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ABSTRACT: The nature and type of local indoor resting wall surfaces to certain level influences the residual bio-efficacy of insecticides used in indoor residual spraying programs. Knockdown and mortality effects of an organophosphate Actellic 300 CS and pyrethroid K-Othrine WG 250 insecticides on the field-collected *Culex quinquefasciatus* were assessed bimonthly from July to November 2014, using World Health Organization (WHO) cones bioassay test. Knockdown and mortality rates were subjected to statistical analysis using χ^2 and Student *t* tests. Result of the bioassay test on *C quinquefasciatus* showed that plywood surfaces had the best residual knockdown activity of Actellic 300 CS with knockdown rate above the WHO-recommended threshold limit of ≥95% for 30 days after treatment. This was followed by mud surface with knockdown rates ≥95% threshold limit 15 days (97%) after treatment. The lowest knockdown rates of less than 95% were observed on cement surface throughout the assessment period. However, the knockdown rates of mosquitoes on deltamethrin WG 250–treated cement and plywood surfaces were 100% and ≥95%, respectively, at 30 days after treatment. But the knockdown activity was below the recommended threshold limit on mud surface during the 17 weeks trial. Knockdown activities varied significantly ($p < .05$), and it is a function of exposure periods, different surfaces, and insecticide formulations. The 24-hour mortality rates of Actellic 300 CS and K-Othrine WG 250 at 120 days after treatment were 83.6% and 86.7%, and 80% and 83.3%, on plywood and cement surfaces, respectively. A maximum residual period of 75 and 45 days were recorded for Actellic 300 CS and K-Othrine WG 250, respectively, on mud surface. Both Actellic 300 CS and K-Othrine 250 WG were highly effective against *Culex* mosquito. The extended residual activity of *p*-methyl CS compared with deltamethrin WG 250 makes it a suitable alternative insecticide against pyrethroid-resistant mosquitoes in Southwest Nigeria.

KEYWORDS: Indoor residual spraying, Actellic 300 CS, K-Othrine WG 250, *Culex quinquefasciatus*, vector control

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Introduction

Culex quinquefasciatus Say is commonly found in urban localities as a pest with many breeding sites around man.[1] This species is prevalent in several urban and semi urban settings in Nigeria, where it thrives in varieties of organic-rich water in pit latrines, septic tanks, block drains, canals, and abandoned wells.[2–5] In Ibadan city, Southwest Nigeria, breeding sites of *C quinquefasciatus* are open drains containing highly polluted water during the dry season, pit latrines and water-logged damaged soak away during the wet season.[6] Besides the biting nuisance caused, *C quinquefasciatus* is a potential vector for Zika virus and the principal vector of filarial parasite *Wuchereria bancrofti*, a causal agent of urban lymphatic filariasis (LF).[7,8] Lymphatic filariasis causes a major public health burden among tropical countries including Nigeria[9–11] and is one of the most debilitating neglected tropical diseases in the world. The disease is predominantly transmitted in the rural areas in Nigeria by the major vectors of malaria (*Anopheles gambiae* and *Anopheles funestus* complexes) and by *C quinquefasciatus* Say in urban communities. Consequently, long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) control interventions are being used to complement mass drug administration campaign in the control of LF in Nigeria. The country has made tremendous progress in achieving the target of free mass distribution of 63 million LLINs with at least 80% utilization. Also, the assistance from the US President Malaria Initiative (US PMI) has greatly boosted the scaling up of several IRS pilot projects across different geopolitical zones in Nigeria, thereby reducing malaria burden in the country.[12]

Indoor residual spraying intervention relied greatly on pyrethroid insecticides because they are cheap and environmentally friendly, with a relatively long-lasting action and low toxic effect on humans.[13] Moreover, they are effective in controlling the major malaria vector *A gambiae* complex, with high residual efficacy of 3 to 6 months that effectively covers the long transmission period with only one round of spraying necessary.[14] These proven efficacies led to the extensive deployment of 2 formulations of K-Othrine wettable powder and water-dispersible granules (WG) in the ongoing IRS pilot projects in Nigeria. However, the huge deployment of pyrethroid insecticides via IRS scaling up, mass LLIN distribution, and agriculture has led to the wide spread of pyrethroid resistance in mosquito vectors across different ecological zones of Nigeria.[15–22] To reduce this menace of insecticide resistance, a rotational application of insecticides

has been recommended by the Global Plan for Insecticide Resistance Management.[23] Actellic 300 CS is among the recommended insecticides by World Health Organization's (WHO) Pesticide Evaluation Scheme (WHOPES).[24] It was recommended due to its long-lasting effects (6-12 months) against *A gambiae* and *C quinquefasciatus* which are resistant to pyrethroids.[13,25,26]

Also critical to the success of IRS operations is the nature and type of indoor sprayed wall surfaces. Previous reports assessing the influence of different types of wall surfaces on the residual effectiveness of deltamethrin against mosquito vectors have been patchy. A report of field study in South Africa revealed 2 to 3 months residual effectiveness of deltamethrin WG 250 (K-Othrine) on mud and cement surfaces, whereas deltamethrin SC-PE had a prolonged residual efficacy for 12 months on both surfaces.[27] In Tanzania, the result of laboratory evaluation of residual efficacy of deltamethrin SC-PE and WG formulations against *Anopheles arabiensis* showed residual performance of 5.2 and 10.1 months on mud and concrete surfaces, respectively. But its residual efficiency on the plywood was 16 months postspraying.[13] A study conducted in a south Cameroonian community showed that deltamethrin WG 250 (K-Othrine) sprayed on concrete surfaces had a longer residual effect (6 months) when compared with mud and wood surfaces with 5 and 4 months, respectively.[28] Similarly, reports from a number of studies in African countries have established the long-lasting activities of Actellic 300 CS in IRS programs. The potency ranged between 3 and 9 months on different indoor wall surfaces.[13,26,29]

However, there is dearth of information on the impacts of various common indoor surfaces found in human dwellings in Southwest Nigeria, on the long-lasting effectiveness of insecticides deployed for vector control programs. The recent renewed interest and scaling up of support of the National Malaria Elimination Programme, US PMI, Department for International Development, Global Funds, and other partners for IRS programs in Nigeria have made such studies important and appropriate.

With this background, this research study was set out to provide a simple technique to evaluate insecticide residual effectiveness preceding the field application and also to provide information that will guide IRS programs, in the context of Nigeria. The objective was to evaluate the residual effect of K-Othrine WG 250 and Actellic 300 CS considering their knockdown and mortality qualities on *C quinquefasciatus*, on different indoor surfaces under laboratory conditions.

Materials and Methods

Collection and rearing of field populations of C quinquefasciatus

Mosquito breeding habitats were scouted for *C quinquefasciatus* larvae and pupae in Ibadan metropolis (Latitude 07˚27.72′ North and Longitude 3˚55.41′ East). Larval collections were conducted during the rainy season (July-November 2014), using standard dippers.[30] Larvae were fed with fine powdered yeast and nonoily biscuits, reared to adulthood and maintained in the insectary under the environmental conditions of 25˚C ± 2˚C and 70% to 80% relative humidity (RH). The emerged adults were identified as *C quinquefasciatus* based on the standard morphological features[31] and separated into sexes.

Preparation and treatment of artificial surfaces

Two absorbent substrates, cement and mud, were molded into blocks with thickness of 1.5 cm in 20-cm diameter plastic bowls. The blocks were allowed to dry at 25˚C ± 2˚C and 70% to 80% RH, temperature and RH, respectively. A nonabsorptive substrate, plywood, was cut into the same sizes with the sorbent substrates, in such a way that the bioassay cones can be fitted suitably on it. The preparation of the surfaces was done according to the method of Vatandoost et al.[32] In total, 24 blocks of substrates were prepared: 8 substrates were made of unpainted cement blocks, 8 substrates with block made of unpainted mud, and the remaining 8 substrates made of unpainted plywood. In each batch of 8 substrates, K-Othrine 250 WG (deltamethrin WG 250 g/kg; Bayer CropScience, Co., Isando, South Africa) was used to spray 3 surfaces, whereas another 3 surfaces with Actellic 300 CS (pirimiphos-methyl capsule suspension 300 g/L; Syngenta, Basel, Switzerland). Water solutions of K-Othrine and Actellic at 0.02 and 1 g ai/m^2, respectively, were applied on the surfaces as recommended by WHOPES.[33] Hudson X-Pert Compression Sprayer (10 L capacity) fitted with HSS-8002 nozzle tips and with regulator adjusted pressure of 24 to 55 psi range.[25] Insecticide-treated surfaces were kept in the laboratory during the exposure period of the research.

Bioassay test

Contact bioassay to evaluate the long-lasting effectiveness of K-Othrine WG 250 and Actellic 300 CS on cement, wood, and mud surfaces were conducted every 2 weeks, using standard WHO cones.[30,34] The cones were fitted vertically on each insecticide-treated surface with masking tape (Figure 1). In each cone, 10 to 12 sugar-fed, 2 to 5 days old female mosquitoes were gently released and exposed to the insecticide-treated surface for 60 minutes, which was a period longer than the 30 minutes specified by WHO for IRS cone bioassay, not minding the insecticide in use.[35] The cone opening was plugged with cotton wool to prevent escape of the mosquitoes, and the number of mosquitoes knocked down within 60 minutes were immediately recorded. At the end of exposure period, the mosquitoes were placed in a small paper cups provided with 10% sugar solution and kept in the insectary for 24 hours at 25˚C ± 2˚C and 70% ± 10% RH. Paper cups were checked for mortalities and % mortalities calculated after 24 hours.

Figure 1. Cone bioassay test on different surfaces using deltamethrin at 0.02 g ai/m² and pirimiphos-methyl at 1 g ai/m² against *Culex quinquefasciatus*.

Data analysis

The mortality and knockdown rates of mosquitoes in different bioassays were calculated as the proportion of dead and knockdown mosquitoes against the total number exposed to treated surfaces. If the mortality of the control group was 5% to 20%, results were corrected by the Abbott formula, and if this was more than 20%, tests were repeated.[34] Comparative measure of 24-hour postexposure mortality and 1-hour exposure knockdown for the 2 insecticides between the treated surfaces and among exposure periods was performed by Kruskal-Wallis χ^2 test. Differences in mortality and knockdown between Actellic and K-Othrine were tested using Student *t* test. The precision about the mean and proportion was determined at 5% significance level using SPSS software for Windows (version 20) (SPSS Inc., Chicago, IL, USA).

Results

Residual bio-efficacy of Actellic 300 CS (pirimiphos-methyl) on various surfaces

The residual bio-efficiency of the insecticide on plywood, cement, and mud surfaces against field populations of *C quinquefasciatus* was monitored for over a period of 120 days (Figures 2 and 3). Different patterns of mean knockdown and mortality rates of the mosquito populations were depicted among the 3 surfaces and by the duration of the treatment. The overall mean knockdown rate of *C quinquefasciatus* populations on the Actellic 300 CS–treated surfaces was 56.3%. Significant differences ($\chi^2 = 11.5$, $df = 2$, $P = .003$) in knockdown activities were observed among the treated surfaces, with plywood surface showing the highest mean knockdown efficacy of 67.5%. This was followed by cement and mud surfaces with knockdown rates of 57.6% and 43.6%, respectively (Figure 2). The results of the 24-hour mortalities indicated that there was high susceptibility of *C quinquefasciatus* (>80% mortalities) to Actellic 300 CS maintained on all surfaces. Over the 120 days of trial, pirimiphos-methyl CS killed 95%, 92.1%, and 86.2% of mosquitoes when applied at 1 g ai/m² on cement, plywood, and mud surfaces, respectively (Figure 2). The difference in bio-efficacy of the

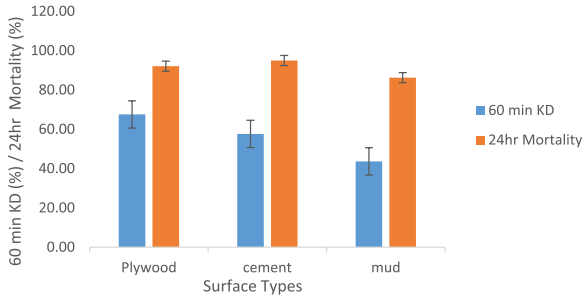

Figure 2. Percentage knockdown and mortality of field-collected populations of *Culex quinquefasciatus* exposed to different *p*-methyl (Actellic 300 CS)–treated surfaces. Mean ± SE, N = 21.

insecticide between the 3 insecticide-treated surfaces was statistically significant ($\chi^2 = 8.0$, $df = 2$, $P = .02$).

As shown in Figure 3, the durations of effective knockdown and mortalities of field-collected *C quinquefasciatus* varied significantly ($P < .05$) among the different Actellic 300 CS–treated surfaces. The results revealed that the highest knockdown rates (>95% knockdown in test mosquitoes) were reported for 30 and 15 days on plywood and mud surfaces, respectively. In contrast, the residual knockdown rate was below 95% on cement surface during 120 days assessment. The results further revealed that the period of efficacy of Actellic 300 CS that kills more 80% of *C quinquefasciatus* was 120 days postexposure on cement surface. Similar trend of activity was observed on plywood surface except for a sharp drop in efficacy (<80% mortality) at 90 days postspraying and thereafter it increased to 83.6% at 120 days postspraying. In contrast, the length of residual efficacy (>80% mortality in test mosquito) of same insecticide was 75 days on mud surface. The mortality rates decrease significantly over time ($P < .05$) on all the treated surfaces except on cement surface, where the mortality rates was uniform ($\chi^2 = 10.2$, $df = 6$, $P > .12$) during the period of trials life span.

Residual bio-efficacy of deltamethrin WG 250 (K-Othrine) on different surfaces

Results of cone bioassay tests on adult female *C quinquefasciatus* mosquitoes to evaluate the effectiveness and duration of effectiveness of deltamethrin WG 250 sprayed on common

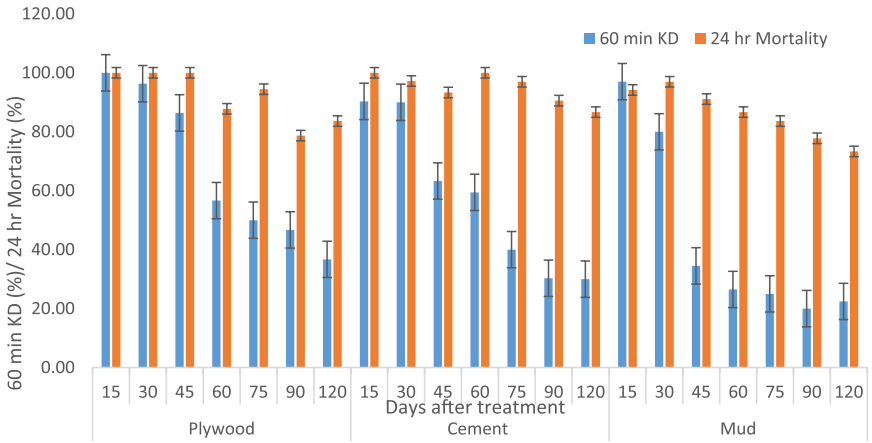

Figure 3. Percentage knockdown and mortality of field-collected *Culex quinquefasciatus* exposed to different *p*-methyl (Actellic 300 CS)–treated surfaces at various posttreatment periods. Mean ± SE, N = 21.

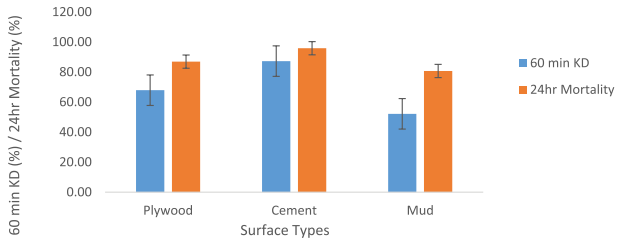

Figure 4. Percentage knockdown and mortality of field-collected populations of *Culex quinquefasciatus* exposed to different deltamethrin WG 250 (K-Othrine)–treated surfaces. Mean ± SE, N = 21.

indoor surfaces are shown in Figures 4 and 5. The overall mean knockdown rates of *C quinquefasciatus* populations exposed to different surfaces sprayed with insecticide was 69.1%. Kruskal-Wallis test revealed that knockdown activities of *C quinquefasciatus* varied significantly ($\chi^2 = 19.8$, $df = 2$, $P < .05$) between K-Othrine–treated surfaces. The lowest knockdown was 52.2% recorded on mud surface followed by 67.9% on plywood surface, whereas the highest knockdown (87.3%) was on cement surface. From the results, it was evidenced that the knockdown rates of the test mosquito populations on the 3 common indoor surfaces were generally less than WHO-recommended least value of ≥95% for effective knockdown. On the contrary, the results of the 24 hours mortality bioassay showed high residual potency of K-Othrine. The mean mortality of the mosquito vector remained above 80% during the period of posttreatment assessment regardless of the surface type. As shown in Figure 4, the effectiveness of deltamethrin WG 250 applied at 0.02 g ai/m^2 on 3 common indoor surfaces varied significantly ($\chi^2 = 15.8$, $df = 2$, $P < .05$) based on the mean *C quinquefasciatus* mortality and can be summarized in the following order: cement (95.9%) > plywood (87%) > mud (80.7%).

The percentage mortalities and knockdown versus time interval posttreatment on 3 different deltamethrin-sprayed surfaces are shown in Figure 5. The result revealed a decrease in knockdown activities of the insecticides on the treated surfaces with time. High knockdown efficacy (>95% knockdown) of K-Othrine was maintained on cement and plywood surfaces during the first 30 days postspraying, with 100% and 97% of test mosquitoes exposed knocked down, respectively. However, the bio-efficacy test conducted at subsequent postspraying time intervals showed knockdown rates to be <95% for cement and plywood surfaces. In contrast, the knockdown activities on the mud surface sprayed with same chemical was largely below the recommended threshold level for effective knockdown, during the period of postspraying assessment (Figure 5).

The 24 hours mortality results revealed that the best residual efficacy (>80% mortality) and maximum duration of effectiveness of K-Othrine WG 250 were observed on cement surface. Complete 100% mortality in tested mosquitoes was maintained on cement surface in the first 75 days postspraying, and thereafter, the death rate progressively and significantly ($P < .05$) declined until the end of the 120 days postspraying assessment when an overall mortality was 83.3%. Similarly, effective residual efficacy (>80% mortality) of deltamethrin was retained on plywood surface throughout 120 days trial period except at 75 days postspraying when the mortality rate declined to 77.8%. But afterward, the mortality rates increased to 83.6% and 80% at 90 and 120 days postspraying. On the contrary, when sprayed on mud surface, K-Othrine had a short residual action against *C quinquefasciatus* by killing more than 80% of tested mosquitoes not exceeding 45 days, followed by a decline to less than 70% mortalities 90 and 120 days after spraying. Unsurprisingly, mortality rates of the mosquito vector decreased significantly and steadily over time ($P < .05$) on all the treated surfaces except on plywood surface, where the mortality rates did not vary ($\chi^2 = 11.04$, $df = 6$, $P = .09$) throughout the study period.

Comparing the efficacy of the 2 candidate insecticides on different surfaces, there was no significant difference between knockdown effects due to Actellic and K-Othrine on all the surfaces except on cement. On cement surface, K-Othrine produced a significant ($t = -4.82$, $df = 40$, $P = .00$) higher knockdown effect (87.3%) compared with Actellic 300 CS (57.6%).

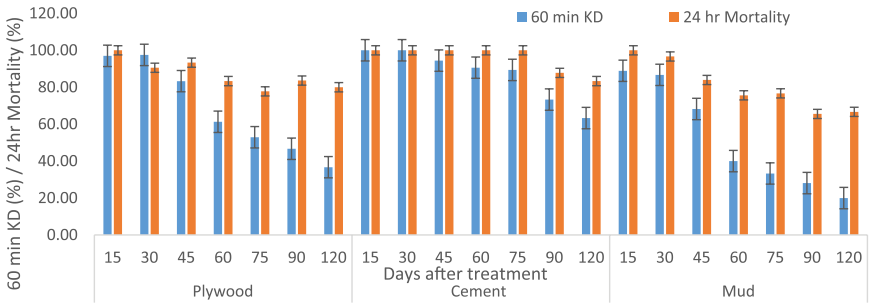

Figure 5. Percentage knockdown and mortality of field-collected *Culex quinquefasciatus* exposed to different deltamethrin WG 250 (K-Othrine)–treated surfaces at various posttreatment periods. Mean ± SE, N = 21.

On the whole, deltamethrin displayed a considerably ($t = -2.66$, $df = 124$, $P = .01$) higher knockdown effect (69.1%) compared with pirimiphos-methyl (56.3%). However, the result of 24-hour mortality revealed that the 2 insecticides had similar lethal effects ($P > .05$) on *C quinquefasciatus*, on all the surfaces. This indicates that both pirimiphos-methyl CS and deltamethrin WG 250 were equally effective on the 3 common surfaces.

Discussion

Apart from the established toxic effects of insecticides on target mosquito vector species, the period of persistence and cost-effectiveness of the insecticides are very critical to the success and sustainability of any insecticide-based vector control program.[36] The durability of an insecticide on any sprayable surface depends not only on the insecticide type and formulation but also on the nature of the surface. Actellic 300 CS and K-Othrine, the 2 products used in these bioassays, were among the 12 insecticides available for IRS programs.[38] These insecticides, however, differ in their efficiency and duration of effectiveness on different sprayed surfaces. This study demonstrates the efficacy and persistence of Actellic 300 CS at 1 g ai/m^2 to control *C quinquefasciatus* for a period of 75 to 120 days on most common indoor surfaces. Based on these data, the spraying cycle for Actellic 300 CS may last for a minimum of 120 days on cement and plywood surfaces, whereas that on mud surface may not exceed 75 days. The observed periods of effectiveness fall within 2 to 6 months of potency recommended for the insecticide by the WHO[37]. The result of this study, however, showed that Actellic 300 CS had a longer residual efficacy on cement and plywood surfaces when compared with mud surface against a susceptible field population of *C quinquefasciatus*. The differences in residual efficacy as indicated by differences in mortality in experimental mosquitoes may be attributed to the porosity of the surfaces. It is apparent from these results that plywood and cement surfaces, which were less porous, are effective in extending the bio-efficacy of Actellic 300 CS. This corroborates the report of Hadaway and Barlow[39] that organophosphates and carbamate insecticides rapidly lose their effectiveness on porous surface, such as mud, than nonporous or less porous surfaces, such as plywood and cement. In addition, insecticide applied on mud surfaces is absorbed into the body of mud, reducing its availability on the surface.[40] The outcomes of our study are similar to previous studies conducted in Zanzibar,[24] Benin,[26] Zambia,[41] and Tanzania.[13] In these different epidemiologic settings, Actellic 300 CS provided longer but effective control on *C quinquefasciatus* and *A gambiae* s.l. for 2 to 10 months, on common indoor surfaces. Also, the insecticide exhibited extended efficacy on cement and wood surfaces compared with mud surface. However, in this study microencapsulation formulation of Actellic 300 CS greatly enhanced the surface bioavailability and improved longevity of the insecticide on mud surface. Consequently, besides killing >80% of *C quinquefasciatus* for more than 60 days, *p*-methyl CS sustained control above 50% mortality for at least 120 days period when test was terminated on mud surface.

Our results also demonstrates that K-Othrine, deltamethrin WG 250, was persistent and effective on plywood and cement surfaces, killing >80% of *C quinquefasciatus* for 120 days. On the contrary, the activity of the insecticide was effective for only 45 days on mud surface. Based on these results, the rounds of spraying of K-Othrine in IRS may be at best in every 1.5 months on mud surface and at least for every 4 months on both cement and plywood surfaces. This study revealed that K-Othrine residual efficacy on mud surface was below the threshold of ≥80% after 45 days postspraying. However, the insecticide was still effective in killing more than 50% of *C quinquefasciatus* population during the 120 days test. This finding was in line with previous studies which reported that porous surfaces such as mud had shorter residual effect (2-3 months) with pyrethroids against different mosquito species, whereas nonporous surfaces such as wood and cement showed longer residual effects (>6 months).[28,31,42,43] These studies concluded that the low durability of deltamethrin on mud surfaces observed compared with wood and cement surfaces was apparently due to fast absorption of the insecticide by porous nature of mud surfaces. This study revealed that both Actellic 300 CS and K-Othrine were effective in controlling *C quinquefasciatus* on the 3 common indoor surfaces for at least 120 days except for mud surface (45-75 days). Nevertheless, both insecticides can be used on these common indoor surfaces in areas where the

mosquito vectors were still susceptible to pyrethroids, provided the insecticides are applied in cycle of 120 to 180 days. Also, due to the lasting malaria transmission in southern part of Nigeria and the increasing threat of mosquito resistance to pyrethroid insecticides, Actellic 300 CS can be deployed for IRS program to complement the ongoing mass distribution of pyrethroid LLINs' campaign, for malaria prevention in Southwest Nigeria.

Conclusions

The conclusion, populations of *C quinquefasciatus* in Ibadan metropolis, Southwest Nigeria, are still susceptible to organophosphates and pyrethroids. Actellic 300 CS and K-Othrine are highly effective with best knockdown effect and prolong residual activity on cement and plywood surfaces compared with mud surface. Mud dwellings are frequently found in rural areas of Nigeria; therefore, communities may be guided to use coats or local materials to smooth their walls to decrease the sorption rate and increase bioavailability of insecticides. This study has provided baseline data that can be used as a guide in the ongoing IRS pilot studies in various regions of Nigeria. Further research on the influence of common sprayable surfaces on the effectiveness of IRS program using other registered candidate insecticides, such as carbamates, under local conditions should be considered. Although Actellic 300 CS is yet to be included in the list of insecticide for IRS programs in Nigeria, it represents a useful alternative to pyrethroids or use in rotation with pyrethroid and other insecticides as a strategy for managing mosquito vector resistance. Because the prolonged effect of *p*-methyl 300 CS could only be assessed for 120 days in this study, field evaluation of its residual effectiveness against pyrethroid-resistant *A gambiae* s.l. beyond 120 days should be performed.

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Author Contributions

KOP and KTI designed the study. KOA and KTI carried out the experiment, analysed the data and drafted the manuscript. KOP critically revised the manuscript. All authors read and finally approved the manuscript.

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