

RESEARCH ARTICLE

ANALYSIS OF INSECTICIDE SUSCEPTIBILITY DATA FOR DENGUE VECTORS USING INSECTICIDE RESISTANCE MANAGEMENT SYSTEM (IRMS)

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ABSTRACT

Laboratory testing generates data, and data analysis contributes to the production of results that aid in problem resolution. As more samples are tested, more data is generated, and the complexity of data management increases. Surveillance monitoring and real-time assessment are crucial in managing insecticide resistance; consequently, manual management of insecticide resistance data requires digital transformation. The Insecticide Resistance Management System (IRMS) was developed in this study to analyse and manage 244 insecticide susceptibility data. This system permits immediate analysis following the entry of laboratory data. Red dots dominated green dots on a map displayed in IRMS depicting the prevalence of insecticide-resistant mosquito populations in both *Aedes* species. In addition, the heatmap revealed that the majority of *Aedes aegypti* were resistant to all tested insecticides during all monitoring cycles. This system circumvents the time-consuming limitations of manual data analysis by enabling rapid result visualisation and report generation.

KEYWORDS

insecticide resistance, data management, *Aedes* mosquito, system, analysis.

1. INTRODUCTION

Dengue is a global health problem. The disease is prevalent in countries with tropical climates that support the growth of *Aedes* mosquitoes (Yang et al., 2009). Population density and mosquito frequency accelerate dengue virus transmission (Gubler, 2011). Insecticide-based interventions are the preferred method for dengue control, but their long-term use can result in insecticide resistance (Ranson et al., 2008). Therefore, it is essential to monitor mosquito susceptibility to ensure that insecticides used in vector control are effective and efficient. The detection of resistant mosquitoes should be intensified so that subsequent resistance monitoring and vector control actions can be significantly improved (WHO, 2012). The WHO insecticide resistance management plan includes a comprehensive list of activities, with the second pillar of the five list of strategies emphasising entomological and insecticide resistance monitoring, together with efficiently manage the data obtained (WHO, 2012). The reports on the insecticide susceptibility status of *Aedes* in Malaysia are remarkably extensive and supported by the vast number articles published (Ishak et al., 2015; Rosilawati et al., 2017, 2018, 2021; Siti-Futri et al., 2020; Wan-Norafikah et al., 2013; Zuharah et al., 2021). The majority of studies recommend for insecticide resistance management since findings indicated that *Aedes* mosquitoes are resistant to insecticides at diagnostic doses, particularly mosquitoes sampled in urban areas and hotspots.

In accordance with WHO guidelines, insecticide resistance is determined using bioassay tests (WHO, 2016). The susceptibility status of tested mosquitoes is classified as susceptible, possible resistance, or confirmed resistance if the test mortality recorded with >98%, 90% to 98%, or <90%,

respectively (WHO, 2022). Despite the fact that the current research discovered widespread resistance in *Aedes* mosquitoes in Malaysia, the screening is against the diagnostic dose, which is double the lethal dose against the susceptible population (Ishak et al., 2015; Rasli et al., 2018, 2021; Rosilawati et al., 2017; Siti-Futri et al., 2020; Zuharah et al., 2021). Nonetheless, this evaluation is necessary to ensure the efficacy of insecticide intervention, presenting a signal to conduct additional monitoring in a population that exhibits possible or confirmed resistance via an intensity bioassay at five to ten times the diagnostic dose, as well as the detection of resistance mechanisms, in order to provide definitive confirmation of resistance occurrence. It is important to prevent the severity of insecticide resistance to field application dosage. Before making a well-informed decision to address a resistance issue, it is imperative to manage susceptibility data. For the future direction of susceptibility data management, therefore, a digital transformation is required.

The insecticide susceptibility profiles are essential data. There will be an abundance of data generated during routine operational monitoring, especially as more mosquito samples are tested against various insecticides. In handling the insecticide resistance data, preparing the data analysis requires direct data entry using Microsoft Excel and translate the findings to a paper-based report, and it was noted that Microsoft Excel is frequently used for data collection and analysis (Elliott et al., 2006). However, in the past year, technology has aided in the management of scientific data, particularly in health management, to save time and ensure proper data management (Abhyankar et al., 2012; Obodai et al., 2021; Wilkerson et al., 2015). To the author knowledge, a system known as IR Mapper has been recognized to collect information from publications on

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mosquito susceptibility profiles and mechanisms. This system displays the status of resistance in mapping by pulling information from articles published all over the world.

It is worth noting, Geographic information systems (GIS) have been shown to be one of the most prevalent and useful tools for visualising results to aid in management and planning decisions (Cheong et al., 2008; Keenan, 2008). As insecticide resistance management involves surveillance monitoring, the evaluation of resistance situation should evaluate in real time manner (WHO, 2012). In order to support this, the Insecticide Resistance Management System (IRMS) aims to facilitate the management of data at the earliest stages of the process, beginning with the management of data after laboratory work has been completed, followed by the analysis and summarization of results, and finally the generation of

final reports. This system will not only map the distribution of insecticide resistance status using a GIS application, but it will also integrate the Lavarel PHP in the development of an electronic form to allow the entry of laboratory data and use of JavaScript's to project the resistance results in multiple graphical displays.

2. MATERIALS AND METHOD

2.1 Project Workflows

The insecticide susceptibility of *Aedes* populations was monitored for three cycles. Each cycle takes approximately 6 months to complete all activities beginning with field sampling and ending with laboratory data analysis (Figure 1).

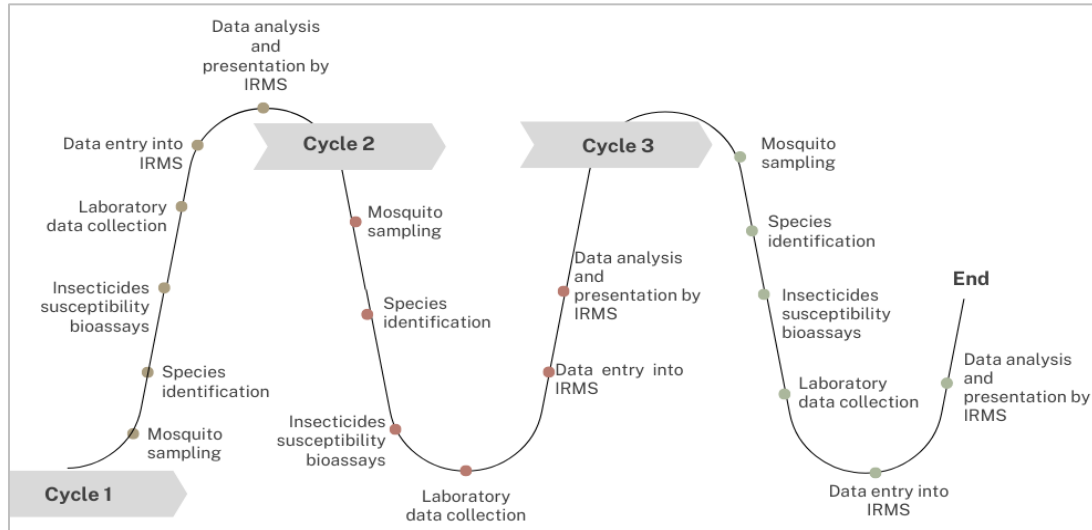


Figure 1: Study design and experimental activities for detection of insecticide resistance in dengue vectors.

2.2 Mosquito Sampling and Species Identification

Aedes mosquitoes were sampled using ovitrapping techniques (Lee, 1992). Each pre-selected site was deployed with a total of 30 ovitraps. During the sampling, Pestrapp was utilised to capture the coordinates of each ovitraps in the field (Cheong et al., 2021). After 5 days of placement in the field, the ovitraps were collected and brought to the laboratory for species identification. The third instar larvae were identified under a compound microscope (Nikon Eclipse E200, Tokyo, Japan) using taxonomy key (Christophers SR, 1960).

2.3 Site Detail

Aedes populations were collected in 12 pre-selected dengue hotspots in Selangor. The sites selection were captured through I-dengue, a community's website, was utilized to capture data about hotspots (MOSTI, 2019). The sites were reported with dengue cases for up to 32 days (Table 1).

No.	Site Name
1	Kampung Kerdas 3
2	Taman Koperasi Polis
3	Pangsapuri Bukit Baru
4	Kampung Sungai Tua Batu Caves
5	Taman Seri Melati, Batu Caves
6	Kampung Changkat Z7
7	Taman Garing Utama
8	Rumah Murah Kampung Padang
9	Saujana Impian (Jalan Impian 2&3)
10	Jalan Dayang, Bandar Mahkota
11	Seksyen 3, Bandar Baru Bangi
12	Kampung Baru Ampang

2.4 Mosquito Colonization

Aedes aegypti and *Aedes albopictus* mosquito were reared in the insectarium of the Institute for Medical Research, Medical Entomology Unit at a temperature of 26°C and relative humidity of 85%. For the purpose of rearing wild-caught *Aedes* mosquitoes to be used for testing, identified larval stages at the third instar stage were pooled in a tray according to species and collection site. Third-instar larvae were fed finely crushed liver powder, whereas the late third and fourth-instar larvae were fed partially cooked cow liver. Pupae were carefully transferred to a dry dish in preparation for their development into adults. Adult mosquitoes were kept in a 32cm X 32cm X 32cm cage and were provided with a 10 % sucrose solution. Adult mosquitoes were blood-fed with mice in order to get the eggs of the next offspring.

2.5 Monitoring Insecticide Susceptibility Status of Dengue Vectors Against Pyrethroids and Organophosphates.

2.5.1 Test Insecticides

The insecticides permethrin and deltamethrin, as well as organophosphates malathion and temephos, were used at diagnostic doses to determine insecticide susceptibility status of each *Aedes* population (2016). These insecticides were supplied from the WHO Vector Control Research Unit, Universiti Sains Malaysia (VCRU, USM).

2.5.2 Insecticide Susceptibility Bioassays

There were two bioassays conducted in this study, comprising of larval and adult mosquito bioassay. The test was conducted in a laboratory at which temperature was 27°C ± 2°C and the relative humidity was 75% ± 10%. Both tests were conducted in accordance with WHO guidelines for susceptibility testing (WHO, 2016). For the larva bioassay, a total of 25 early fourth instar larvae were placed for 24 hours in a 250ml test solution of temephos at a diagnostic concentration of 0.012 mg/L. Together with control assay involved mixture of 1ml ethanol in 249ml distilled water. Larval mortalities were observed at 24 hours post-exposure.

For the adult bioassay, female mosquitoes aged 5 to 7 days were employed. The treatment groups were exposed for 1 hour to permethrin, deltamethrin, or malathion impregnated paper at the diagnostic concentrations, whilst the control groups were subjected to the respective

control paper. Each assay was performed with five replicates for the treatment group and two replicates for the control group. After an hour of exposure, the mosquitoes were transferred to holding tubes and given cotton pads containing 10% sucrose for a 24-hour recovery period. Finally, adult mortalities were observed and recorded.

2.6 Data Input and Data Sources

This study utilized a total of 244 datasets from insecticides bioassays of field collected *Aedes* mosquitoes. These are the monitoring data generated over three cycles. The initial surveillance data were released previously, but the subsequent surveillance data have not been published (Rasli et al, 2021). The data collected in this study reflects a subset of *Aedes* strains from hotspots and is not representative of the *Aedes* population in Selangor as a whole.

2.6.1 Data Elements

The insecticide resistance data collected during testing on paper-based records were transferred into the IRMS system. The information was entered into a customised electronic form within the IRMS. This electronic form was created using Lavarel PHP. The information collected during testing as outlined in the Annexes from WHO guideline (WHO, 2016). Table 2 depicts the data elements captured in IRMS.

Table 2: Detail of Information Collected During Conduct of Insecticide Susceptibilities Bioassays.	
Title	Data elements
Site Detail	Name of location, district, state, history of insecticide usage.
Collection method	Sampling method, date of sampling.
Test sample and control sample	Name of species tested, filial, stages of mosquito used in testing, age (days) used physiological stage.
Test insecticide information	Name of insecticide tested, impregnated paper prepared by, date of expiry, number of times paper is used, concentration.
Test condition	Temperature starting and ending, relative humidity starting and ending,
Results observation of mosquitoes in treatment and control tubes.	Number of exposed mosquitoes used in each replicate, number of knocked-down mosquitoes within 60 minutes exposure, number of dead mosquitoes after 24h recovery period, number of live mosquitoes after 24h recovery period.
Observation of mosquitoes in treatment and control cups.	Number of exposed mosquitoes used in each replicate, number of pupae observed, number of dead larvae observed, number of alive larvae observed

2.7 Data Analysis Via Irms

After entering data from the bioassay, IRMS analysed and processed the data, enabling the execution of ad hoc queries. IRMS allows for the analysis of 24-hour mortality results, as well as the projection of result output in mapping, table, and report formats.

2.7.1 Determination Of Mortality at Post 24-Hours Exposure

The IRMS analysed the mean mortality in accordance with the WHO guidelines (WHO, 2016). The average mortality of each bioassay was determined via average the mortalities observed in 5 replicates of the treatment group (sum of r_1 nd/n, r_2 nd/n, r_3 nd/n, r_4 nd/n, and r_5 nd/n mortalities divided by 5, where r represents replicate, nd is the number of mortal mosquitoes observed, and n is the total number of mosquitoes exposed). For control mortality, mortalities observed in 2 replicates of control group (sum of c_1 nd/n and c_2 nd/n mortalities divided by 2, where c represents control's replicate, nd is the number of mortal mosquitoes observed, and n is the total number of mosquitoes exposed). The average of mortality was converted to a mortality rate in percentage. If the control mortality rate was less than 5%, IRMS stipulates that the obtained mean mortality will not be adjusted. If control mortality falls between 5% and 20%, IRMS will executed correction mortality. The corrected mortality rate follows the WHO-described formula (WHO, 2022).

3. RESULT AND DISCUSSION

3.1 Participating Sites

Figure 2 depicts an overview of the locations where *Aedes* populations were monitored. To visualise the updated number of monitored sites, the primary interface of IRMS displayed purple dots on maps. As this project focuses on 12 locations, twelve purple dots were displayed on the map. To the author's knowledge, IRMS provides an important feature where a zooming the purple dots allowed visualization of mosquito sampling coverage based on ovitraps coordinates obtained from PesTrapp, a mobile apps that digitally collected the ovitraps details projecting the area where ovitraps were placed in the field. Despite the fact that the primary function of IRMS is to aid in the management of insecticide resistance data, this feature is believed to aid decision - makers in establishing correlations between the resistance status of mosquito samples and the location of dengue cases. Certainly, this system enables the collection, storage, and prevention of ovitraps data loss.

3.2 Insecticide Susceptibility Status of Aedes Populations

Numerous scientific publications have incorporated mapping to illustrate resistance dispersion across map locations. Visualization of outputs via mapping is interactive and representative. Generally, the data feeds on the maps displayed multiple pin points on monitoring sites with three colours coded that corresponded to a range of susceptibility levels ranging from susceptible to possible resistance and resistance. The results depicted in Figures 4 and 5 revealed the insecticide susceptibility status for the third cycle, based on the most recent data fed into the IRMS system, revealing the insecticide susceptibility status of both *Aedes* species to deltamethrin, permethrin, malathion, and temephos at diagnostic concentrations. The result was found to be significantly variable between *Aedes aegypti* and *Aedes albopictus* strains. There were a greater number of red dots on the Figure 4 maps than in Figure 5, indicating a higher prevalence of resistance in *Aedes aegypti* strains (Figure 4) than in *Aedes albopictus* strains (Figure 5). In detail, all *Aedes aegypti* strains were resistant to permethrin (Figure 4C), while 91% of *Aedes aegypti* strains were resistant to deltamethrin (Figure 4B), malathion (Fig 4D), and temephos (Figure 4A) respectively. This mapping revealed widespread multiple resistance in the majority of the *Aedes aegypti* strains collected, except for one strain that exhibited a cross-resistance pattern (see blue arrow in Figure 4).

For *Aedes albopictus* strains, 64% of strains were resistant to temephos, while the remaining 36% were susceptible to temephos (Figure 5A). On the other hand, all *Aedes albopictus* strains were resistant to malathion and exhibited variable susceptibility patterns to permethrin and deltamethrin. Based on this outcome, 45% of *Aedes albopictus* strains exhibited possible resistance to deltamethrin, 36% exhibited confirmed resistance, and 27% exhibited susceptibility to deltamethrin (Figure 5B). Susceptibility testing of *Aedes albopictus* strains to permethrin revealed a high level of resistance, with 72% of strains being resistant and 27% being susceptible (Figure 5C).

3.3 Insecticide Susceptibility Profiling by Specific Site Monitored

In general, mapping of the insecticide resistance status permitted observations by one type of insecticide and one type of species over the selected time period, and often in-depth assessment of insecticide resistance profiling by specific site is required to address the resistance issue. To support the visualisation of susceptibility profiling of insecticide tested over time and across species, IRMS allows graphically presenting the results in a bar chart and a table on the map.

The table summarises both *Aedes* species' insecticide susceptibility, as indicated by the red arrow insert in the map (Figure 6). The information in the table are based on the users selection (see the blue arrow in the map shown in Figure 7). Apart from the table format for presenting the findings, IRMS enhanced the ability to view all insecticide susceptibility tests conducted at a particular site by simply clicking on the site name indicated by the green arrow on the map. This will display a bar chart with the results of all insecticide susceptibility tests conducted in the selected site (Figure 8).

3.4 Insecticide Susceptibility Pattern of All Monitored Sites.

The heatmap in IRMS enables comparisons of all tested insecticide resistance across species and strains. The present study's IRMS analysis of insecticide resistance data revealed widespread resistance to deltamethrin, permethrin, malathion, and temephos at discriminating doses at all 12 sites monitored across three monitoring cycles (Figure 9). The heatmap is an additional feature included with IRMS, which aids in

projecting all results over the selected surveillance period. The heat map depicts susceptibility levels ranging from resistance to susceptible level, with red representing resistance and green representing susceptible. Besides this, white cells indicate that no samples were collected during the sampling. In this study, the heatmap with white cells indicated that no *Aedes albopictus* was collected from S13 during three monitoring cycles. On the other hand, *Aedes aegypti* was absent from S2 and S20 during cycles 2 and 3, as well as from S4, S6, S12, S17, and S25 during the third cycle (Figure 8).

By incorporating a heat mat into IRMS, it is possible to detect the susceptibility profile over time in an easily understandable time-series format. The resistance pattern can be extrapolated from the heatmap color projection of susceptibility findings. Findings that are completely red or

shift from green to red should be treated with the utmost caution, and immediate countermeasures are required. In this study, the results of all 12 sites monitored over three cycles indicate widespread resistance to deltamethrin, permethrin, malathion, and temephos at discriminating dosages, with the majority of findings indicating consistent resistance throughout the cycle, as indicated by the red colour display throughout the three-cycle monitoring, and green to red color display as seen in *Aedes albopictus* from S4, S9, and S20; with the exception of *Aedes albopictus* from S23, which was only susceptible to deltamethrin (Figure 9). The heat map function illustrates in real-time the reversion of resistance to the susceptible population. *Aedes albopictus* strains S2, S6, S7, and S19 were resistant to deltamethrin in cycle 1 but susceptible in cycles 2 and 3. A similar pattern was discovered in the S2 strain, which was discovered to be resistant to permethrin during the surveillance period (Figure 9).

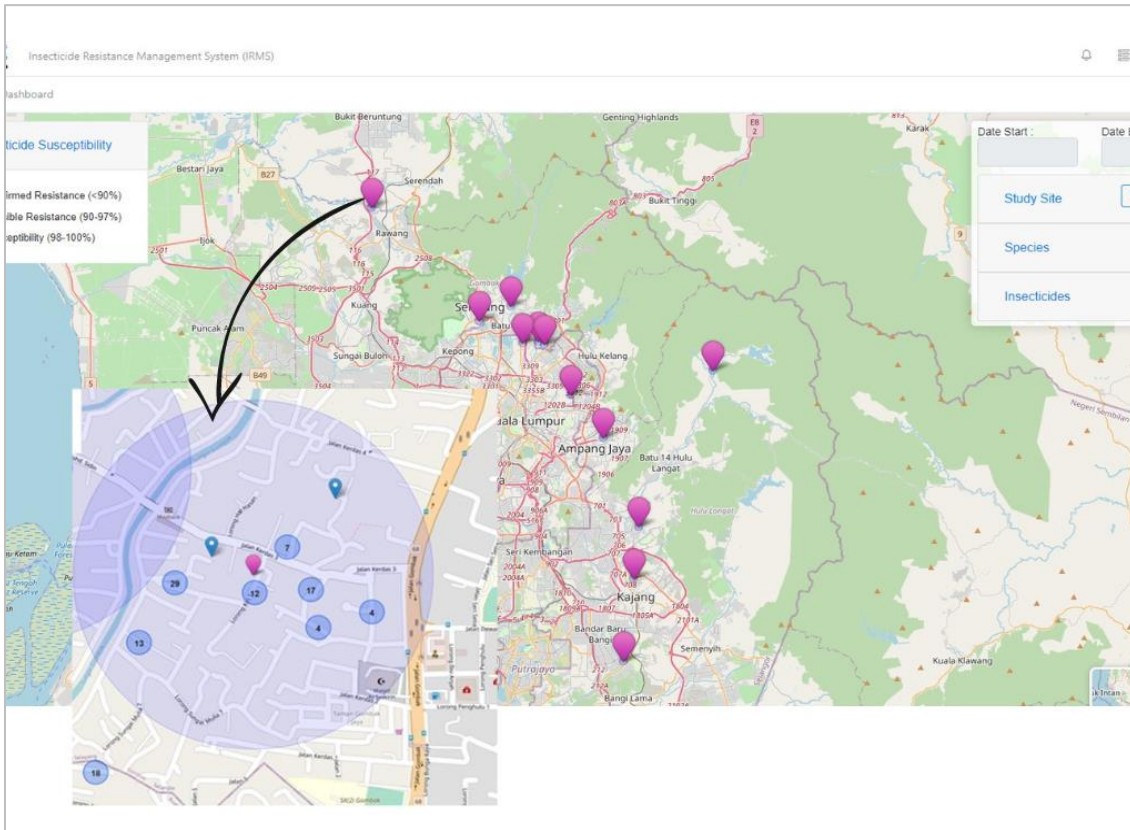


Figure 2: The main interface of IRMS with purple dots on maps indicates the location of collected *Aedes* populations, while zooming into the monitored site reveals information regarding the placement of ovitraps during sampling.

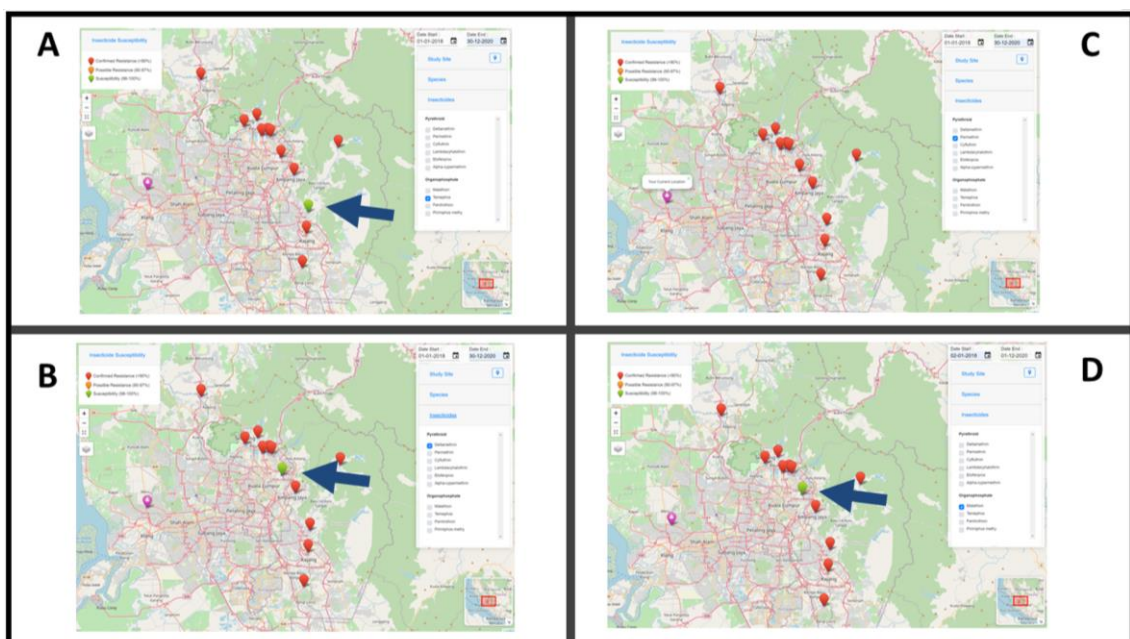


Figure 4: A screenshot of the IRMS interface illustrates the results of the insecticide resistance status of *Aedes aegypti* strains against all insecticides tested.

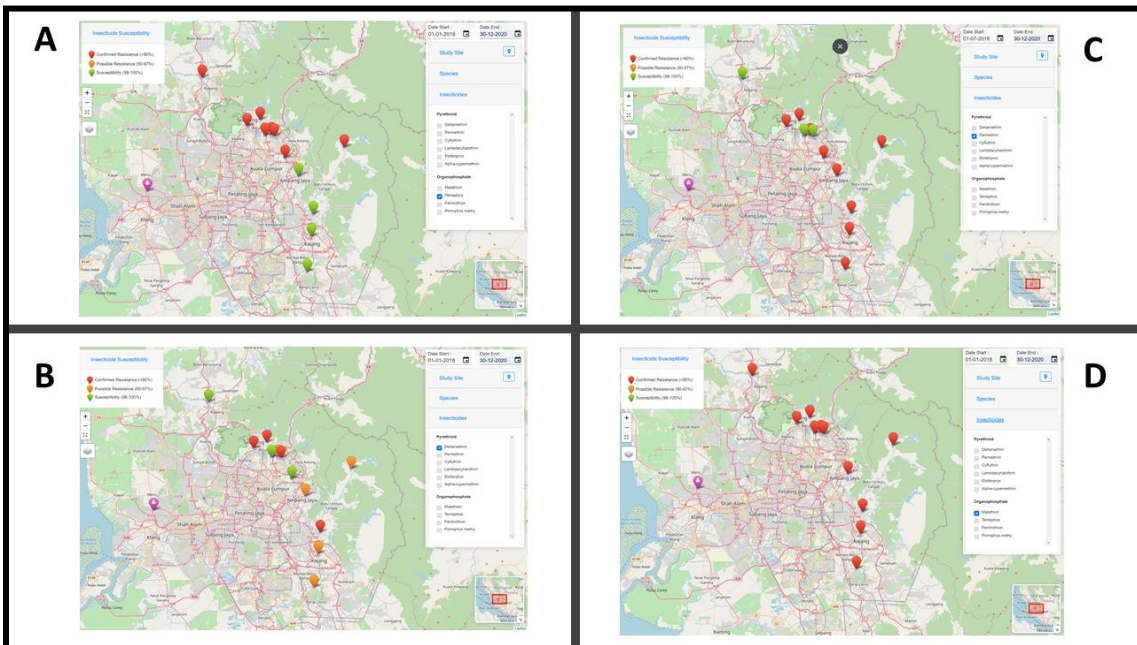


Figure 5: A screenshot of the IRMS interface shows outcomes of insecticide resistance status of *Aedes albopictus* strains against all tested insecticides.

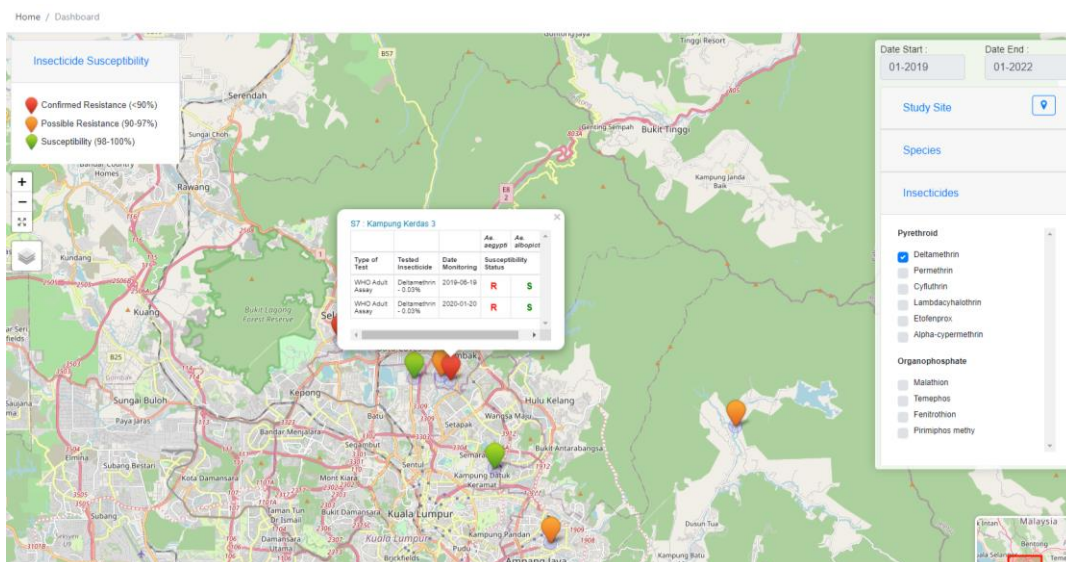


Figure 6: Resistance profiling of both *Aedes* species to one type of insecticide.

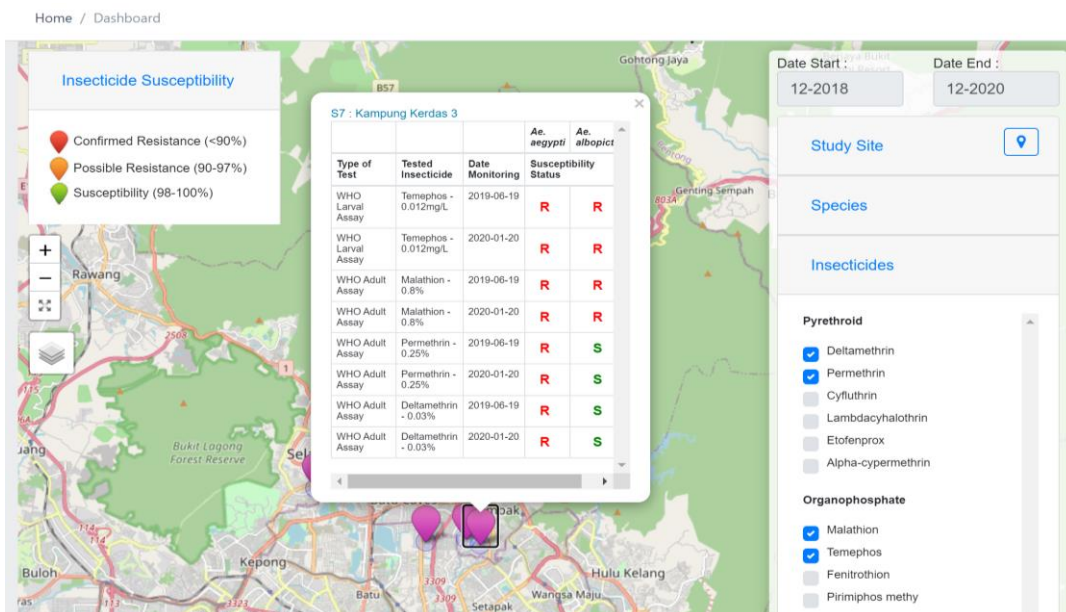


Figure 7: Resistance profiling of both *Aedes* species to more than one type of insecticide.

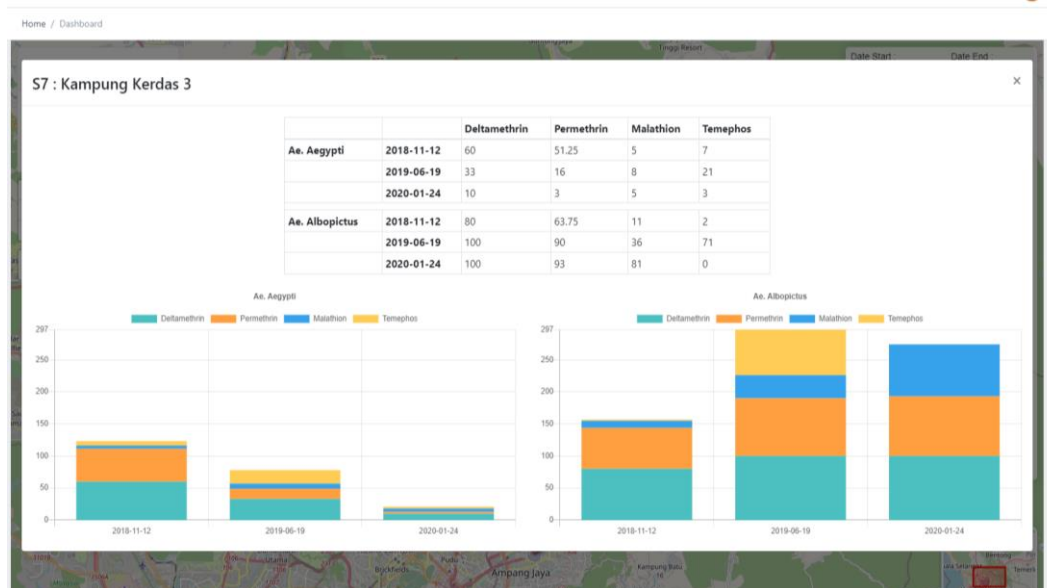


Figure 8: A screenshot from IRMS illustrating the insecticide resistance status of *Aedes* mosquito collected from a site against all tested insecticides.



Figure 9: Heat map of *Aedes aegypti* and *Aedes albopictus* insecticide resistance profiles.

3.5 IRMS Reporting of Insecticide Resistance

Resistance to insecticides is managed in practice through site-specific analyses. The collection of surveillance data illustrating resistance patterns enables a more complete understanding and resolution of this problem. Monitoring data is critical because it acts as a warning system for the implementation of initial insecticide resistance management as a preventative measure against widespread resistance. As a result, reporting activity is a critical first step prior to stakeholders being able to take corrective action. Using IRMS to manage insecticide resistance data enables rapid generation of insecticide resistance output, removing the step of transcribing resistance data in Excel solely for the purpose of creating a graphical representation and enabling the export of IRMS findings in the fix-format reports.

3.6 Integrating A System For Managing Insecticide Resistance Into The Management Of Insecticide Resistance Data

It is critical to monitor insecticide resistance in order to avoid dengue control measures failing, which would have a detrimental effect on current dengue control measures. To ensure the insecticide's effectiveness, thoroughly interpreted data on the development of resistance in the *Aedes* population over time enable prudent decisions about insecticide resistance countermeasures, particularly in preventing future resistance development. More importantly, IRMS improves accuracy and reduces time spent manually retracing raw data when determining resistance trends in a locality. This innovative insecticide resistance management system (IRMS) represents a significant step forward in the digital transformation of the entomologist, academician, scientist, researcher,

and stakeholder communities involved in the management of insecticide resistance in dengue control programmes. Additional features will be added in the future, most notably the ability to integrate data on resistance mechanisms and the capacity to integrate data from multiple platforms into a centralised hub.

3.7 Limitations and Future Directions

Researchers and personnel involved in insecticide susceptibility analysis as well as those responsible for monitoring insecticide susceptible profiles will benefit from the IRMS. In IRMS, users are required to enter data obtained from the lab into electronic forms; however, if this system is to be used to manage historical records, capturing the historical data will require the insertion of an excel format to be uploaded in IRMS, under the condition that the excel format adheres to the standard data elements and guidance established by the WHO guideline. This feature can be modified to accommodate future needs and the most recent update regulation. Currently, the system focuses on the primary management of susceptibility profiling, resistance mechanisms involving enzyme detoxification and mutation at the target site will be the next data element to be captured and incorporated into IRMS. As the IRMS is now ready for user use, future research will focus on user experience in order to identify any gaps that need to be filled.

4. CONCLUSION

The development of the IRMS represents a proactive effort to manage comprehensive insecticide resistance data. Generally, raw data from insecticide resistance tests were collected on paper and then entered into

an Excel spreadsheet to determine insecticide susceptibility status. The data were then imported into a paper-based reporting system. A high volume of monitoring requires prompt analysis and reporting of results. Consequently, it is believed that the IRMS is advantageous to the efficient monitoring of insecticide resistance data, as it reduces the likelihood of time delays in combating widespread insecticide resistance, particularly in dengue hotspots.

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