Improving the efficiency of household insecticide testing against mosquitoes

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Abstract

Background

Developing robust, standardized approaches for testing mosquito populations against insecticides is a vital step for understanding the effectiveness of new active ingredients or formulations. Methods for testing mosquito susceptibility against contact insecticides or products, such as those delivered through public health programmes are well-established and standardized. Nevertheless, approaches for testing volatile or aerosolized insecticides used in household products can be challenging to standardize and implement efficiently.

Methods

We adapted WHO guidelines for household insecticides to develop a standardized and higher-throughput methodology for testing aerosolized products in Peet Grady test chamber (PG-chamber) using caged mosquitoes and an efficient decontamination method. The new approach was validated using insecticide resistant and susceptible Aedes and Anopheles mosquito colonies. An added feature is the inclusion of cage-facing cameras to allow real-time quantification of knockdown following insecticide exposure.

Results

The wipe-based decontamination method was highly effective for removing pyrethroids’ aerosolized oil-based residues from chamber surfaces, with < 2% of recorded mortality for susceptible mosquitoes tested directly on the surfaces. There was no spatial heterogeneity for knockdown or mortality of caged mosquitoes within the PG chamber, and the dual-cage approach applied effectively discriminate susceptible and resistant mosquito colonies tested side-by-side.

Conclusions

The dual-cage based assay that we implement yields eight-times the throughput compared to a free-flight protocol and allows simultaneous testing of different mosquito strains.

Background

Insecticide-based interventions are the primary approach to tackle the burden of widespread vector-borne diseases, including Aedes-transmitted viral diseases, such as dengue, chikungunya and Zika, and malaria transmitted by Anopheles mosquitoes. Widespread reports of insecticide resistance indicate threats to public health anti-vector programmes (1, 2). In response, at least in part, alternative insecticide-based tools for public health have been further explored, with increasing interest in pyrethroid-based ambient
insecticides such as spatial repellents (3-5). Herein, ambient insecticides refer to aerosolized and volatile insecticides (spatial repellents) deployed by candles, mats, emanators, sprays and coils.

To date, aerosolized insecticides have been applied primarily as household products intended for personal protection, with limited use in public health programmes. Nevertheless, recent studies have revealed extensive use of domestic insecticides by householders in vector-borne disease-endemic regions (6-8). Whether or not this is motivated by nuisance-biting or disease prevention, there is a clear need for standardized and tractable approaches for susceptibility screening of endemic mosquito populations against household formulations. This is a vital step for both predicting local insecticide effectiveness and understanding how household insecticide usage drives selection for the evolution of insecticide resistance (9).

Improving tests for the effectiveness of ambient insecticides and deployment devices could assist in early-stage testing of multiple products against local vectors for utility as public health interventions. Though most ambient insecticides have pyrethroids as their active ingredient, volatile pyrethroids provide a dual mode of action via killing activity and repellent effects, acting through alternative pathways. Pyrethroids’ volatile phase interferes mostly with olfactory receptors to disrupt mosquito host-seeking behaviour and locomotion, while the residual and aerosolized droplets cause knockdown by disrupting the gating function of the voltage-gated sodium channel (Vgs) target-site (5, 10, 11).

Despite their widespread use and potential for further market growth, few studies have assessed the bioecacy of spatial repellents and aerosolized formulations, especially when compared to IRS and ITNs products which act primarily as contact insecticides (2, 5). In part, this discrepancy reflects that methodologies for contact insecticides have efficient and well-established protocols that are widely applied, such as the WHO tube test and CDC bottle assay to assess insecticide susceptibility (12, 13), and the WHO cone bioassay to evaluate net or residual spray effectiveness (14). In contrast guidelines for testing aerosols and volatile insecticides are less well developed and have technical limitations which limit testing capacity, such as the need for controlled environments such as the Peet Grady chamber (PG-chamber) (15). The PG-chamber is a sealed compartment (180 cm x 180 cm x 180 cm) with smooth stainless steel internal inner walls. The chamber features include extracting fans and extract ducts to remove aerosol vapour after each test, and four large glass windows on three sides for screening mosquito knockdown.

Testing of household products' bioecacy against vector mosquitoes is expected to follow the WHO guidelines published in 2009 (15). However, the recommended approach has very low throughput, especially considering the demanding but critical decontamination step after every test, by thorough internal chamber washing to remove insecticide residuals. Moreover, several recommendations in the guidelines lack precision, which is likely to impact reproducibility. To address these limitations, we developed and validated a more standardized methodology based on the WHO guidelines, but with improved reproducibility and throughput to characterize mosquito susceptibility against aerosolized insecticides.
Our design uses a cage-based approach, which is already recommended for other ambient insecticides (coils, vaporizing mats, ambient emanators and liquid vaporizers), as an alternative to the free-flying bioassay (15), and includes three specific innovations 1. a fast wipe-based decontamination procedure, 2. a dual-cage assay to increase throughput and 3. video cameras to collect behavioural data including mosquito knockdown.

**Results**

**Effectiveness of decontamination**

Applying our novel PG-chamber decontamination approach, we detected an overall mortality of only 1.86% (n=268) in the insecticide-susceptible *An. gambiae* Kisumu colony in stringent 24h-exposure cone tests (Table S1, Additional file 3). This demonstrates the excellent effectiveness of the method, which offers a reduction of decontamination time to 20 min compared to 1 hour for the WHO methodology. For cage decontamination no mortality was recorded in the control cages based on four replicates performed on distinct days with two cages per replicate: again, indicating good effectiveness.

**Aerosol deployment**

Whereas no specification for the automatic aerosol dispenser is provided by the WHO guidelines (15), our assembled RCAD device represents a feasible design, which performed consistently and deploys a spray burst equivalent to manual spraying (Fig. 1A). It is also important to bear in mind that the volume of a spray burst may vary between aerosol cans due to total size as shown in Fig. 1A and 1B, based on a 3-sec burst of aerosol spray. In our experimental conditions, this variation was addressed by defining a burst length to achieve 5 - 7 g per deployment (Fig. 1B).

Based on spraying burst duration vs volume of product delivered, our aerosol dispenser also shows good accuracy to scale the amount of aerosol deployed, at least within the tested 1- to 4-fold range (Fig. 2A). This feature is particularly important as demonstrated in Fig. 2B, C, as it will allow the characterisation of dose-response relationships for new aerosol formulations or to establish an insecticide resistance discriminating dose.

**Dual Cage-based bioassay**

The dual-cage based assay effectively discriminates resistant and susceptible tested colonies (Fig. 3A – C and Fig. 4A, B). For both susceptible strains, 100% mortality was recorded, following three replicates, with a fast knockdown effect, within 5 min, after aerosol deployment, indicating rapid contact with insecticide droplets (Fig. 3A – C). The steady knockdown rate for *Ae. aegypti* resistant colony against each aerosol tested (Fig. 3A, B) from approximately 15 min after aerosol deployment suggests that both mosquitoes' contact with cages insecticide-impregnated walls and circulation of residual droplets have a
low impact for accumulating knockdown individuals in the resistant strain for 1h-exposure, allowing to discriminate the effectiveness of aerosolized insecticide formulations for mosquito's knockdown.

**Dual-cage assay validation**

Our results based on the *Aedes* Cayman resistant strain revealed that an uneven fan set-up could impact aerosol bioassay reproducibility (Fig. 3A), such as for average knockdown rate ($\chi^2 = 4.875, P = 0.03, df = 1$), presumably due to heterogeneous insecticide droplet dispersion. However, this was readily corrected by the use of a spirit level for fan horizontal orientation, following which there was no significant difference ($\chi^2 = 4.671, P = 0.700, df = 7$) in mortality between cage halves or among cages positioned next to the different observation windows (Fig. 3B, C and Fig. 4A, B). Thus, fan calibration is important for even aerosol dispersion and resultant assay reproducibility.

Our results also revealed that fan airflow plays an important role in cage-based assays. There was a difference between the mortality rates for cage-confined mosquitoes assayed under conditions of 1-min or 1-hour fan ventilation, with 27% higher mortality in the latter condition ($P < 0.001$), though this remained 16% lower than for free-flying mosquitoes (Fig. 4C, Table 1).

In the free-flight assays, there was a notable clustering of knocked-down mosquitoes around the fan position (strata C and D within the grid in Fig. 4D) for 1-hour fan ventilation. However, this was not linked with mortality, with similar rates to those for 1-min (Fig. 4C), suggesting a little impact of airflow disturbance on knockdown. This indicates that either short or long-duration ventilation is suitable to overcome the problem of poor spray dispersion in unventilated conditions.

**Discussion**

We show that our revised protocol for household aerosolized insecticides assessment using wipe-based decontamination, dual-cages, controlled-remote spray device and bioassay recording with action cameras are practical alternatives to enable higher throughput in the WHO current guidelines (15). For chamber decontamination, the 20 min wipe-based approach, is a major time-saving compared to the alternative (1 hour per chamber in our routine). Moreover, it also diminishes the length of user time within full protective equipment usage (e.g., respirator helmet and antistatic overalls). Furthermore, the approach minimises the likelihood of contaminating the room within which the PG chamber is housed as it produces less contaminated liquid and clothes for disposal compared to a full chamber's washing. The wipe-based is also a worthwhile approach for semi-field testing room decontamination, in which a minimal furniture setup in distinct room layouts is applied (15, 16).

The wipe-based decontamination was effective for removing residual aerosolized insecticides, as evidenced by < 2% mortality of susceptible mosquitoes (< 20% threshold for unsatisfactory decontamination (15)) exposed to the chamber surface directly using cone tests (4). The wipe-based effectiveness could result from the scrubbing steps, which together with the 5% detergent solution act directly in the impregnated oil-based residuals on the chamber's surfaces. In contrast, after standard
washing (detergent solution spraying, followed by a hosepipe rinse), we often recorded mortality higher than 20% for satisfactory decontamination, which spurred the investigation of alternatives. The detergent solution was also effective for removing pyrethroid-based residuals from cage fabric and metal frames (based on 2 hours of soaking), as indicated by zero mortality of confined susceptible mosquitoes in control cages after 24 hours.

A critical point addressed in our study and elsewhere (5, 17), is the challenge of standardizing exposure dose for ambient insecticides over testing procedures. As shown in Fig. 1B, despite a fixed burst length, the spray volume discharged varied across aerosol cans, which likely reflects manufacturing features (e.g., variable interior pressure, propellant volume, nozzle configuration, etc). Furthermore, the absence of a commercial remote spray device with the required features for research purposes (flexible spraying burst-length), imposes further challenges for inferring formulations dose-response on mosquitoes' knockdown. Manual spraying through the chamber's door or access ports (where fitted) is likely to result in varying exposure doses. Indeed, our results highlight the need for, and importance of, reducing these sources of variation: for the A. aegypti resistant colony we observed a relationship between aerosol dose and whether susceptibility or resistance would be concluded (Fig. 2B). Ideally, the aerosol dose should be standardised by grams delivered rather than burst-length, facilitating comparison across studies.

To minimise the impact of technical variation, the RCAD is an alternative for future studies. Also, we are aware that normalising the density of an aerosol burst is not a feasible task, although based on our experience a calibration of the burst length can approximate the mass of aerosol delivered in a repeatable manner for different aerosol cans (Fig. 1B, 2A). Such standardisations to aerosol concentration delivery within any testing chamber are vital to improving the reproducibility of bioefficacy screening across mosquito strains and formulations.

Our dual-cage approach could facilitate aerosolized insecticide testing routines (e.g. tracking the evolution of insecticide resistance for household formulations) by boosting test capacity by 2- or 8-fold compared to the standard cage and free-flying assays, respectively. Our study design provided experimental evidence as shown in Fig. 4A, B, that the cage-based approach did not trigger heterogeneities in knockdown rate for insecticide resistance, prevent knockdown in the susceptible colonies, or delay knockdown (Fig. 3A-C).

Nevertheless, our results also revealed that the fan airflow is an important feature to improve the cage assay efficiency compared to the standard free-flight assays (Fig. 4C and Table 1). By contrast, the fan airflow has no impact on the free-flying mortality rate although was observed a gathering of mosquitoes knockdown around the fan for 1-hour operating (Fig. 4D). We observed that the knockdown gathering was linked to a vacuum effect instead of an airflow turbulence. It is important to bear in mind, that our results from free-flying are based on an assay set-up with an operating fan for aerosol dispersion, which is not applied by the current WHO guideline. However, a previous study testing vaporizing mats (17), also discussed the critical impact of airflow circulation on reproducibility and bias in result, as demonstrated
by delayed and variable knockdown patterns underlined by the presence/absence of an operating fan and airflow direction.

In our assay set-up, fan level also impacted the bioassay results from different parts of the chamber; almost two-fold for the resistant Cayman strain (Fig. 3A). The solution to this issue is the simple, use of a spirit level to ensure a straight upward direction to avoid uneven aerosol dispersal. Similarly, a previous study also attributed inconsistencies in estimated mosquito coil efficacy between labs – and field-based testing due to limited ventilation and insecticide dispersion (5). Taken together, these insights also raise concerns about the absence of a formal guideline for semi-field study design, as often such studies (9, 16, 18) have adapted a test room without standardizing (e.g., room size, airflow circulation and manner of aerosol deployment), as well as using a cage-based assay without ventilation to disperse the insecticide.

In our experience and that of other research groups (5, 17), the recommendations for testing aerosol assays against free-flying mosquitoes in the current guideline (14), present substantial logistical challenges for laboratory and semi-field assessments. For instance, the free-flying approach has very low throughput (including the time-consuming recapture of free-flying mosquitoes) and presents technical drawbacks, such as the impaired ability to determine real-time knockdown effect over semi-field testing (17, 18).

While our validated dual-cage assay, which addresses such throughput limitations, is a feasible approach for screening mosquitoes’ susceptibility against aerosolized insecticides, it is important to highlight that the recorded mortality for cage-assayed Cayman mosquitoes was significantly lower compared to free-flying (Fig. 4C, Table 1). The difference could reflect a reduced insecticide dose within the cages, as fewer aerosol droplets penetrate the mesh to the cage's interior. Further testing using fabrics with wider mesh aperture, fan airflow strength and cage layouts such as cylindrical configurations (9, 16), could be performed to bring closer alignment between assay types.

Whether such quantitative differences observed in our present dual-cage assay set-up are important or not likely depends on the objective. When testing a new product, at least initially, the free flight assays may be preferred to allow the most precise assessment of quantitative effectiveness. However, if work involves the comparison between mosquito strains, the simultaneous testing in a more homogenized set-up presented by caged assays is likely to be advantageous, whereas a hybrid approach is also possible.

Remarkably, despite recent reports of fuelled use of household insecticides for personal protection in vector-borne diseases endemic countries (7, 9, 19), likely due to the low availability of PG-chambers for research purposes and additional challenges in field-based testing, there is a dearth of published studies to permit insights into issues encountered when testing household insecticides. To this end, our experiential study provides suggestions to address limitations in the current guidelines (15) and to improve efficiency and throughput. Furthermore, the use of new features for the study design, like the use of action cameras in our routine, not only improved and refined the real-time data collection of the knockdown effect while screening up to eight strains in parallel but also would facilitate further studies of
insect behaviour exposed to ambient insecticides. An additional footage file shows this in more detail (see Additional file 4).

The dual-cage assay set-up, which allows testing multiple strains in parallel, represents a time and cost-effective platform for the study of evolving insecticide resistance to household formulations in mosquitoes from vector-borne diseases endemic regions, which could also threaten the effectiveness of public health anti-mosquito programmes.

**Conclusion**

Taken together, the approaches described and validated here, facilitate the throughput and reproducible testing of household insecticides at higher throughput, and in a way that far improves comparisons between separate strains or species, compared to a free-flying approach.

**Materials And Methods**

**Mosquito colonies**

Four mosquito colonies with well-characterised susceptibility/resistance profiles against non-volatile contact pyrethroids (using WHO tube assays) and against volatile pyrethroids using custom plate bioassays (12, 20), were used to validate the approaches developed in this study. All colonies, two susceptible; *Ae. aegypti* (New Orleans) and *An. gambiae* (Kisumu) and two resistant; *Ae. aegypti* (Cayman) and *An. gambiae* (Tiassalé) are maintained and were provided by LITE (Liverpool Insect Testing Establishment). Colonies are maintained under insectary conditions: 27\(^\pm\)2\(^\circ\)C, 80\%+10\% relative humidity and a 12h light:dark photoperiod.

**Aerosol insecticidal formulations**

All aerosol cans used for the present work were purchased in a retail store and are household pyrethroid-based insecticide formulations. Since this work is concerned with methodology, manufacturer and product names are omitted to avoid commercial interest. The cans used are from two manufacturers and comprise household insecticides for personal protection: (A) 300 ml can with isobutane 20 – 30%, naphtha (petroleum) 10 – 20%, 1R-trans phenothrin 0.10 - 0.50% and prallethrin 0.10 - 0.50%; or (B) 380 ml can with BHT (butylated hydroxytoluene) 0.005%, polyglycerol oleate 0.90%, butane and propane 30%, N – paraffin 7.5%, imiprothrin 0.040%, permethrin 0.056%, D-trans allethrin 0.108% and water 61.40%.

**Aerosol insecticidal testing**

The step-by-step of our protocol for testing aerosol insecticides is provided in additional file 1.

All aerosol insecticidal bioassays were performed in the PG-chamber located in the Liverpool Insect Testing Establishment (LITE). The chambers were designed as outlined by WHO (15) and manufactured by Atlas Clean Air Ltd, United Kingdom. The PG-chamber has an interior measurement of 180 x 180 x 180
cm, with all internal wall panels made from polished stainless steel, for easy cleaning of insecticide or solvent residues (Fig. 5A, B). Insecticide vapour after each test is vented through an extractor duct located in the ceiling connected to a remote extractor fan. Screening of mosquito mortality and/or behaviour throughout testing was performed through glass observation windows at the front and on each side of the chamber (Fig. 5A and 5C).

For aerosol deployment and air circulation, an automatic aerosol dispenser facing the back wall and a 30-cm diameter fan facing upwards were sited at the centre of the chamber (Figure 5B). For the fan set-up, a spirit level was used to check that the fan was horizontal to ensure even air-flow circulation.

To recover mosquitoes after aerosol testing and for chamber decontamination, full personal protective equipment was worn including a white antistatic coverall, disposable overshoe, respirator helmet (3M™ Versaflo™ M-206 Helmet) or safety face mask and goggles and disposable gloves.

**Assembly of remote-controlled aerosol dispenser (RCAD)**

Although the use of an automatic aerosol dispenser is recommended by the WHO guideline, specifications are not provided. Applying an automatic dispenser is crucial to address as manual spraying - in addition to being physically difficult - may create spatial bias and introduce variation in the duration of spraying.

Commercial automatic aerosol spray dispensers (Fig. 1S- A, additional file 2), can deploy the recommended WHO standard burst of 0.65 ± 0.10g. However, fixed spraying burst length (single click burst), is not feasible for testing formulations dose-response knockdown effect. To overcome this limitation, we assembled the RCAD to operate on switch on/off mode (Fig. S1-B, Additional file 2). For this purpose, we modified a commercial automatic aerosol dispenser by replacing the receiver relay with a universal wireless relay module (Fig. S1-C, Additional file 2), which allows us to pair the spray device to an on/off transmitter.

The RCAD reproducibility was tested for consistency in the aerosol deployment of insecticide within a fume hood by weighing the can before and after spraying either 3 or 5 bursts for 3 seconds of the aerosols described above. Using the same spray can, the discharged burst in grams from the RCAD was then compared to manual spraying using the same criteria. This allowed us to identify whether the primary source of variation in the burst density was the result of variation in the interior pressure of the can, the propellant concentration, or heterogenous application by the manual operator.

**Validation of the alternative approach for testing aerosol insecticidal**

**Chamber and equipment decontamination**
Chamber decontamination must be performed after each test, but the thorough internal washing method recommended by WHO guideline (15) uses water piped through a hose; in our routine, this approach was the most time-consuming step for bioassay's set-up so we designed and tested a wipe-based decontamination procedure.

Briefly, the wipe-based decontamination is performed by spraying 5% of detergent solution (Decon 90) and surface scrubbing using a sponge with the following scrubbing with a stainless-steel squeegee window cleaner. Then, any detergent residue was removed by rinsing all surfaces with deionized and then applying a stainless-steel squeegee window cleaner.

To verify the effectiveness of the wipe-based approach, assuming < 20% threshold for unsatisfactory decontamination as WHO guideline (15), after each PG–chamber decontamination, susceptible mosquitoes (Kisumu) were tested using WHO cone bioassays (14). For each chamber, six cones with 10 mosquitoes each, one per surface, were fixed onto walls. To provide a stringent test, cone tests were performed for an exposure period of one hour, applied to WHO tube assays (12). After exposure, mosquitoes were transferred to a holding cup and provided with 10% glucose for a 24-hour period after which mortality was recorded. Holding cups were kept at 27 ± 2 °C temperature, 80 ± 10% humidity and 12:12 hour photoperiod (light: dark).

Cages and other movable equipment were decontaminated by soaking in 5% Decon 90 solution for a minimum of 2 hours then rinsed thoroughly with tap water and deionized water. To verify the effectiveness of cage decontamination, two cages with 10 susceptible mosquitoes (Kisumu) were kept at 27 ± 2 °C temperature, 80 ± 10% humidity and 12:12 hour photoperiod (light: dark) for scoring the 24 hours mortality. Only batch of clean cages with zero mortality was used for bioassays.

The removable parts of the fan were treated as above whilst the fan blades and wireframe were cleaned with 5% volume of Decon 90 on a sponge.

**Dual-Cage bioassay approach**

To enable aerosol droplets diffusion to the interior of cages, all-around mesh cages are recommended. To provide this we modified a 24.5 x 24.5 x 24.5 cm (650 µm mash aperture - BugDorm-4M2222 Insect Rearing Cage), by using the cage’s meshed sleeve to replace the plastic on the bottom. Since PG–chamber assays are time-consuming and could be prone to locational heterogeneity, we also incorporated a split wall at the centre of the cage (Figure 5C). This doubles assay throughput, and also allows side-by-side testing of strains for comparison (e.g. resistant and susceptible mosquitoes).

To assess whether this added internal dividing wall might lead to an uneven aerosol droplet spreading within the cages, the knockdown of mosquitoes from the same colony was tested in parallel in each side. In addition to knockdown at the end of the trial, we scored knockdown every 5 min for 60 min, following direct observation at each of the four chamber’s glass windows as well as based on bioassay footage recorded at 60 frames per second with 1.1 x zoom using an action camera (The Xtreme I+ 4K, Campark).
In some tests, at the same time as cage assays, 50 mosquitoes were released within the same chamber for direct comparison between the standard free-flying and cage-based approaches.

**Dual-cage assay validation**

Despite a fan for insecticide dispersion being applied for testing volatile products using a cage-base assay, this airflow is not required for testing free-flying mosquitoes against aerosolized insecticides following the WHO guidelines (14). For commercial formulations, manufacturer instructions recommend a spray burst for 3 - 6 seconds, which in our testing system corresponds to 5 - 9 grams. Such a spray duration created residual droplets and gathering of spray foam on the chamber's surface facing the aerosol deployment direction, suggesting poor homogeneity in dispersal.

To facilitate homogenous aerosol dispersion, we implemented a fan with a set-up as described beforehand. To assess whether the fan affected mosquito knockdown, two alternative conditions were tested: a) fan ventilation for 1 min at the start of the assay and b) fan ventilation for the full 1-hour assay duration. For each setting, a pairwise comparison between the free-flying and cage-based approach was performed through side-by-side assays using 50 free-flying resistant mosquitoes (Cayman colony) and four cages with 25 mosquitoes each containing either susceptible (New Orleans) or resistant (Cayman) colonies. One cage from each colony was placed into the opposing chamber corners.

To investigate the impact of the fan's airflow disruption on the free-flying mosquito's knockdown a covering was applied to the chamber’s floor with a grid of 36 squares of 30 X 30 cm. The number of knocked-down mosquitoes within each square was recorded after 1h-exposure to aerosol. At the end of the trial, both free-flying and cage-confined mosquitoes were transferred to a holding cup and provided with 10% glucose for a 24-hour period after which mortality was recorded. Holding cups were kept at 27 ± 2 °C temperature, 80 ± 10% humidity and 12:12 hour photoperiod (light: dark).

Analysis used a binomial generalized linear model (GLM) using the IBM SPSS v26 software, with knockdown or mortality as the independent variable and airflow ventilation length and bioassay type (cage-based and free-flying mosquitoes) as factors.

**Declarations**

**Acknowledgements**

We would like to thank LITE (Liverpool Insect Testing Establishment) for supporting the tests in the Peet-Grady chamber and for providing the mosquito colonies used in this study.

**Authors’ Contributions**
WFSM perform the study design, collection, and interpretation of the data, writing of the manuscript and submission for publication. ER contributed to data collection. ST contributed to design, assemble, and validate of the RCAD device. GE contributed to data collection. GJ and AG provided the mosquitoes colonies and Peet Grady facility in LITE. MD contributed to study design and data interpretation. DW contributed to study design, data analysis and interpretation.

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**Availability of Data and Materials**

All the data supporting the results are included in the article

**Ethics Approval and Consent to Participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and materials**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Tables**
Table 1. Binomial Generalized Linear Model (GLM) summary performed on mosquitos’ mortality exposed to aerosolised insecticides using alternative bioassay set-up (cage-base and free-flying mosquitoes) and airflow ventilation time-length.

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References


**Figures**

![Figure 1](image)

**Figure 1**

Validation and calibration of the assembled remote-controlled aerosol dispenser. **A** Comparison of spraying reproducibility within and between manual and automatic burst deployment. **B** Burst length calibration to normalise spraying density across aerosol cans. The red dashed line is a baseline concentration to calibrate spraying burst length across variable spray can weights.
Figure 2

Concentration-dose and dose-response curve for spraying burst and mosquito’s knockdown effect. A Diagrammatic representation of the spraying burst density deployed by automatic dispenser for 1 to 4 bursts each of 3 seconds. B Dose-response curve of the knockdown effect in pyrethroids-resistant colonies: *Aedes aegypti* – Cayman and *Anopheles gambiae* – Tiassale.

Figure 3

knockdown effect across aerosol exposure time for *Aedes aegypti* and *Anopheles gambiae* susceptible and resistant colonies. A and B, C are instances of the fan's impact on aerosol droplets dispersion without and with spirit level calibration, respectively. Susceptible (*Ae. aegypti* - New Orleans and *An. gambiae* – Kissumu) and resistant (*Ae. aegypti* – Cayman and *An. gambiae* – Tiassale).
Figure 4

Dual-cage approach validation for screening mosquitos’ susceptibility against aerosolized insecticides. A and B comparison of mortality rates for susceptible (Ae. aegypti - New Orleans and An. gambiae – Kissumu) and resistant (Ae. aegypti – Cayman and An. gambiae – Tiassale) mosquitoes depend on cage structure and location in the chamber. C Impact of fan airflow on mortality in dual-cage and free-flying assays. D Summary of the space grid mapping for the mosquito’s knockdown distribution in the presence/absence of fan airflow. Error bars represent 95% confidence intervals. S-cage (Standard cage) and D-cage (Dual-cage) are cages with and without an internal wall, respectively. Cage - number and letter; numbers represent the cage’s clockwise location in the Peet-Grady windows and letters - A and B, dual-cage left or right halve.
Figure 5

Peet-Grady chamber's external and internal overview. **A** Chamber's lateral profile showing glass observation windows, electrical control panel and extract duct at the ceiling's rear. **B** Set-up of the automatic aerosol dispenser and a 30-cm diameter fan at the chamber's centre. **C** Viewing from a chamber's glass observation window with a sited action camera to assist with the scoring of mosquitoes' knockdown.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.pdf
- Additionalfile2.pdf
- additionalfile3.pdf
- additionalfile4.mp4