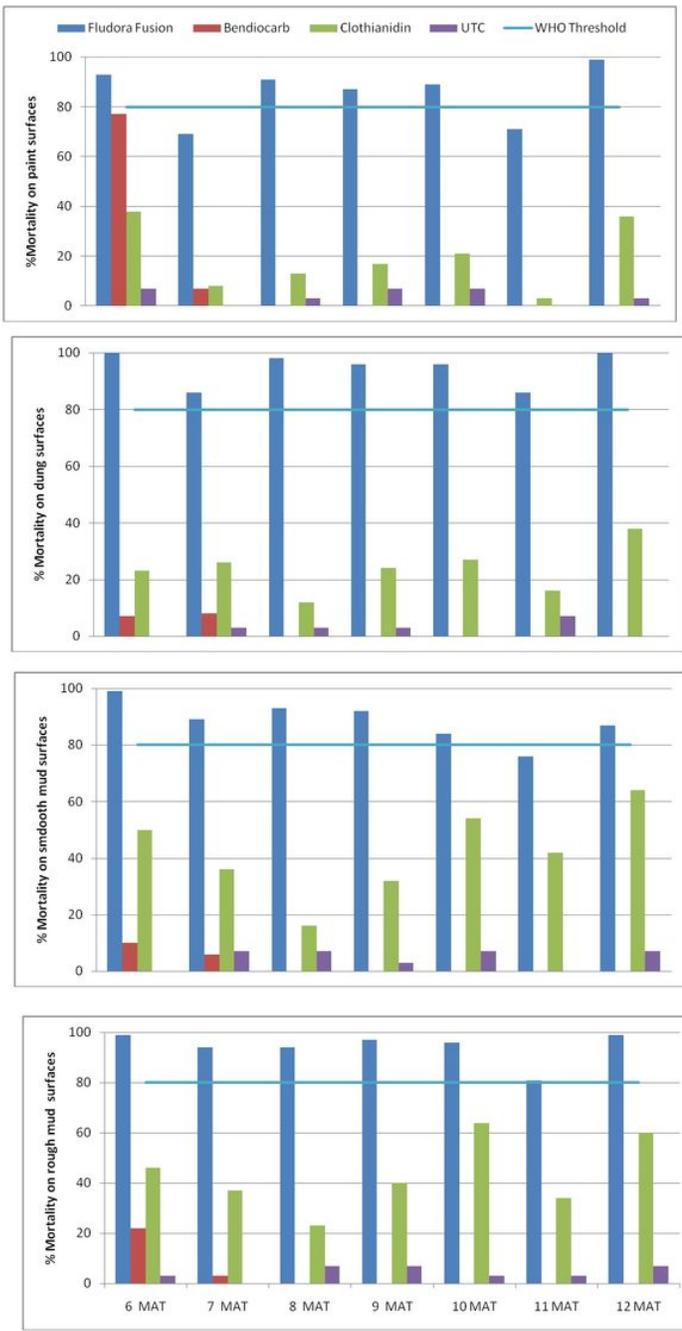


Figure 3

Residual activity of insecticides against susceptible *Anopheles arabiensis* on four surface types of simple huts wall cone bioassays at 48 hours holding time post exposure, Ethiopia

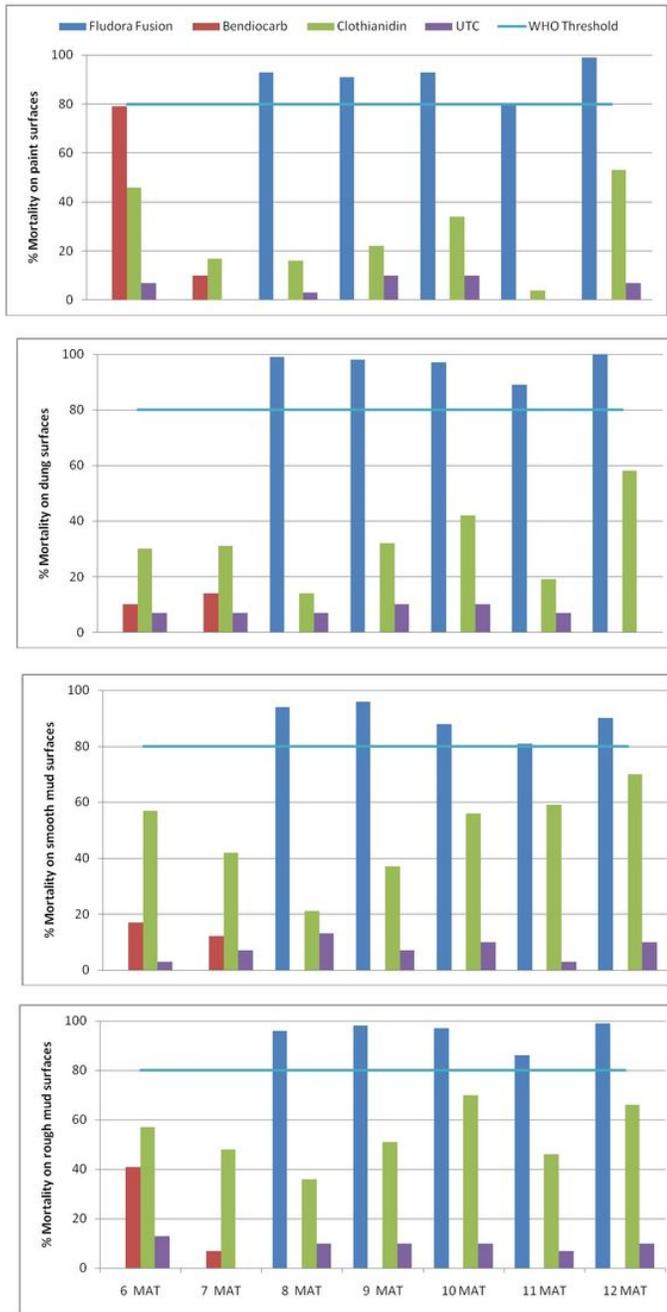


Figure 4

Residual activity of insecticides against susceptible *Anopheles arabiensis* on four surface types of simple huts wall cone bioassays at 72 hours holding time post-exposure, Ethiopia