INSECTICIDE RESISTANCE PREVALENCE OF DENGUE VECTORS IN MALAYSIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Abstract. A comprehensive understanding of insecticide resistance in dengue vectors is crucial, especially given the increasing global incidence of dengue. This concern underscores the need to assess the resistance status of *Aedes aegypti* and *Aedes albopictus*, the vectors responsible for transmitting the virus. In Malaysia, chemical-based insecticides remain the primary intervention for prevention efforts. Preventing the failure of these interventions requires an understanding of the prevalence of insecticide resistance. To provide an overview of resistance reported in *Aedes* mosquitoes, this review analyzed the reported resistance status of *Ae. aegypti* and *Ae. albopictus* in Malaysian *Aedes* mosquitoes from 30 eligible studies published from 2005 to 2022. The resistance and susceptibility trends were analyzed according to location, insecticide class and over time, revealing widespread resistance of *Ae. aegypti* to deltamethrin, malathion and permethrin, the insecticides most studied. On the other hand, *Ae. albopictus* exhibits variable susceptibility trends to these insecticides, with resistance to the carbamates and organochlorines being more pronounced than in *Ae. aegypti*. A meta-analysis with a random-effect model was also conducted to assess publication bias. Substantial variations were noted, possibly due to the sampling methods and number of samples screened, but the results confirm the abovementioned conclusions. The data obtained from the literature review will help fill the gaps in knowledge of the insecticidal susceptibility status of *Aedes* mosquitoes in Malaysia.

Keywords: *Aedes* spp, dengue vector, insecticide resistance, carbamate, dichlorodiphenyltrichloroethane, organochlorine, organophosphate, pyrethroid

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INTRODUCTION

Arthropod-borne diseases pose a significant global health challenge, with dengue ranking among the most critical. Dengue infection places approximately over 40% (3.9 billion) of the world's population at risk. The number of annually reported dengue cases has surged from 500,000 in 2000 to 5.2 million in 2019 (WHO WPRO, 2023). Dengue transmission is predominantly concentrated in tropical and subtropical regions, where dense populations of *Aedes* mosquitoes act as the primary vectors. Besides the presence of a high population of *Aedes*, the rise in dengue cases is attributed to such factors as an absence of a viable tetravalent vaccine, increased urbanization, a growing population, and proliferation of water containers for *Aedes* breeding in the environment (Gubler, 2011).

It is recognized that people residing in 128 countries, located in Africa, the Eastern Mediterranean region, South and Southeast Asia, the Americas, and the Western Pacific, are at risk of dengue infection, with Asia alone accounting for more than 50% of the disease burden (WHO, 2023). In Malaysia, Selangor stands out as the most heavily affected area, accounting for 50% of the cumulative cases in the country (Table 1). In 2023, Selangor continues to report the highest number of cases in Malaysia, followed by Kuala Lumpur, Putrajaya, Pulau Pinang, Johor, and Sabah (MOH, 2023b).

Dengue control relies on various preventive measures, including insecticide fogging, ultra-low volume (ULV) space spraying and larviciding. However, repeated and prolonged application of insecticides can contribute to the development of insecticide resistance in the *Aedes* mosquito populations (WHO Expert Committee on

Table 1
Cumulative dengue cases in Malaysia 2020-2022

Areas (Federal Territories/States)	Cumulative number of dengue cases by year		
	2020	2021	2022
Johor	11,622	1,775	4,458
Kedah	780	644	2,753
Kelantan	3,889	223	908
Kuala Lumpur and Putrajaya	10,631	3,067	6,127
Labuan	7	5	51
Melaka	2,843	611	483
Negeri Sembilan	2,891	649	1,788
Pahang	3,220	428	599
Perak	2,665	557	1,698
Perlis	80	21	409
Pulau Pinang	1,043	406	4,956
Sabah	4,078	1,763	4,253
Sarawak	1,516	426	347
Selangor	44,635	15,741	28,688
Terengganu	404	49	121

Source: MYSA, 2022; MOH, 2023a

Note: Areas include the Federal Territories (Kuala Lumpur, Putrajaya, and Labuan), while the remaining areas are the States in Malaysia.

Insecticides and WHO, 1970; WHO, 2011b). The first documented study on insecticide resistance in *Ae. aegypti* dates back to the Malayan era, associated with using dichlorodiphenyltrichloroethane (DDT). DDT was commonly used to control vector-borne diseases such as malaria and dengue fever during the mid-20th century, starting in the 1940s (Teng and Singh, 2001; WHO, 2011b). Its widespread use peaked in the 1950s and 1960s, particularly in malaria eradication programs. However, its use declined in the 1970s due to environmental and health concerns, as well as the development of resistance in mosquito populations. Resistance to DDT was reported in the *Ae. aegypti* population from Sijangka, Malaya (Shindawi, 1957), suggesting that exposure to DDT results in resistance development.

Since 1999, DDT insecticides have not been used for dengue control in Malaysia, replaced by pyrethroids, a class of insecticides known for their effective mosquito knockdown property and low toxicity to humans, and have become the preferred choice. The first pyrethroid insecticide, bioresmethrin, successfully contained the 1974 Malaysian dengue outbreak in Penang (Shekar and Huat, 1992). To date, pyrethroids remain the favored insecticide option, especially for space spray application. In Malaysia, health policy mandates that vector control measures such as fogging or space spray, must be implemented within 7 days of a reported dengue case, with repeat spraying within 2 weeks if additional cases emerge in the same locality (MOH, 2022). Given the vital role of insecticides in vector control programs, their extensive use is particularly observed in areas designated as hotspots for ongoing reported dengue cases lasting more than 32 weeks (Tham, 1993; MOH, 2022).

The study of insecticide resistance in Malaysia highlights the significant contributions from research institutions and universities, reflected in publication affiliations. Under the National Resistance Management Program (MOH, 2022), health authorities are mandated to conduct insecticide resistance detection and establish susceptibility profiles for *Aedes* spp, primarily from localities with reports of persistent dengue cases. Resistance detection uses the WHO tube assay protocol (WHO, 2022), classifying specimens as either resistant or susceptible. Coordinated efforts aim to enhance resistance monitoring, address knowledge gaps and deepen understanding of resistance patterns in dengue vector populations. This activity is critical for supporting evidence-based vector control strategies, focussing on managing the risk of insecticide resistance in intervention programs.

In this review, we analyzed resistance and susceptibility data of *Aedes* spp reported in eligible studies, providing a comprehensive overview of resistance patterns among dengue vectors in Malaysia. The evolving patterns of resistance in *Aedes* spp to various insecticide types and across different locations over time were thoroughly reviewed. Additionally, we examined the guidelines used in the eligible studies for conducting the WHO adult bioassay to determine insecticide resistance status. Challenges and limitations associated with studies of insecticide resistance in mosquito vectors of dengue infection were also briefly discussed within the scope of the review. A comprehensive analysis of insecticide resistance patterns in *Aedes* mosquitoes provides valuable data, emphasizing the need for routine surveillance and standardized resistance monitoring protocols to mitigate the risk of insecticide resistance and sustain effective dengue prevention measures.

MATERIALS AND METHODS

Search strategy

Two reviewers independently performed the identification process for inclusion of the selected publications. The Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guided the selection of eligible publications (Moher *et al*, 2009). The search was performed using three databases, namely PubMed, Scopus and Web of Science. The search keywords were derived from key concepts of the following research question: "To what extent have studies been conducted on the insecticide susceptibility of *Aedes* mosquitoes in Malaysia?". The identified key concepts were "Aedes mosquito", "insecticides" and "insecticide resistance". The keywords for the search query to select eligible articles were

"*Aedes aegypti*", "*Aedes albopictus*", "deltamethrin", "insecticides", "insecticide resistance", "insecticide susceptibility", "malathion", "organophosphates", "permethrin", "pyrethroids", and "temephos". The search covered articles published between 2005 and 2022, focusing on studies conducted in Malaysia.

Article eligibility

The research articles were converted into a Comma-separated values (CSV) file format and duplicated articles were removed. Included in the search were publications on the determination of insecticide resistance status of wild-caught *Ae. aegypti* and *Ae. albopictus* specimens collected in Malaysia. Excluded were reports on the insecticide susceptibility status of laboratory-bred *Aedes* spp, bioassay conducted on wild-caught *Aedes* spp from non-Malaysia origins, *Anopheles* mosquitoes, and insecticide resistance studies of *Aedes* spp via selection pressure, field assessments on the bio-efficacy of insecticides and unpublished data. The process employed in selecting the eligible articles is shown in Fig 1.

Data extraction and validation

The data extraction process for the synthesis of quantitative meta-analysis was performed separately by the reviewers, with disagreements resolved through discussion. The review aimed to address two significant questions: (i) "What is the distribution of insecticide resistance patterns in *Aedes* mosquitoes according to the chemical classes of insecticides tested in studies conducted in Malaysia?", and (ii) "What are the specific insecticides most frequently associated with resistance in *Aedes* spp collected in Malaysia according to geographical locations and reported over the specified period?". To address these questions, data on insecticide susceptibility, specifically 24-hour mortality rates in wild-caught *Ae. aegypti* and *Ae. albopictus* against test insecticides, including the numbers of susceptible and resistant specimens reported in the eligible publications, were recorded. Variables included the names of authors, publication year, types of insecticides tested, selection of insecticide studies according to class and type of insecticides, discriminating diagnostic concentrations of insecticide tested in each publication (Table 2), and information on the sampling locations (Supporting Data 1).

Data analysis

The meta-analysis of proportion evaluates the frequency of resistant specimens detected relative to the total number of specimens screened in each study. The primary result was to determine the proportion of a resistant population. A random-effects model was applied to address publication bias and assess heterogeneity among the reviewed studies, estimating prevalence rate and a 95% confidence interval (CI) (Henmi and Copas, 2010). Insecticide resistance patterns, indicated by the proportion of resistant strains reported, were visualized using a heat map and forest plot. Additionally, temporal resistance trends according to locations were illustrated through a bubble chart.

RESULTS

Selection of publications

Two hundred and seventy-two publications were retrieved from the three databases (PubMed, Scopus and Web of Science), among which 152 duplicates were identified and removed to obtain 120 unique articles (Fig 1). The inclusion criteria were the full text articles studying *Aedes aegypti* and *Aedes albopictus* collected from Malaysia, utilizing test protocols that followed WHO or CDC guidelines for laboratory evaluation against a single concentration of the tested insecticide, and reporting published susceptibility data. Therefore, among these 120 unique articles, 60 were excluded because they contained only Abstracts and no full texts were available. Furthermore, 30 articles were excluded as they did not meet the inclusion criteria, resulting in a final selection of 30 articles for a full review.

The insecticides described in the reviewed eligible publications showed a significant increase in

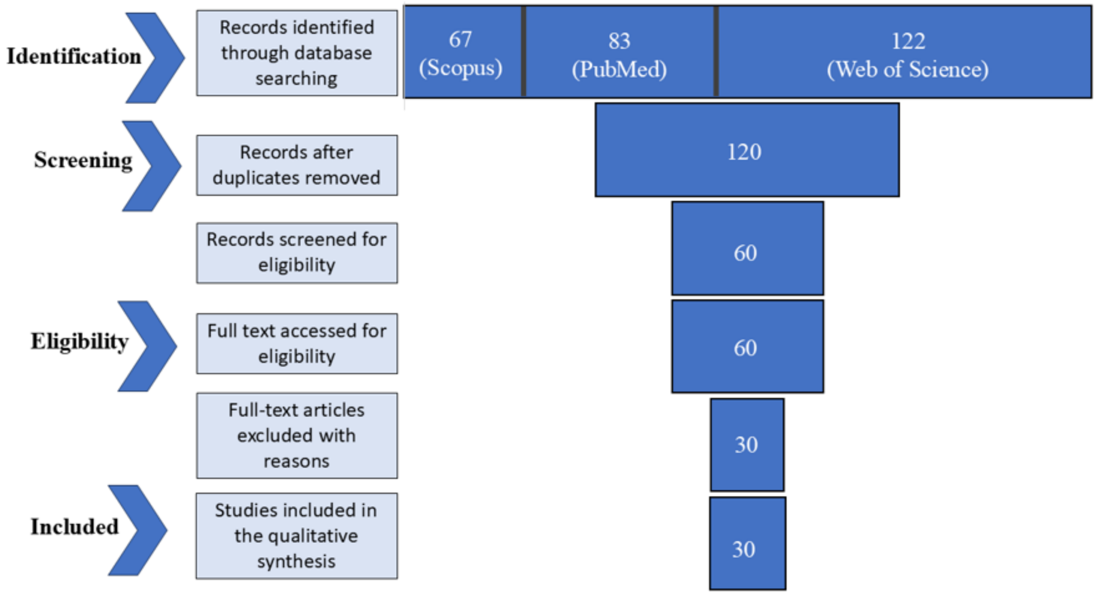

Fig 1 - Search strategy of articles included in the review based on preferred reporting items for systematic reviews and meta-analyses (PRISMA)

resistance among *Aedes* spp from 2005 to 2022 (Fig 2). The highest number of studies was reported in 2021, with pyrethroids emerging as the most studied insecticide class. Throughout the review period, organophosphates were the most frequently selected insecticide class in the studies (n = 23), followed by pyrethroids (n = 21), organochlorines (n = 10), and carbamates (n = 9). Since multiple insecticide classes could be examined within the same studies, these numbers highlight the strong research interest in each class.

The earliest documented study was in 2005 on temephos. Among pyrethroids, deltamethrin and permethrin were the most extensively studied compared to other pyrethroids, such as cyfluthrin, etofenprox and lambda-cyhalothrin (Fig 3). As for organophosphates, malathion and temephos were the most frequently

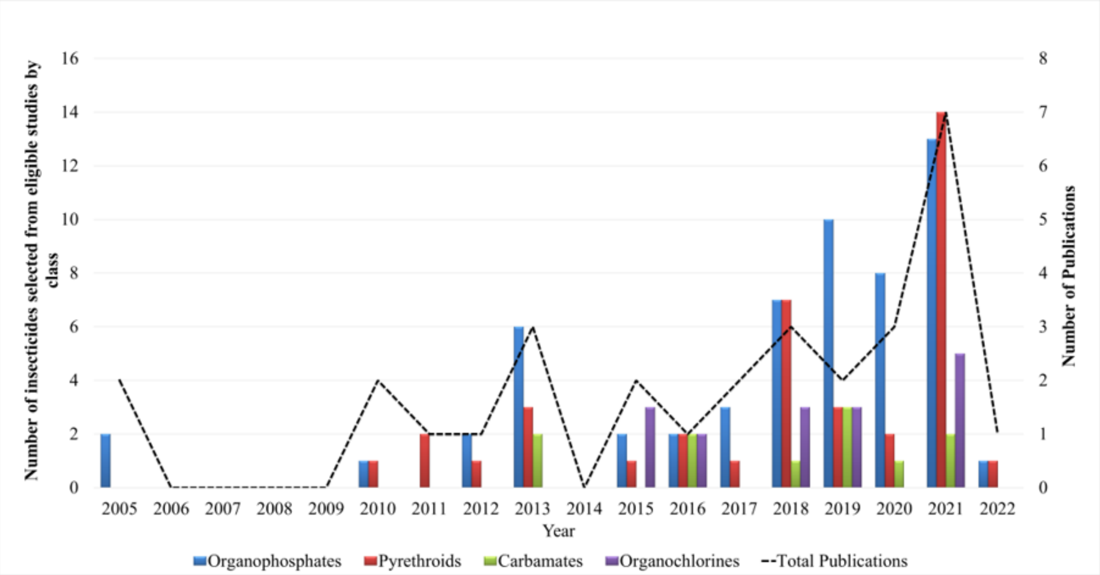

Fig 2 - Publications on insecticide resistance classified according to four insecticide classes 2005-2022

Note: The publications were from PubMed, Scopus and Web of Science.

studied compared to bromophos, chlorpyrifos, fenitrothion, fenthion, and pirimiphos-methyl. On the other hand, carbamates and organochlorines receive relatively less mention, as these classes of insecticides are not widely used in dengue vector control programs.

Study sites and resistance patterns

The majority of studies included geographic coordinates of their sampling locations, with 11/30 studies without this information, but referred the sites as either dengue or non-dengue areas or as "Site A" and "Site B" (Supporting Data 1). Providing locality coordinates is essential for operational teams, as it helps target areas requiring intensified efforts to manage insecticide resistance. It was noted that Selangor is the primary focus compare to other areas in

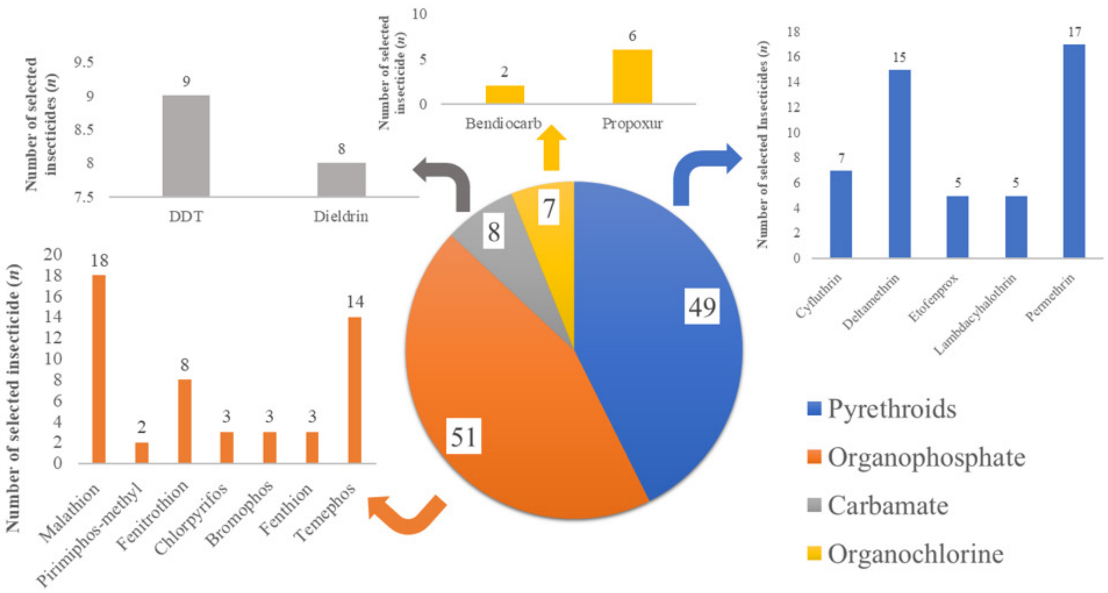

Fig 3 - Insecticides used in monitoring *Aedes* mosquito resistance in Malaysia reported in 30 eligible publications 2005-2022

Malaysia due to its high number of dengue cases throughout the year (Supporting Data 1). Identifying the sampling regions where *Aedes* mosquitoes are collected remains an underreported aspect of the reviewed published reports. Notably, space-spray interventions for dengue control typically mandate 85% coverage of areas surrounding the reported dengue cases (MOH, 2022). Consequently, reporting the exact locations of sampling zones for insecticide-resistant dengue vectors is of significant value for the Ministry of Health (MOH), particularly if these zones can be directly associated with dengue outbreaks.

The ability to trace resistance patterns over time enables knowledge of temporal trends in resistance with specific locations. The reported frequency of resistant *Aedes* in the eligible publications according to the study areas and insecticide classes from 2008 to 2024 is shown in Fig 4. Resistance patterns exhibit distinct geographical trends, particularly in areas such as Johor Bahru, Kuala Lumpur and Selangor, where insecticide usage is extensive, primarily in dengue-endemic regions. Notably, *Ae. albopictus* is generally more susceptible to deltamethrin whereas *Ae. aegypti* is more susceptible to permethrin (Figs 4A and 4B). On the other hand, *Ae. albopictus* shows greater resistance to organophosphates than *Ae. aegypti* in all locations (Figs 4C and 4D). These phenomena reflect the application practices in Malaysia, where temephos is widely used outdoors for larviciding, whereas the pyrethroids, such as deltamethrin and permethrin, are predominantly applied indoors (Lee *et al*, 2015). *Ae. aegypti* predominantly occupies indoor environments while *Ae. albopictus* is more prevalent in outdoor settings (Rudnick, 1967).

Nearly all articles conducted only a one-time collection, with only four articles reporting multiple monitoring samplings (Supporting Data 1). For instance, Chen *et al* (2005b) investigating the resistance of *Aedes* spp to temephos through weekly samplings in Taman Samudera, Gombak; Kampung Banjar, Gombak; Taman Lembah Maju, Cheras; and Kampung Baru, Kuala Lumpur, revealing variations in resistance in *Aedes* spp collected at different times, indoors and outdoors. Notably, *Ae. aegypti* collected outdoors shows an increase in mortality rates compared to the initial surveillance. However, Rong *et al* (2012) monitored temephos resistance over a year in Seksyen 17, Shah Alam and observed increased resistance in *Aedes* populations compared to sites where *Bacillus thuringiensis israelensis* (Bti) interventions are implemented. These findings underscore the need for temporal surveillance as the prevalence of insecticide resistance measured at a single time point may not provide a comprehensive understanding of the resistance patterns, as susceptibility can fluctuate over time and with types of control measures. In addition, continuous, long-term monitoring

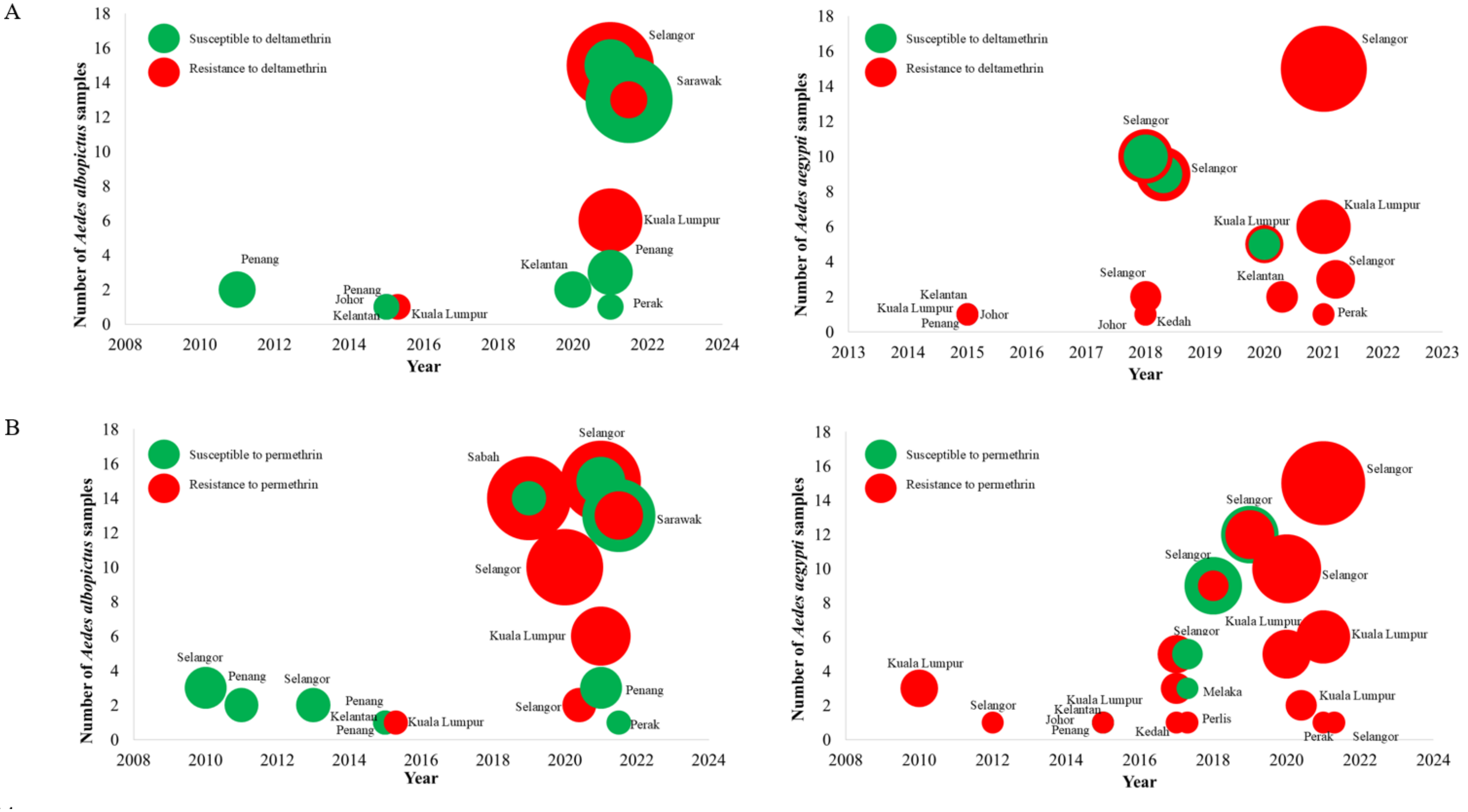

Fig 4 - Insecticide susceptibility and resistance of *Aedes* mosquitoes in the States of Malaysia 2008-2024

Note: Size of circle indicates prevalence proportion with larger circles indicating more samples and smaller circles indicating fewer.

A: Deltamethrin; B: Permethrin

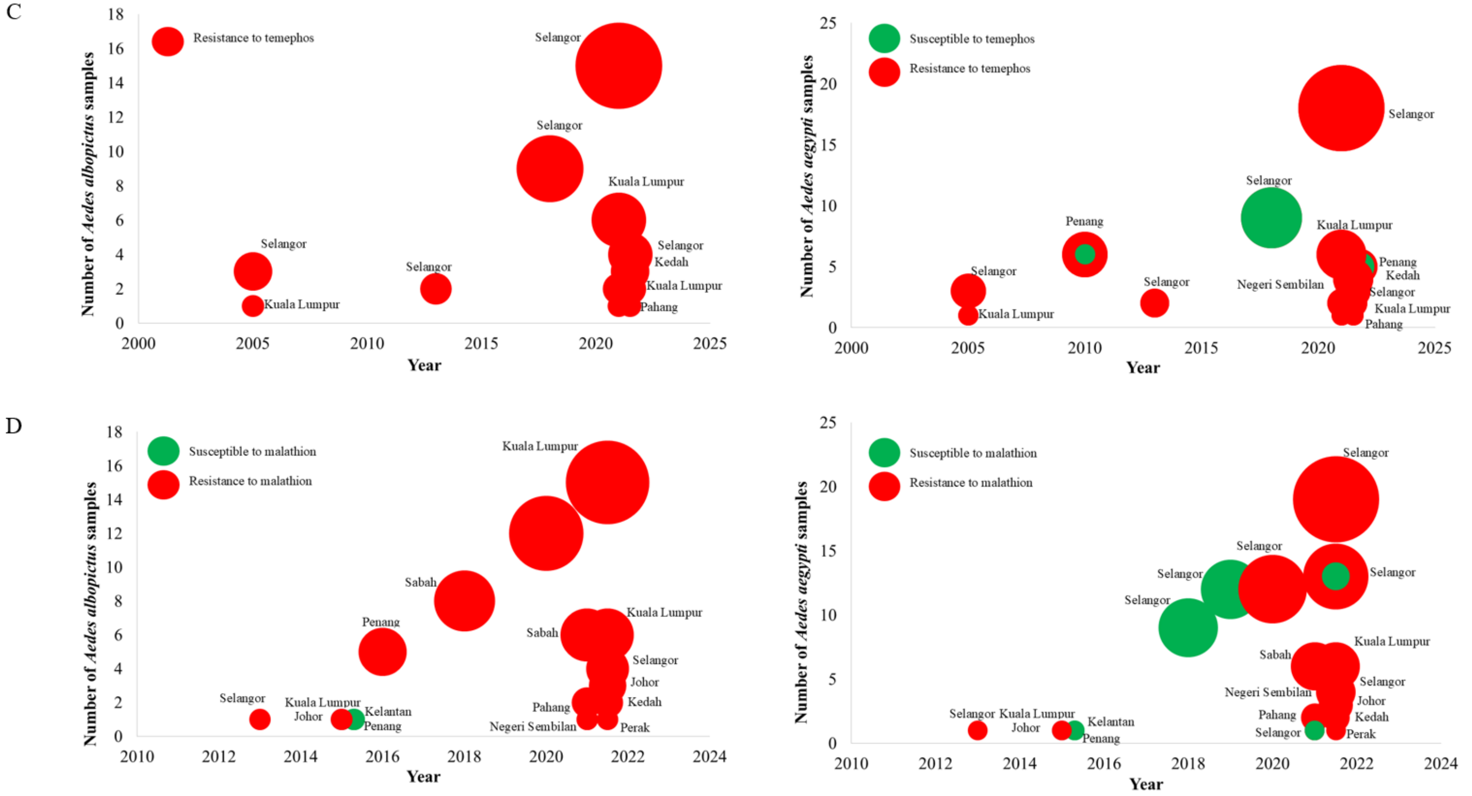

Fig 4 - (cont)

Note: Size of circle indicates prevalence proportion with larger circles indicating more samples and smaller circles indicating fewer.

C: Temephos; D: Malathion

is important to help guide strategies for mitigating insecticide resistance at sites recognized as areas of concern for dengue outbreaks.

Insecticide resistance of wild-caught *Ae. aegypti* and *Ae. albopictus* in Malaysia

We assessed the susceptibility status of wild-caught *Ae. aegypti* and *Ae. albopictus* in Malaysia reported in the eligible publications. *Aedes* spp exhibiting observed mortality rates <98% after 24 hours of exposure to an insecticide are classified as resistant. Mosquitoes displaying an effective response against a test insecticide, with mortality rates >98% after 24 hours of exposure, are categorized as susceptible. The discriminating concentrations of the test insecticides employed in each study are listed in Table 2.

Analysis of resistance trends in wild-caught *Ae. aegypti* and *Ae. albopictus*, based on adult mortality data from 2005 to 2022, reveals intriguing patterns. Variability in resistance patterns to the four insecticide classes, namely carbamate, organochlorine, organophosphate, and pyrethroid, is observed among the tested *Aedes* samples. Meta-analysis was used to assess the prevalence of resistant strains reported in each study, utilizing a scale ranging from 0 to 1, with a value of 0 indicating an absence of resistant samples and a value of 1 a high proportion of resistant samples. A heat map was then constructed to visualize the resistance patterns among the *Aedes* spp across all four classes of insecticides reported in the legible publications (Fig 5). The significant variability between studies reflects the diversity of mosquito populations analyzed in the respective studies, which were classified and analyzed by grouping them based on the types of insecticides tested. The meta-analysis of resistance patterns across all eligible studies revealed substantial heterogeneity for most of the tested insecticides in both *Ae. aegypti* and *Ae. albopictus*. However, low to moderate heterogeneity was observed in studies assessing resistance of *Ae. albopictus* to the

Table 2

Descriptive data on mosquito species and insecticide testing guideline and tested insecticides from eligible publications 2005-2022

Reference	*Aedes* sp	Tested insecticide	Guideline
Chen *et al*, 2005a	*Ae. aegypti* *Ae. albopictus*	OP: Temephos 0.012 mg/l	WHO (1981c)
Chen *et al*, 2005 2005b	*Ae. aegypti* *Ae. albopictus*	OP: Temephos 0.012 mg/l	WHO (1981c)
Loke *et al*, 2010	*Ae. aegypti*	OP: Temephos 0.2 mg/l	WHO (1981c)
Wan-Norafikah *et al*, 2010	*Ae. aegypti*	PY: Permethrin 0.75%	WHO (1981b)
Chan *et al*, 2011	*Ae. albopictus*	PY: Deltamethrin 0.2%, Permethrin 0.7%	WHO (2009)
Rong *et al*, 2012	*Ae. aegypti*	C: Bendiocarb 0.1%, Propoxur 0.1% OC: DDT 4% OP: Fenitrothion 1%, Malathion 5% PY: Cyfluthrin 0.15%, Permethrin 0.75%	WHO (1981a); WHO (1981b)
Wan-Norafikah *et al*, 2013	*Ae. albopictus*	PY: Permethrin 0.75%	WHO (1981b)
Chen *et al*, 2013a	*Ae. albopictus*	C: Bendiocarb 0.1%, Propoxur 0.1% OC: DDT 4%, Dieldrin 4% OP: Fenitrothion 1%, Malathion 5% PY: Cyfluthrin 0.15%, Deltamethrin 0.05%, Etofenprox 0.5%, Lambdacyhalothrin 0.05%, Permethrin 0.75%	WHO (1981b)
Chen *et al*, 2013b	*Ae. albopictus*	OP: Temephos 1.0 mg/l	WHO (1981b)
Low *et al*, 2015	*Ae. albopictus*	C: Dieldrin 4%	WHO (1981a)

Table 2 (cont)

Reference	*Aedes* sp	Tested insecticide	Guideline
Ishak *et al*, 2015	*Ae. aegypti*	C: Dieldrin 4% OC: DDT 4% OP: Malathion 5% PY: Deltamethrin 0.05%, Permethrin 0.75%	WHO (1998)
Rahim *et al*, 2016	*Ae. albopictus*	OP: Malathion 0.2 mg/l, Temephos 0.02 mg/l	WHO (2005)
Rahim *et al*, 2017	*Ae. albopictus*	OP: Malathion 2.4% PY: Deltamethrin 0.28%, Permethrin 0.95%	WHO (1981b)
Rosilawati *et al*, 2017	*Ae. aegypti*	PY: Permethrin 0.75%	WHO (2016a)
Rasli *et al*, 2018	*Ae. aegypti*	OP: Fenitrothion 1%, Malathion 0.8% PY: Cyfluthrin 0.15%, Deltamethrin 0.03%, Lamdacyhalothrin 0.03%, Permethrin 0.25%	WHO (2016a)
Leong *et al*, 2018	*Ae. aegypti*	C: Propoxur 11.653 mg/ml OC: DDT 11.21 mg/ml OP: Malathion 0.629 mg/ml, Temephos 0.082 mg/ml PY: Cyfluthrin 0.003 mg/ml, Deltamethrin 0.003 mg/ml, Etofenprox 0.161 mg/ml, Lambdacyhalothrin 0.022 mg/ml, Permethrin 0.033 mg/ml	WHO (1981c); WHO (1998)
Elia-Amira *et al*, 2018	*Ae. albopictus*	OC: DDT 0.012 mg/l, Dieldrin 0.05 mg/l OP: Bromophos 0.05 mg/l, Chlorpyrifos 0.012 mg/l, Fenitrothion 0.02 mg/l, Fenthion 0.025 mg/l, Malathion 0.125 mg/l, Temephos 0.012 mg/l	WHO (2016a)

Table 2 (cont)

Reference	*Aedes* sp	Tested insecticide	Guideline
Elia-Amira *et al*, 2019	*Ae. albopictus*	C: Bendiocarb 0.1%, Propoxur 0.1% OC: DDT 4%, Dieldrin 0.4% OP: Malathion 5%, Fenitrothion 1% PY: Cyfluthrin 0.15%, Deltamethrin 0.05%, Etofenprox 0.5%, Lambdacyhalothrin 0.05%, Permethrin 0.25%	WHO (2016a)
Leong *et al*, 2019	*Ae. aegypti*	C: Propoxur 1 µg/bottle OC: DDT 150 µg/bottle OP: Malathion 50 µg/bottle PY: Cyfluthrin 150 µg/bottle, Deltamethrin 0.5 µg/bottle, Etofenprox 5 µg/bottle, Lambdacyhalothrin 3 µg/bottle, Permethrin 1 µg/bottle	CDC (2011)
AhbiRami *et al*, 2020	*Ae. aegypti* *Ae. albopictus*	PY: Deltamethrin 0.05% OP: Pirimiphos-methyl 0.25%	WHO (2016a)
Ali *et al*, 2020	*Ae. aegypti* *Ae. albopictus*	PY: Lamdacyhalothrin 0.03%, Permethrin 0.25% C: Propoxur 0.1% OC: DDT 4% OP: Malathion 0.8%	WHO (2016a)
Siti-Futri *et al*, 2020	*Ae. aegypti*	PY: Cyfluthrin 0.15%, Deltamethrin 0.05%, Lambdacyhalothrin 0.05%, Permethrin 0.25%	WHO (2016a)

Table 2 (cont)

Reference	*Aedes* sp	Tested insecticide	Guideline
Lau *et al*, 2021	*Ae. albopictus*	C: Bendiocarb 0.1%, Propoxur 0.1% OC: DDT 4%, Dieldrin 0.4% OP: Fenitrothion 1%, Malathion 5% PY: Cyfluthrin 0.15%, Deltamethrin 0.05%, Etofenprox 0.5%, Lambdacyhalothrin 0.05%, Permethrin 0.25%	WHO (2016a)
Zuharah *et al*, 2021	*Ae. albopictus*	OP: Malathion 5% PY: Deltamethrin 0.05%, Pemethrin 0.75%	WHO (2016b)
Dinesh *et al*, 2021	*Ae. aegypti* *Ae. albopictus*	PY: Deltamethrin 0.05%, Pemethrin 0.75% OP: Malathion 5%, Pirimiphos-methyl 0.25%	WHO (2016b)
Zuharah and Sufian, 2021	*Ae. aegypti*	PY: Deltamethrin 0.05%, Pemethrin 0.75%	WHO (2016b)
Elia-Amira *et al*, 2021	*Ae. albopictus*	OC: Dieldrin 0.05 mg/l, Diphenytrichlo-roethane 0.012 mg/l OP: Bromophos 0.05 mg/l, Chlorpyrifos 0.012 mg/l, Fenitrothion 0.02 mg/l, Fenthion 0.025 mg/l, Malathion 0.125 mg/l, Temephos 0.012 mg/l	WHO (1981c)
Wan-Norafikah *et al*, 2021	*Ae. albopictus*	OC: DDT 0.012 mg/l, Dieldrin 0.05 mg/l OP: Bromophos 0.05 mg/l, Chlorpyrifos 0.012 mg/l, Fenitrothion 0.02 mg/l, Fenthion 0.025 mg/l, Malathion 0.125 mg/l, Temephos 0.012 mg/l	WHO (2016a)

Table 2 (cont)

Reference	*Aedes* sp	Tested insecticide	Guideline
Rasli *et al*, 2021	*Ae. aegypti*	OP: Malathion 0.8%, Temephos 0.12 mg/l, Temephos 1.0 mg/l PY: Deltamethrin 0.03%, Permethrin 0.25%	WHO (2016a)
Akhir *et al*, 2022	*Ae. aegypti*	OP: Pirimiphos-methyl 0.25% PY: Permethrin 0.25%	WHO (2016a)

C: carbamate; DDT: dichlorodiphenyltrichloroethane; mg/l: miligram per liter; mg/ml: miligram per mililiter; OC: organochlorine; OP: organophosphate; PY: pyrethroid; μg: microgram

organophosphates bromophos, cyfluthrin, etofenprox, and lambda-cyhalothrin (Fig 5B); similarly, low heterogeneity was noted for *Ae. aegypti* tested with pirimiphos-methyl (Fig 5A). The low level of heterogeneity for pirimiphos-methyl likely reflects the influence of a limited dataset available on resistance patterns, as the small number of tested samples limits conclusions regarding resistance prevalence. In contrast, significant levels of heterogeneity for other insecticides highlight a larger and more consistent dataset of tested samples, minimizing publication bias and reducing instances of non-significant levels of heterogeneity.

The meta-analysis of the prevalent resistance against tested insecticides was also displayed in a forest plot (Fig 6; Supporting Data 3-6). The resistance patterns to deltamethrin and permethrin show a contrasting pattern of resistance between the two species (Figs 6A and 6B). *Ae. aegypti* exhibits a trend of resistance to deltamethrin with the majority of the studies showing of a value 1.0, indicating

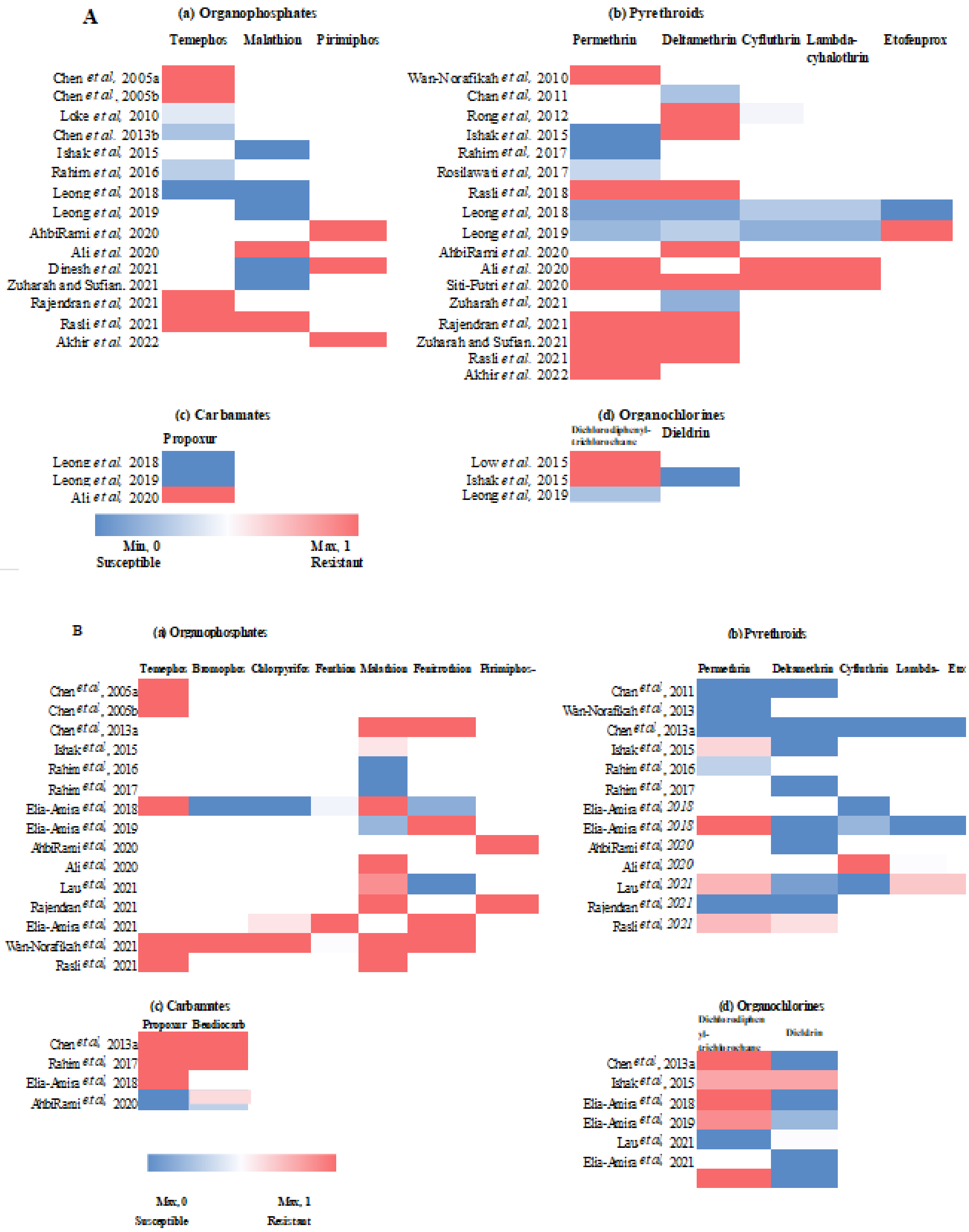

Fig 5 - Susceptibility of *Aedes aegypti* (A) and *Aedes albopictus* (B) samples to four classes of insecticides listed chronologically

Note: Data were from 30 eligible publications from 2005 to 2022. Heat map shows the range of resistance, from minimum (Min) (dark blue) to maximum (Max) (dark red).

all samples are resistant to deltamethrin at the discriminating concentrations (Fig 6A). The majority of the publications from 2011 to 2021 reported susceptibility of *Ae. albopictus* to deltamethrin with values of 0, except for the study by Rasli *et al* (2021), which report variable resistance of 0.6% proportion resistant and 0.4% proportion susceptible (Fig 6A). For permethrin resistance in *Ae. albopictus*, studies exhibit variable trends over time (Fig 6B). In the early years (2011-2013), a clear pattern of susceptibility was observed. However, permethrin resistance prevalence becomes more variable in later years, with resistance proportions ranging from 0.2 to 1 observed in 2015, 2019 and 2021 (Fig 6B). Only Rajendran *et al* (2021) and Zuharah *et al* (2021) report susceptibility of *Ae. albopictus* to permethrin, the former study being limited to a single site, while the latter included three sites. It is important to note that the findings of Zuharah *et al* (2021) are based on a diagnostic concentration of 0.75%, higher than the diagnostic concentration of 0.05% recommended by WHO (2016b). Variations in diagnostic thresholds may explain (in part) the significant heterogeneity in the data.

Limited research has been conducted on the resistance patterns of *Ae. aegypti* to the pyrethroids cyfluthrin, etofenprox and lambda-cyhalothrin (Supporting Data 3). The resistance patterns of *Ae. aegypti* and *Ae. albopictus* to these three insecticides are variable, with a clear and distinct pattern of resistance observed only in *Ae. albopictus* (Ishak *et al*, 2015; Low *et al* 2015; Supporting Data 3).

In studies on organophosphate insecticides, there is a notable focus on determining susceptibility profiles against malathion and temephos, aligning with the prevaling public health practices of using malathion as adulticides and temephos as larvicides. The resistance pattern of *Ae. aegypti* to temephos exhibits variability, with a proportion value of 1 reported by

Chen *et al* (2005a), Wan-Norafikah *et al* (2021) and Rasli *et al* (2021), while Loke *et al* (2010) and Rahim *et al* (2016) report values ranging from 0.6 to <0.9; on the other hand, Leong *et al* (2018) report all tested samples susceptible, with a proportion value of 0 (Fig 6D). Whereas, *Ae. albopictus* showed resistance in all tested samples (Fig 6D).

Susceptibility studies on the organophosphates bromophos, chlorpyrifos and fenthion highlight a notable gap in research in *Ae. aegypti*, as most studies are predominantly focused on *Ae. albopictus*. Consequently, no resistance data for *Ae. aegypti* against these larvicides have been published. The available resistance findings of *Ae. albopictus* for these larvicides are contributed by Elia-Amira *et al* (2018), Elia-Amira *et al* (2019) and Wan-Norafikah *et al* (2021) (Supporting Data 4). The organophosphates fenitrothion, malathion and pirimiphos-methyl are used as adulticides. Detection of resistance to these insecticides in *Ae. aegypti* has been significant after 2020, with most studies reporting resistance to malathion (Fig 6C). The discriminating concentrations used against malathion vary among the reports, except those of Ali *et al* (2020) and Rasli *et al* (2021), which apply a concentration of 0.8%. Even at the highest concentrations, resistance to malathion is observed (Fig 6C). Studies on profiling fenitrothion resistance are focused solely on *Ae. albopictus*. Chen *et al* (2013a), Elia-Amira *et al* (2019), Elia-Amira *et al* (2021), and Wan-Norafikah *et al* (2021) report a prevalence of resistance with a proportion value of 1, while Elia-Amira *et al* (2018) indicate a low proportion of resistance and Lau *et al* (2021) report susceptibility (Supporting Data 4). Regarding pirimiphos-methyl, AhbiRami *et al* (2020) report that *Ae. aegypti* and *Ae. albopictus* are susceptible but studies thereafter indicated both *Aedes* spp tested are becoming resistant (Supporting Data 4).

Regarding carbamate and organochlorine insecticides, more attention is placed on *Ae. albopictus*

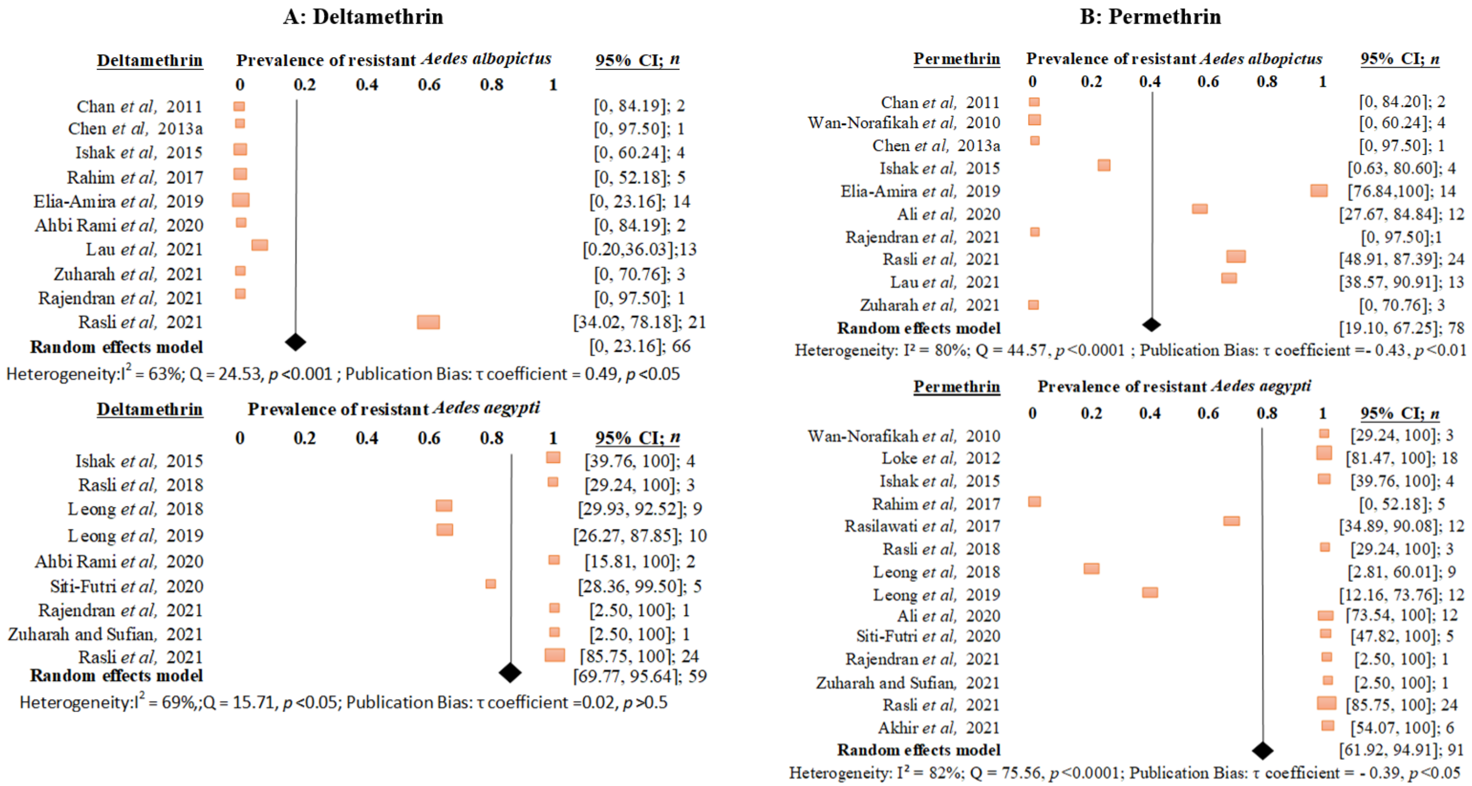

Fig 6 - Forest plots of meta-regression analysis on proportions of resistant *Aedes aegypti* and *Aedes albopictus* to various insecticides

Note: Rectangle (pink) represents the size of the samples involved, with a larger size indicating more samples and a smaller size indicating fewer samples.

CI: confidence interval; I^2: I-squared statistic measuring the inconsistency or heterogeneity between studies; Q: chi-square distribution

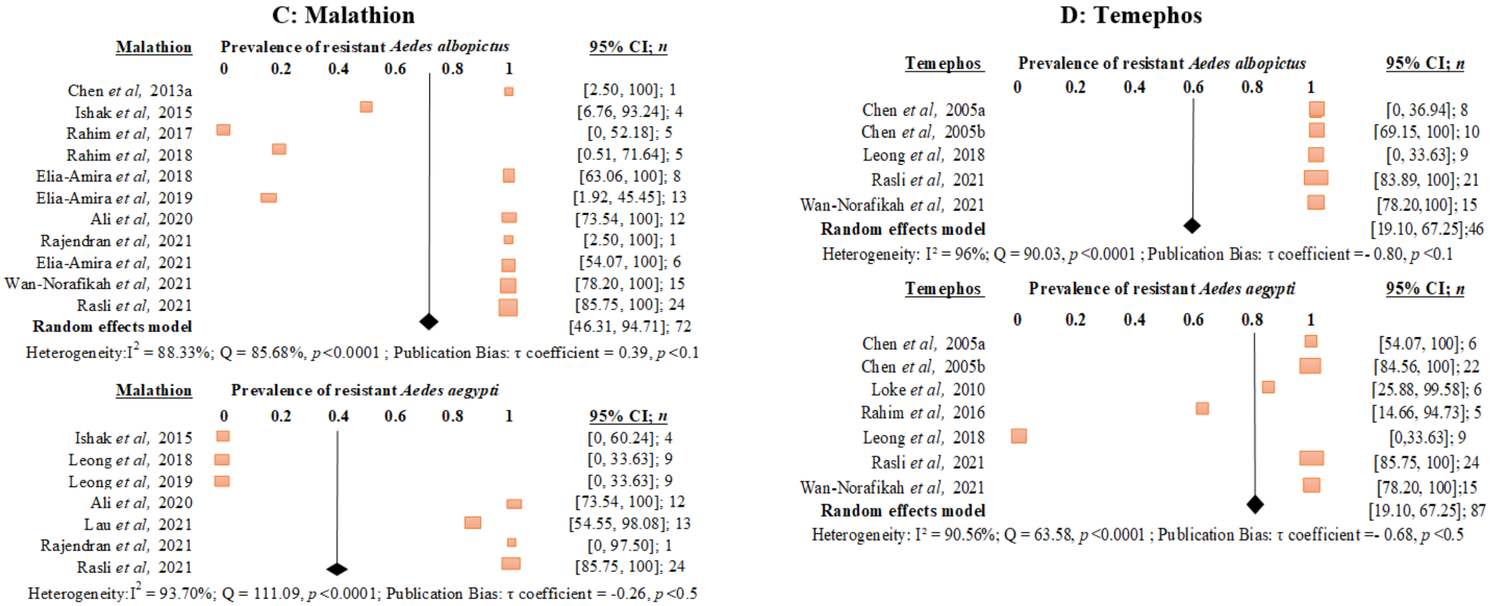

Fig 6 - (cont)

Note: Rectangle (pink) represents the size of the samples involved, with a larger size indicating more samples and a smaller size indicating fewer samples.

CI: confidence interval; I^2: I-squared statistic measuring the inconsistency or heterogeneity between studies; Q: chi-square distribution than *Ae. aegypti* (Supporting Data 5 and 6). Susceptibility studies of carbamates reveal a distinct resistance pattern between the two species, with the majority of studies reporting resistance of *Ae. albopictus* against propoxur while *Ae. aegypti* is susceptible, but a study by Ali *et al* (2020) demonstrate a population of *Ae. aegypti* resistant to propoxur (Supporting Data 5). Research on bendiocarb susceptibility is exclusively focused on *Ae. albopictus*, indicating a concerning trend of resistance development (Supporting Data 5).

The majority of studies shows that *Ae. albopictus* exhibits resistance to DDT and dieldrin, while research on *Ae. aegypti* remains less extensive (Supporting Data 6). Resistance to DDT by *Ae. aegypti* is observed for all samples tested by Ishak *et al* (2015), in contrast to the varying resistance patterns reported by Leong *et al* (2018) and Leong *et al* (2019). Conversely, the resistance pattern to dieldrin in *Ae. aegypti* is opposite to that of DDT (Supporting Data 6).

DISCUSSION

Insecticide-based interventions, such as space spraying and larviciding, are commonly employed to control dengue in many endemic countries, including Malaysia (WHO, 2011a). Four main classes of insecticides are used in vector control efforts (WHO, 2011a). Among these, pyrethroids are the most widely utilized for targeting adult mosquitoes, with applications including space spraying through fogging, residual spraying and ULV techniques. Meanwhile, organophosphates, particularly temephos, are predominantly used for larviciding via direct application (van den Berg *et al*, 2021). In most dengue-endemic countries, insecticide-based strategies remain the primary approach to dengue control. However, despite their effectiveness, sole reliance on these chemical-based methods poses a significant risk of resistance development in the mosquito vector populations, especially in areas with persistent dengue outbreaks (Hemingway *et al*, 1989; Ranson *et* *al*, 2010). Therefore, containment of insecticide resistance is a crucial element in ensuring the long-term effectiveness and sustainability of insecticide-based interventions for dengue vector control (Nauen, 2007; Dusfour *et al*, 2019).

In our review, we have highlighted the findings of widespread insecticide resistance in *Ae. aegypti* and *Ae. albopictus*. We have compared the patterns of insecticide resistance between the two *Aedes* species according to location and time. Geographically, resistance in *Aedes* spp varies significantly (Fig 4). The occurrences of resistance are localized, with variability in susceptibility to pyrethroids and organophosphates depending on specific locations. Overall, *Ae. albopictus* in screened study areas demonstrates greater susceptibility to pyrethroids compared to *Ae. aegypti*. In contrast, *Ae. albopictus* strains are more resistant to organophosphates compared to *Ae. aegypti* in the different States (Fig 4). These results are related to the application of pyrethroids as adulticides via indoor fogging, to which *Ae. aegypti*, predominantly found indoors, are more exposed compared to *Ae. albopictus* that is primarily found outdoors. *Ae. albopictus* is more commonly associated with direct larvicide applications, such as temephos, hence, the high prevalence of *Ae. albopictus* resistance to temephos.

Among the Malaysian study areas, Kuala Lumpur and Selangor are the most frequently selected study sites for insecticide resistance research throughout the past 17 years. It is noted that Selangor reports the highest number of dengue cases compared to the other areas (Table 1). Higher numbers of dengue cases in both Selangor and Kuala Lumpur, reflecting factors such as high human population density and urban environment (Adnan *et al*, 2021), which may contribute to increased selection pressure for insecticide resistance. This is linked to the site selections in most studies, where *Aedes* spp are collected from dengue-endemic and hotspot areas - locations with high dengue case burdens. These areas are recognized as experiencing intense selection pressure due to repeated insecticide applications for dengue prevention and control. In Malaysia, health policy mandates space spraying at sites with reported dengue cases, aiming for 85% coverage within seven days of the first reported case to interrupt disease transmission (MOH, 2022). If cases persist, repeat fogging is conducted to target the infected adult *Aedes* mosquitoes. Hence, the risk of persistent insecticide exposure is present when there is continuous insecticide use to combat dengue outbreaks.

Ae. aegypti samples collected from hotspot areas are consistently associated with regions characterized by high dengue transmission and elevated populations of *Aedes*, where fogging activities are routinely conducted as part of the vector control strategies aimed at reducing mosquito populations (MOH, 2022). Despite the increasing risk of insecticide resistance, the effectiveness of fogging interventions, primarily involving pyrethroid insecticides in these hotspot areas, appears to outweigh the possibility of the resistance emergence in studies evaluating the use of diagnostic concentrations (Rosilawati *et al*, 2017; Siti-Futri *et al*, 2020; Ali *et al*, 2020; Rasli *et al*, 2021). However, to date, the failure of control efforts using pyrethroid insecticides remains inconclusive. It is important to emphasize that resistance detection at diagnostic concentrations is not intended to represent the operational dose but rather serves as an early screening tool to facilitate more effective resistance management strategies (Bagi *et al*, 2015).

Surveillance of insecticides is critical, highlighting the need for continuous monitoring to effectively track resistance development over time. Regular, long-term surveillance will enable health authorities to determine the temporal development of resistance and identify emerging resistance trends before they become widespread. Continual monitoring should include seasonal assessments to account for variations in mosquito populations and resistance patterns, as well as post-intervention assessments to evaluate the effectiveness of chemical-based vector control interventions. In the Malaysia National Program (MOH, 2022), resistance management begins with assessing localities where resistance is identified at a one-time diagnostic concentration (1xDC). Subsequent monitoring evaluates insecticide-resistant populations exposed to five and ten times the diagnostic dose to determine the magnitude of resistance (MOH, 2022).

Rapid detection of insecticide resistance through the characterization of resistance mechanisms using enzyme bioassay is also among the activities that provide insights into the resistance situation (WHO, 1998). Managing insecticide resistance presents a complex challenge, particularly in susceptibility profiling, where resistance detection using the WHO bioassay requires colony preparation, a process that is both time-consuming and resource-intensive for control programs. Concurrently, efforts are directed towards reducing the high burden of dengue cases. While resistance screening remains an ongoing effort, alternative interventions such as the large-scale application of Wolbachia bacteria have been implemented in Malaysia (Nazni *et al*, 2019). In addition to the aim of reducing the disease burden, this approach also contributes to mitigating insecticide resistance within mosquito populations (Hoffmann *et al*, 2024).

We showed that the resistance frequency of insecticides included in the eligible publications, based on a meta-analysis of resistance data, indicates deltamethrin, malathion, permethrin, and temephos as the four major insecticides studied (Fig 3). Other insecticides among the carbamates, organochlorines, organophosphates, and pyrethroids, contribute less significantly according to the eligible publications (Supporting Data 2-6). Our findings from the review highlight the widespread resistance of *Ae. aegypti* to pyrethroids, particularly permethrin and deltamethrin. As for other insecticides within the pyrethroid group, the scarcity of reported studies leads to variable resistance patterns. However, a clear trend similar to that of permethrin and deltamethrin has emerged, namely resistance to cyfluthrin being more commonly reported in *Ae. aegypti* while *Ae. albopictus* shows higher susceptibility. The results reflect the practice of application using pyrethroids as adulticides via fogging practice in indoor space, thus resulting in *Ae. aegypti*, predominantly found indoors, being more exposed to the pyrethroids compared to *Ae. albopictus*.

In Malaysia, pyrethroids have been used since 1973 following the first nationwide dengue outbreak (Skae, 1902; Rudnick *et al*, 1965). They were incorporated into dengue control programs as adulticides, and preferred over malathion due to their odorless nature, effective knockdown capabilities and low toxicity to humans (Teng and Singh, 2001). Despite the reduced use of malathion in recent years, it remains the most frequently studied insecticide, with a significant number of studies reported in 2021 (Fig 6C). Although its use in dengue control has decreased, malathion continues to be widely used in the agricultural sector.

The resistance pattern of insecticides within the organophosphate group shows a contrasting trend, with resistance to malathion observed in both species (Fig 6C), while resistance to temephos is more pronounced in both *Aedes* spp (Fig 6D). An increasing trend of resistance to malathion in *Ae. aegypti* and *Ae. albopictus* has been detected in hotspot areas, 12 areas by Ali *et al* (2020) and 24 areas by Rasli *et al* (2021), which show all tested samples resistant to malathion.

The resistance to malathion is influenced by the discriminating concentrations used in the WHO (2016a) adult bioassay. The diagnostic concentration set by WHO for malathion insecticide susceptibility testing in *Aedes* is set at 0.8%. Prior to the issuance of this guideline, most studies adopted the diagnostic concentrations intended for *Anopheles* mosquitoes, set at 5% (Table 2). Nevertheless, several studies conducted in 2021 continued to employ the 5 % discriminating concentration (DC) (WHO, 2016b). For instance, Lau *et al* (2021) reported *Ae. albopictus* samples from 11 sites in Malaysia are resistant to malathion at 5% DC, a concentration also used by Rajendran *et al* (2021) and Zuharah *et al* (2021) to determine malathion susceptibility against *Ae. albopictus*. Zuharah *et al* (2021) found one *Ae. albopictus* collection to be resistant to malathion, while two other collections show susceptibility. In contrast, Rajendran *et al* (2021) found resistance to malathion in both *Ae. aegypti* and *Ae. albopictus*. On the other hand, Leong *et al* (2018) assessed susceptibility by establishing a local DC to determine resistance in *Ae. aegypti* at the larval stage, using a DC based on twice the LC_{99} value of the Bora-bora strain. The study reported the susceptibility of *Ae. aegypti* populations to malathion and temephos, while showing variable resistance patterns to other insecticides in the carbamate, organochlorine and organophosphate classes. These data indicate that the choice of DC and mosquito life stage can influence regional resistance assessments. Such variations underscore the need for standardized methods in insecticide resistance monitoring to ensure consistency and comparability among different reports.

Insecticide susceptibility studies on the larval stage generally focus on larvicides, with temephos being widely used in the public health sector. We have showed that *Ae. aegypti* exhibits variable resistance to temephos, while *Ae. albopictus* consistently displays resistance that is likely influenced by the outdoor application of this insecticide. Other types of insecticides reported in our review include bromophos, chlorpyrifos and fenthion. Elia-Amira *et al* (2018) and Elia-Amira *et al* (2021) characterized samples from Sabah, while Wan-Norafikah *et al* (2021) compared collections from agricultural and non-agricultural areas. These studies on larvicides primarily focus on *Ae. albopictus*, as the application of larvicides is predominantly carried out outdoors (Supporting Data 4).

For carbamate and organochlorine insecticides, the eligible publications on resistance are limited (Supporting Data 6). Current findings indicate that while susceptibility to dieldrin is observed in *Ae. albopictus* there is also resistance to DDT and dieldrin (Supporting Data 5 and 6). More research on *Ae. aegypti* should provide a clearer understanding of resistance patterns for this equally important dengue vector.

Although resistance to organochlorines is less frequently documented, the mode of action of DDT that targets the voltage-gated sodium channel (VGSC) is known to contribute to cross-resistance with pyrethroids that act at a similar target (Silver *et al*, 2014). Thus, residual DDT in the environment may influence the efficacy of VGSC-targeting insecticides.

Susceptibility studies of insecticides often involve investigating resistance mechanisms, such as the molecular characterization of knockdown resistance (*kdr*) genes. We did not focus on data related to resistance mechanisms. However, studies on *kdr* mutations by Ishak *et al* (2015), Rasli *et al* (2018), AhbiRami *et al* (2020), Zuhurah *et al* (2021), Zuhurah and Sufian (2021), and Akhir *et al* (2022) have significantly advanced our understanding of resistance mechanisms associated with VGSC-targeting insecticides, particularly DDT and pyrethroids.

In conclusion, the key findings of our study highlight concerns regarding insecticide resistance in *Aedes* mosquitoes, particularly the clear trend of resistance in *Ae. aegypti* compared to the variable resistance and susceptibility observed in *Ae. albopictus*, especially with insecticides from the pyrethroid class. Susceptibility to other insecticides, such as cyfluthrin and lambda-cyhalothrin, reported in recent years, may provide opportunities to rotate other insecticides than to depend solely on deltamethrin and permethrin. However, expanding detection to include other pyrethroid insecticides is necessary to enhance data profiling and gain a more accurate understanding of insecticide susceptibility status. Combining research data on resistance identification with the characterization of genetic and biochemical mechanisms will contribute to a more effective insecticide resistance management. Molecular studies on the detection of resistance genes have been conducted in Malaysia, which are focused on identifying mutations in the voltage-gated sodium channel and gamma-aminobutyric acid receptor genes associated with insecticide resistance (Ishak *et al*, 2015; Low *et al*, 2015; Rasli *et al*, 2018; Zuharah and Sufian, 2021). However, molecular characterization requires specialized skills and equipment; therefore, in addition to screening or detecting resistance status, there is a pressing need for rapid tools or biomarkers to identify resistance mechanisms, enabling timely and effective mitigation strategies. Research should be expanded beyond susceptibility screening at discriminating concentrations. Notably, the eligible publications primarily employ adult bioassays for testing wild strains under controlled conditions using discriminating concentrations designed to reflect the resistance response of laboratory strains. To complement this, incorporating intensity assays should provide deeper insights into the magnitude of resistance at specific sites, thereby better reflecting the properties of field populations. While controlling insecticide resistance in *Aedes* mosquitoes is challenging, the ultimate goal is the reduction of dengue cases. Therefore, implementing non-chemical interventions should help reduce the disease burden and simultaneously decrease pressure on the development of insecticide resistance not only in Malaysia but also in countries where *Aedes* thrives.

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CONFLICT OF INTEREST DISCLOSURE

The authors declare no coflict of interest.

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