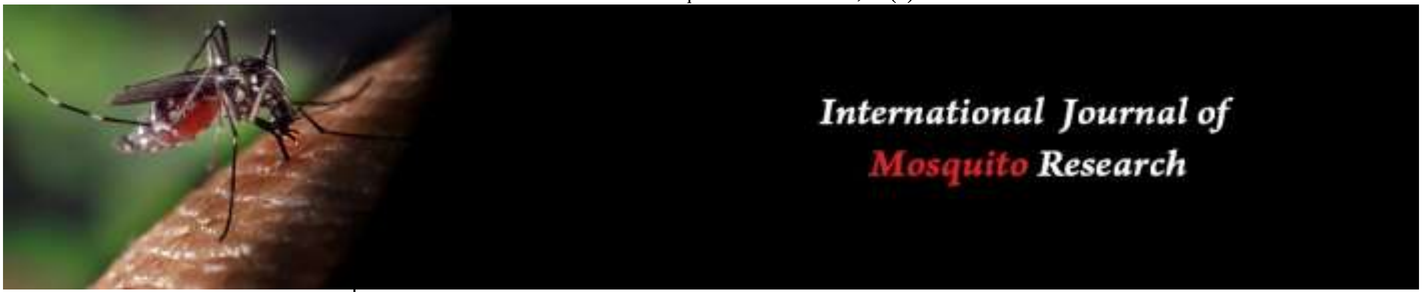

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Giyantolin Giyantolin
Master of Epidemiology Postgraduate School, Diponegoro University, Semarang, Indonesia

Dwi Sutiningsih
[1] Master of Epidemiology Postgraduate School, Diponegoro University, Semarang, Indonesia
[2] Faculty of Public Health, Diponegoro University, Semarang, Indonesia

Mursid Raharjo
[1] Master of Epidemiology Postgraduate School, Diponegoro University, Semarang, Indonesia
[2] Faculty of Public Health, Diponegoro University, Semarang, Indonesia

Yuli Subiakto
Faculty of Pharmacy, Republic of Indonesia Defense University, Indonesia

Martini Martini
[1] Master of Epidemiology Postgraduate School, Diponegoro University, Semarang, Indonesia
[2] Faculty of Public Health, Diponegoro University, Semarang, Indonesia

Onny Setiani
[1] Master of Epidemiology Postgraduate School, Diponegoro University, Semarang, Indonesia
[2] Faculty of Public Health, Diponegoro University, Semarang, Indonesia

Corresponding Author:
Giyantolin Giyantolin
Master of Epidemiology Postgraduate School, Diponegoro University, Semarang, Indonesia

Potential breeding sites, density, and insecticide resistance status (Cypermethrin, Malathion and Temephos) in *Aedes* spp. mosquitoes in central java province, Indonesia

Giyantolin Giyantolin, Dwi Sutiningsih, Mursid Raharjo, Yuli Subiakto, Martini Martini and Onny Setiani

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Abstract
Background: Central Java Province is one of the regions in Indonesia that has a high number of cases and deaths from dengue hemorrhagic fever (DHF). The high number of cases of dengue fever is influenced by the potential for mosquito breeding sites, the density of mosquitoes in the area, and the prolonged use of insecticides, which results in resistance.
Objective: This research aims to determine potential breeding sites, mosquito density, and resistance status in Central Java Province.
Methods: The method used is a literature study referring to the Preferred Reporting Items for Systematic Reviews Meta-Analyses (PRISMA). Data collection was carried out through Google Scholar, PubMed, Science Direct, Scopus, Web of Science, and Biomed Central. Search in Indonesian and English. Data identification is carried out by inclusion and exclusion. Data Analysis using Microsoft Excel and ArcGIS.
Results: High potential breeding sites are tank areas for bathroom, bucket, and toilet tub. Mosquito densities from the House Index (HI), Container Index (CI) and Brute Index (BI) are mostly categorized as moderate-high. Only the Cilacap district is classified as low, namely BI. The resistance status of the study area shows that almost all of the study area is resistant to insecticides such as cypermethrin, Malathion, and temephos. Only three areas in the Semarang region are still classified as tolerant, and that is only malation.
Conclusion: The study area in Central Java Province has the highest potential breeding sites, namely tank for bathrooms. The risk of transmission of dengue fever is in the moderate to high category. All study areas in Central Java Province are in the resistant category, except for the Semarang area which is in the tolerant category for Malathion insecticide.

Keywords: Breeding site, insecticide, resistance status, mosquitoes, *Aedes* sp.

Introduction
Aedes is a mosquito, an animal from the insect group belonging to the Culicidae family. This mosquito is known as a vector-borne disease [1]. *Aedes aegypti* and *Aedes albopictus* are types of mosquito that commonly cause diseases such as dengue fever, zika, yellow fever, and chikungunya [1, 2]. These diseases can cause a significant health burden on society, including death, disability, and high medical care costs [3, 4]. Apart from that, diseases caused by *Aedes aegypti* and *Aedes albopictus* mosquitoes have become endemic in various parts of the world, in tropical and subtropical parts [5, 6].
Indonesia is a tropical country where until now there are still cases of disease caused by *Aedes aegypti* and *Aedes albopictus* mosquitoes. Based on the Indonesian Health Profile (2022) [7], this mosquito causes Dengue Hemorrhagic Fever (DBD) and Chikungunya. In 2021, there were 73,518 dengue fever cases [7]. In 2021, 241 cases of Chikungunya fever were found [7]. Of these cases, what is currently still high is the Indonesian region in Central Java Province with 4,470 cases of dengue fever and 188 chikungunya fever cases [7, 8].
Several things that can cause disease due to *Aedes aegypti* and *Aedes albopictus* are geographical conditions [9], mosquito breeding sites [10], mosquito density [11], and mosquito

resistance status to insecticides [12]. Central Java Province is located in the middle of Java Island, Indonesia, and is one of the provinces with a dense human population. This makes it vulnerable to the spread of mosquito-borne diseases. This is then supported by potential breeding sites in densely populated areas, both urban and rural, such as bathtubs, rainwater reservoirs, pieces of used goods that can hold rainwater, gutters, and others. In addition to that, the density or presence of mosquitoes in an area also plays a role in the transmission of dengue fever [10].
To control the diseases caused by *Aedes aegypti* and *Aedes albopictus* mosquitoes, several government programs have been implemented, such as the use of chemicals ranging from larvicides to insecticides. The continuous use of chemicals can cause *Aedes aegypti* and *Aedes albopictus* mosquitoes to become resistant [13]. Mosquito resistance to insecticides is a problem that affects efforts to control *Aedes aegypti* and *Aedes albopictus* mosquitoes [14]. Thus, understanding the level of resistance in Central Java Province can help plan effective insecticide use.
From this background, epidemiological research on potential breeding sites, mosquito density, and resistance status of *Aedes aegypti* and *Aedes albopictus* mosquitoes in Central Java Province, Indonesia becomes very relevant to help plan and implement more effective and sustainable vector control programs. This also provides benefits in protecting public health from diseases transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes.

Methods

Search Methods

This study used a systematic review that refers to the Preferred Reporting Items for Systematic Reviews Meta-Analyzes (PRISMA) guidelines. The scientific databases used include PubMed, Biomed Central, Scopus, Science Direct, Web of Science, and Google Scholar with a period published from 2018 to 31 October 2023. The research keywords used are "*Aedes aegypti* and *Aedes albopictus* mosquitoes in Central Java Indonesia (English) = nyamuk *Aedes aegypti* dan *Aedes albopictus* Jawa Tengah Indonesia (Indonesian)", "breeding grounds for *Aedes aegypti* and *Aedes albopictus* mosquitoes, Central Java Indonesia (English) = tempat perindukan nyamuk *Aedes aegypti* dan *Aedes albopictus* Jawa Tengah Indonesia (Indonesian)", "Resistance of *Aedes aegypti* and *Aedes albopictus* mosquitoes Central Java Indonesia (Engslish) = resistensi nyamuk *Aedes aegypti* dan *Aedes albopictus* Jawa Tengah Indonesia(Indonesian)". Apart from that, keyword synonyms are used such as "insecticide status of *Aedes aegypti* and *Aedes albopictus* mosquitoes, Central Java, Indonesia (english) = status insektisida nyamuk *Aedes aegypti* dan *Aedes albopictus* Jawa Tengah Indonesia (Indonesia)", and "habitat of *Aedes aegypti* and *Aedes albopictus* mosquitoes, Central Java, Indonesia". "Entomological index of *Aedes aegypti* dan *Aedes albopictus* mosquito in Central Java (English) = Indeks Entomologi nyamuk *Aedes* Jawa Tengah (Indonesian)". The searches were carried out in English and Indonesian.

Study Identification

The selection of articles was carried out for analysis based on the following characteristics (inclusion) (1) region; number of samples, (2) intervention: mosquito breeding/habitat; mosquito density; resistance status (3) comparison: Container Index; House Index; Breteau Index; Insecticides (Cypermethrin, Malathion, Temephos) (4) results: year of publication, type of mosquito, number of mosquito breeding sites, epidemiological index, mosquito resistance category in each area.
The exceptions are (1) repetitive articles, (2) not full text, (3) less informative methods, (4) review articles, and (5) articles on other vectors.
From the identification of studies carried out according to these selection criteria, 18 articles were selected from a total of 15,905 articles and then evaluated (Figure 1).

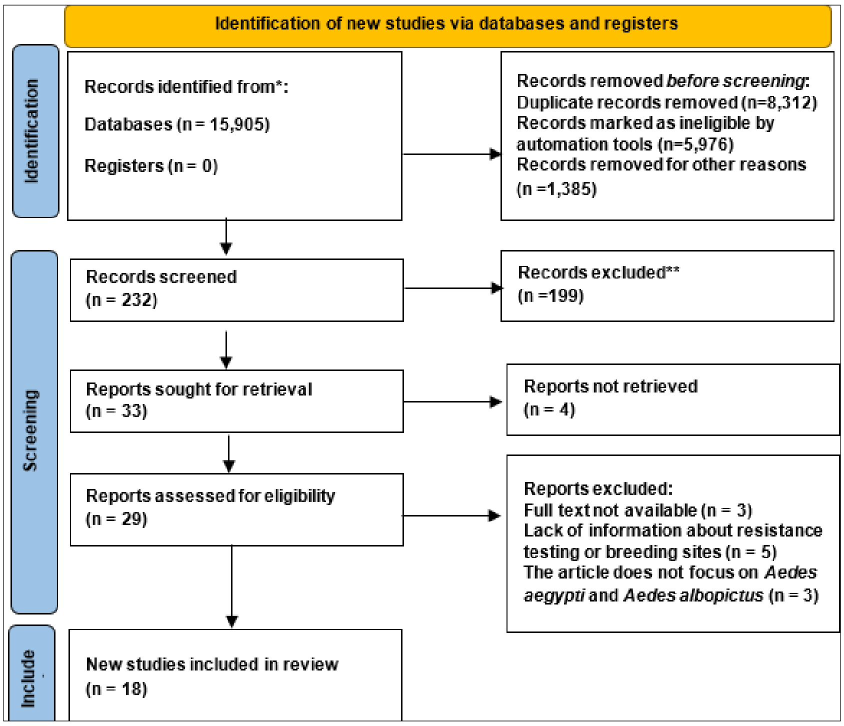


Fig 1: PRISMA flow diagram for study selection

Data analysis
Data analysis was carried out using Excel and ArcGIS applications, namely the number of containers; mapping of the study area; mapping CI, HI and BI conditions; and resistance status in each study area.

Result
Potential breeding sites for *Aedes aegypti* and *Aedes albopictus*: Based on the number of breeding sites that were examined and became breeding sites for *Aedes aegypti* and *Aedes albopictus* mosquitoes, positive containers and numbers were obtained as presented in Figure 2.

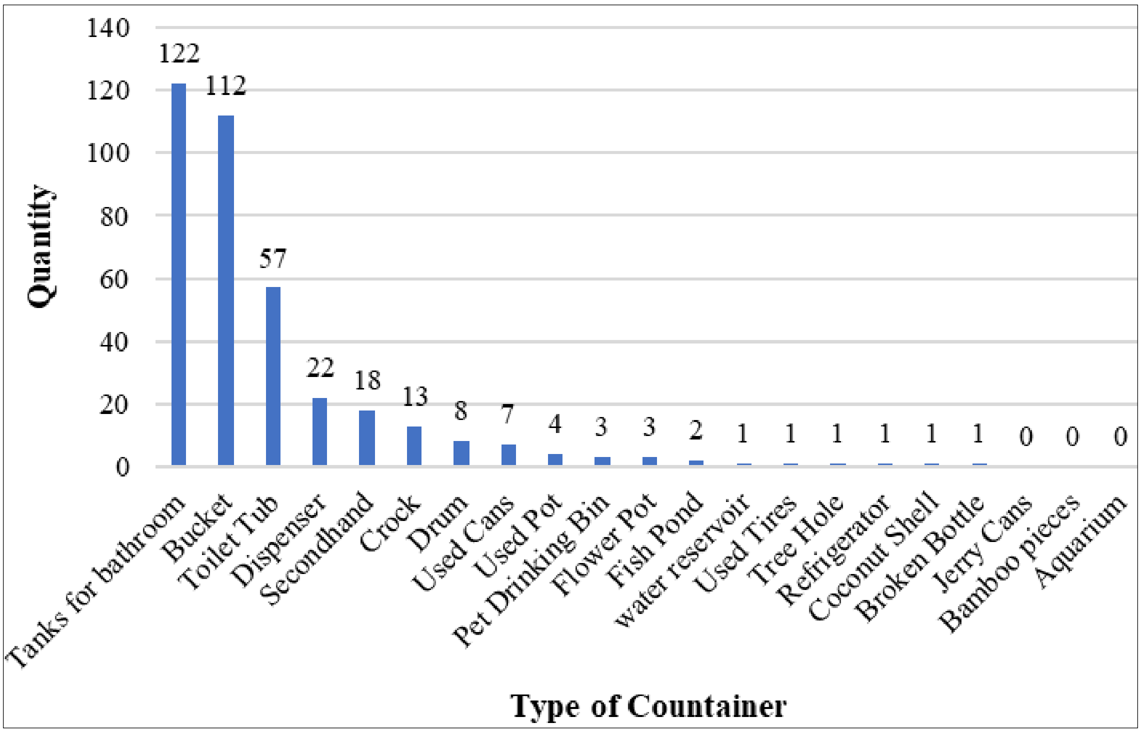


Fig 2: Number of containers tested positive for Aedes mosquitoes

Source: (Wijayanti *et al.*, 2019; Hidajat *et al.*, 2021; Marahema and Binugraheni, 2021; Novaranti *et al.*, 2021) [15-18].
There were 21 types of containers that had the potential to serve as breeding sites, but only three types of containers were not found, namely Jerry Cans, Bamboo pieces and Aquarium. The total number of examined was 3047, 377 mosquito larvae were positive (Figure 2).

Mosquito Density: The study results obtained were that there were 11 districts/cities with 28 study areas. The density of mosquitoes can be seen from the House Index (HI), Container Index (CI), and Breteau Index (BI). The mosquito density study area in Central Java Province is presented in Table 1.

Table 1: Density of *Aedes aegypti* and *Aedes albopictus* mosquitoes based on the House Index (HI), the Container Index (CI) and the Breteau Index (BI).

Studi Area		HI (%)	Risk	CI (%)	Risk	BI (%)	Risk	Author
District/City	Location/Village							
Banyumas District	Kedungrandu	25	Moderate	77.78	High	35	Moderate	Puspitasari *et al.*, 2019 [21]
	Sidamulih	7.69	Moderate	55.55	High	12.8	Moderate	
Kudus District	Tanjungrejo	17.3	Moderate	10	Moderate	19.1	Moderate	Hidajat *et al.*, 2021 [19]
	Undaan	31.8	Moderate	22.8	High	31.8	Moderate	
	Pasuruan Lor	43.8	High	39.9	High	55.2	High	
Jepara District	Demaan	14	Moderate	9.3	Moderate	16	Moderate	Hidajat *et al.*, 2021 [19]
	Ujungbatu	28	Moderate	21.1	High	16	Moderate	
	Bandengan	5	Moderate	4.1	Moderate	7	Moderate	
Semarang City	Karangayar Gunung	5.22	Moderate	2.86	Low	5.72	Moderate	Nurhidayah *et al.*, 2022 [22]
Wonogiri District	Kaliacir	20	Moderate	7.22	Moderate	20	Moderate	Marahema and Binugraheni, 2021 [17]
Cilacap District	Karangpucung	10	Moderate	10	Moderate	1.75	Low	Wijayanti *et al.*, 2019 [15]
	Purwanegara	12	Moderate	17	Moderate	3.39	Low	
	Arcawinangun	14	Moderate	19	Moderate	4.4	Low	
Purworejo District	Pangen Juru Tengah	37.33	High	17.84	Moderate	11	Moderate	Novaranti *et al.*, 2021 [18]
	Mudal	44.44	High	11.86	Moderate	9.67	Moderate	
	Keseneng	28.75	Moderate	13.91	Moderate	7.25	Moderate	
	Sindurjan	17.71	Moderate	4.12	Moderate	18.5	Moderate	
	Pangenrejo	34.67	Moderate	17.74	Moderate	9.5	Moderate	
	Mranti	26.67	Moderate	15.71	Moderate	6	Moderate	

	Doplang	25	Moderate	9.4	Moderate	5	Moderate	
Magelang District	Mertoyudan	73.68	High	21.42	Moderate	94.74	High	Yuliasari *et al.*, 2019 [23]
	Banyurojo	26.08	Moderate	14.1	Moderate	47.83	Moderate	
	Sumberrejo	29.41	Moderate	15.09	Moderate	47.06	Moderate	
	Danurejo	35	Moderate	14.58	Moderate	35	Moderate	
	Donorojo	23.8	Moderate	14.3	Moderate	38.1	Moderate	
Pati District	Wedarijaksa	21.67	Moderate	5.16	Moderate	21.67	Moderate	Massaid *et al.*, 2021 [24]
Salatiga District	Ledok	15	Moderate	5.7	Moderate	19	Moderate	Kumara *et al.*, 2020 [20]
Sukoharjo District	Grogol	36.9	High	31	High	36.9	Moderate	Handayani *et al.*, 2023 [25]

Table 2: Indicators and Risk Categories for DHF Transmission

No	HI (%)	CI (%)	BI (%)	Category	Referensi
1.	HI < 4	CI < 3	BI <5	Low	Padonou *et al.*, 2020 [26]
2.	4 ≤ HI ≤ 35	3 ≤ CI ≤ 20	5 ≤ BI ≤50	Moderate	
4.	HI > 35	CI > 20	BI > 50	High	

Based on entomological parameters and the risk of transmission of dengue fever, all study areas in the HI indicator are in the moderate to high category. In the CI indicator section, there is one area in the low category, namely Karanganyar Gunung, Semarang City. In the BI indicator section, there are three areas in the low category, namely Cilacap Regency (Karangpucung, Purwanegara, Arcawinangun). The determination of this category is based on Padonou *et al.*, (2020) [26].

Mosquito Resistance Status: The study results obtained were 8 districts/cities with 27 study areas. Different numbers of resistance tests were carried out in this study area, namely the pyrethroid group of pyrethrin type in 23 study areas, the organophosphate group of malathion type in 21 study areas, and the group of temephos organophosphate in 17 study areas. This is presented in Table 3.

Table 3: Study area with resistance status for each type of insecticide

Studi Area		Pyrethroid (Cypermethrin)		Organophosphate (Malathion)		Organophosphate (Temephos)		Author
District/City	Location/Village	Mortality (%)	Resistance status	Mortality (%)	Resistance Status	Mortality (%)	Resistance Status	
Semarang District	Karangjati	80	R	100	S	36	R	Sayono *et al.*, 2023[27]
	Gebungan	52	R	99	S	22	R	
	Bandungan	21	R	83	R	6	R	
Pemalang District	Ketapang	65	R	96	PR	24	R	Sayono *et al.*, 2023[27]
	Serang	66	R	91	PR	15	R	
	Gombong	35	R	90	PR	19	R	
Tegal District	Pakembaran	66	R	91	PR	24	R	Sayono *et al.*, 2023[27]
	Pangkah	-	-	-	-	91	PR	Kresnadi *et al.*, 2021[28]
Kudus District	Piji	56	R	86	R	41	R	Sayono *et al.*, 2023[27]
	Maducendana	16	R	86	R	31	R	
	Kaliwungu	20	R	75	R	21	R	
	Tanjungrejo	6	R	4	R	-	-	Hidajat *et al.*, 2021[19]
	Pasuruan Lor	7	R	21	R	-	-	
	Undaan	10	R	5	R	-	-	
Semarang City	Sendangguwo	67	R	97	PR	51	R	Sayono *et al.*, 2023[27]
	Rowosari	86	R	100	S	43	R	
	Tembalang	80	R	80	R	45	R	
	Patemon	-	-	91.7	PR	62.4	R	Hidajat *et al.*, 2021[19]
	Terboyo Wetan	-	-	86.7	R	30	R	
	Kandri	-	-	81.7	R	75.3	R	
Jepara District	Ujungbatu	5	R	28.3	R	-	-	Hidajat *et al.*, 2021[19]
	Bandengan	15	R	20	R	-	-	
Klaten District	Ngawonggo	97	PR	-	-	-	-	Irawati and Putri, 2021[29]
	Kajen	97	PR	-	-	-	-	
	Meger	89	R	-	-	-	-	
Banyumas District	Jatinegara	28.3	R	-	-	-	-	Gunawan *et al.*, 2019[30]
	Karangklesem	23.3	R	-	-	-	-	

Description: R= Resistance; PR = Possible Resistance; S= Susceptibility

Table 4: Categories of Interpretation of Insecticide Resistance Status

No	Category	Information
1.	Confirmed resistance (R)	If mortality (Corrected, if necessary) is <90%, provided that at least 100 mosquitoes were tested, the vector population can be considered resistant to the insecticide.
2.	Possible resistance (PR)	If mortality (Corrected, if necessary) is ≥90% but <98%, the presence of resistance is possible but not confirmed. The test results should be confirmed by repeating the test with a new sample from the same mosquito population. (Note: Avoid using F1 of the tested mosquitoes.) If two tests consistently show mortality <98%, resistance is confirmed.
3.	Susceptibility (S)	If mortality (Corrected, if necessary) is ≥98%, the vector population can be considered susceptible to insecticide.

Table 3 shows areas that are resistant to three groups of insecticides, namely cypermethrin, malathion, and temephos. This study area shows that the type of cypermethrin is in the Possible Resistance-Resistance category. In the malathion-type organophosphate group, 3 areas are tolerant (susceptibility) in the Semarang region, namely the city of Semarang (Rorowosari) and the district of Semarang (Karangjati and Gebungan). For the organophosphate group, the temephos type is in the possible resistance-resistance category. These categories are based on WHO, which is in accordance with Table 4.

Discussion

Mosquitoes are said to be disease-carrying vectors for viruses, microfilariae, and protozoa. *Aedes aegypti* and *Aedes albopitus* are mosquitoes that transmit dengue hemorrhagic fever, where *Aedes aegypti* is the main vector [6]. *Aedes aegypti* and *Aedes albopitus* are still a problem for public health, especially in tropical and subtropical countries [5].
Indonesia is a tropical country where, to date, there are still frequent cases and deaths of Dengue hemorrhagic fever caused by mosquitoes of the *Aedes aegypti* and *Aedes albopitus* types. Almost all provinces or regions in Indonesia experience cases of dengue fever. Central Java province has recently been named the area with the highest DHF case fatality rate (CFR). The CFR from 2017-2021 experienced a quite significant increase [7].

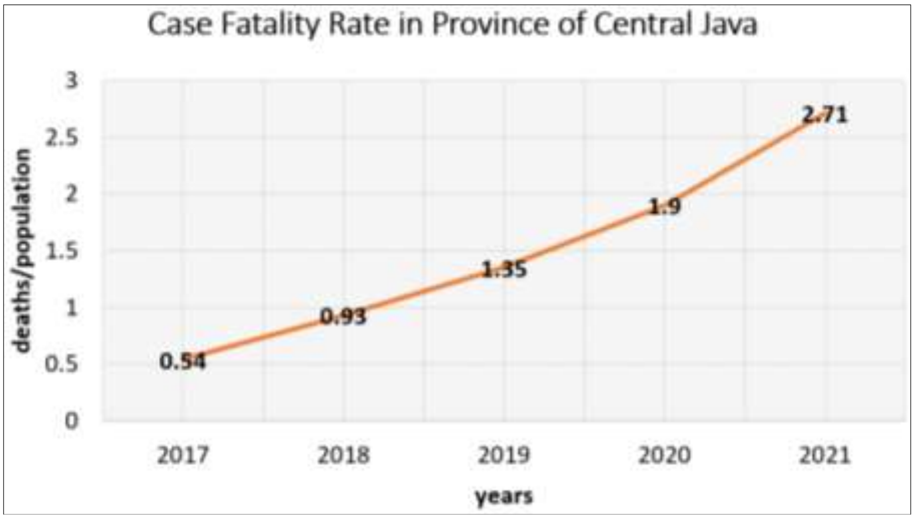
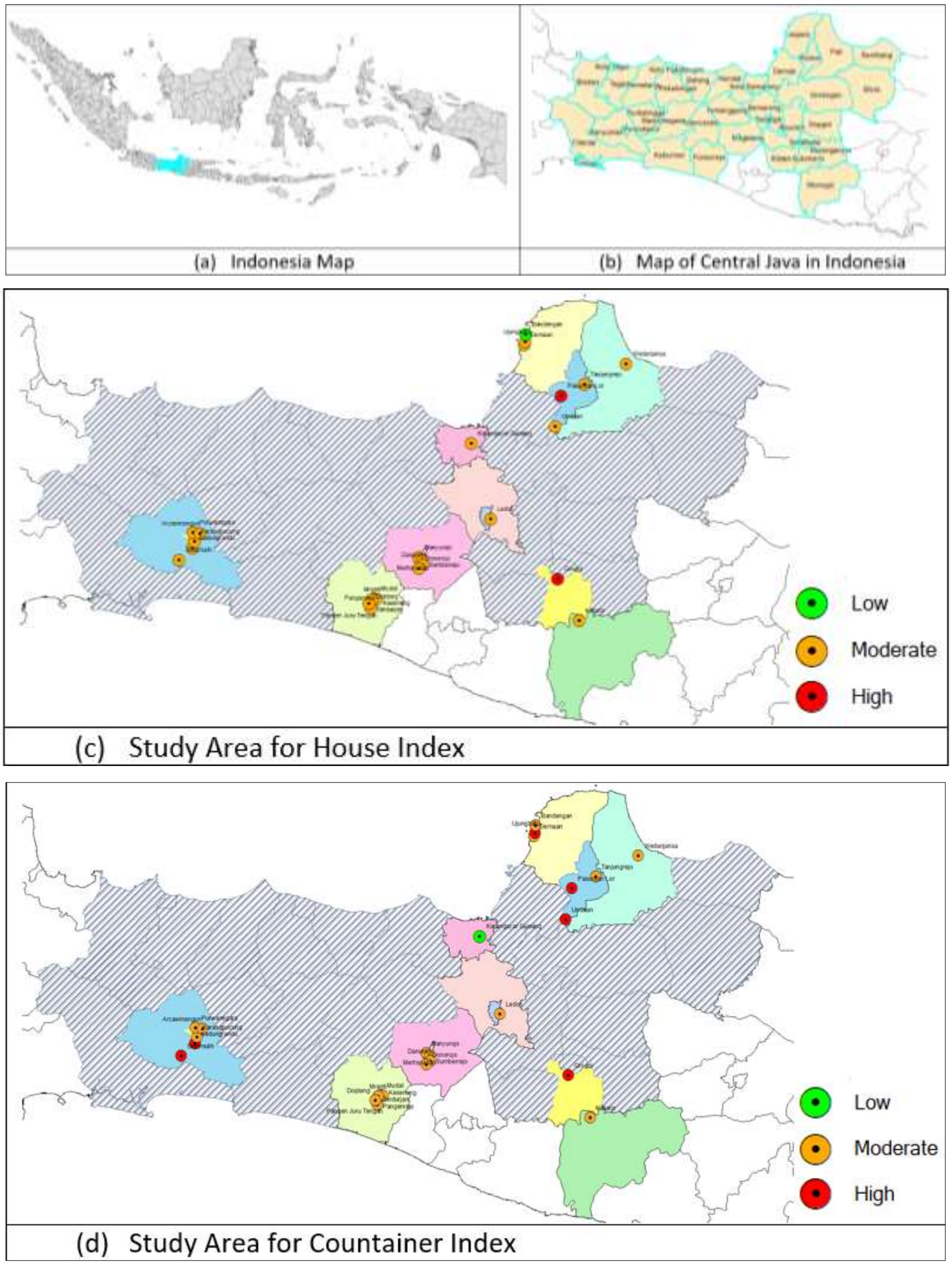


Fig 3: Case Fatality Rate DHF in Province of Central Java

The Central Java Province is a province in the central part of the island of Java with a population of 34.55 million and an area of 34,337 km². As time goes by, the population in this area is expected to continue to increase. With increasing population, regional density will occur and become a factor in the development of dengue fever in this region.
To anticipate the occurrence of cases and deaths of dengue fever, the local government and health services have made various efforts to prevent and control dengue fever. The mosquito prevention and control program at the national to village level is 3 Mplus. 3 Mplus is 3 M "Menutup/Close", "Menguras/drain", "Mendaur ulang/recycle", and the plus part is various efforts that can control mosquitoes (one of which is the use of insecticides). However, until now, dengue fever has continued to occur every year and there has even been an increase in deaths.
To overcome this, it is necessary to analyze several factors that could influence the occurrence of increased cases of dengue fever, such as potential breeding sites, mosquito density, and mosquito resistance status. These three things are closely related to programs that have been implemented by the government or health services. In addition to that, the study area needs to see the potential distribution of dengue fever transmission through the studies that have been carried out. This analysis will be used as a factor for program evaluation and optimizing programs to overcome the problem of dengue fever.
From the studies carried out, it was found that several study areas were related to potential breeding sites, mosquito density, and resistance status (Table 1 & Table 3). Mosquitoes' breeding sites are one of the potential factors for the growth and development of mosquitoes. The high growth of mosquitoes will be a contributing factor to the high transmission of dengue fever. Figure 3 clearly shows that during the inspection, several containers were found in tanks for bathrooms, followed by the largest buckets and toilet tubs. In addition to that, several other types of containers were found, which made it a concern for the public to continue to be aware of potential brooding sites around them, both inside and outside the house. Containers are still often found because

people have not optimized the government program (PSN-3Mplus) and rarely clean containers or pay attention to containers; they should be cleaned at least once a week. This cleaning is carried out because the life cycle of mosquitoes, which may become adults and transmit the Dengue virus, is 5-7 days. Efforts to prevent and control dengue fever are highly recommended to increase awareness through the government program, namely eradication of mosquito nests (Pemberantasan Sarang Nyamuk/PSN).

In addition to knowing potential breeding sites, mosquito density is also very necessary to see the risk of transmitting dengue fever. The risk of transmission can be seen through HI, CI, and BI. In the analysis carried out, it was found (Table 1) that in HI and CI all regional studies were classified as Moderate to High, except for the part CI of the Karanganyar Gunung area, which was classified as low (Figure 3). Although the CI category is low, the HI and BI categories are moderate, so the risk of transmission of dengue fever can still occur and must be taken into account. Then in BI, most of them were in the moderate to high category, but it was found that there were still low categories, namely three areas in Cilacap district and one area in Purworejo district. This is also the same as BI is low, but CI and HI are moderate, so there is still a risk of transmission. None of the study areas showed low HI, CI and BI. Therefore, the region of Central Java Province has a moderate to high risk of transmission.



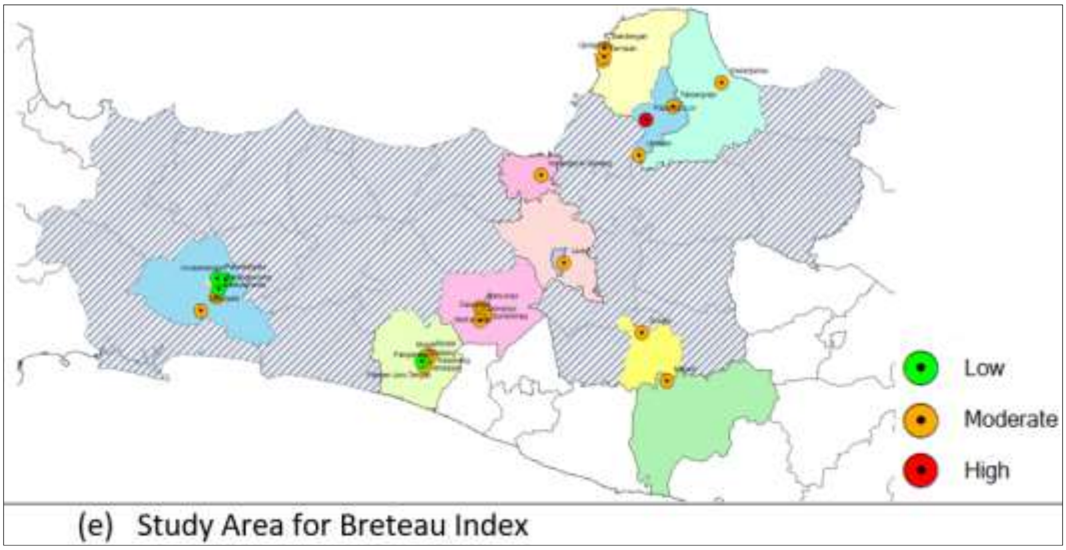
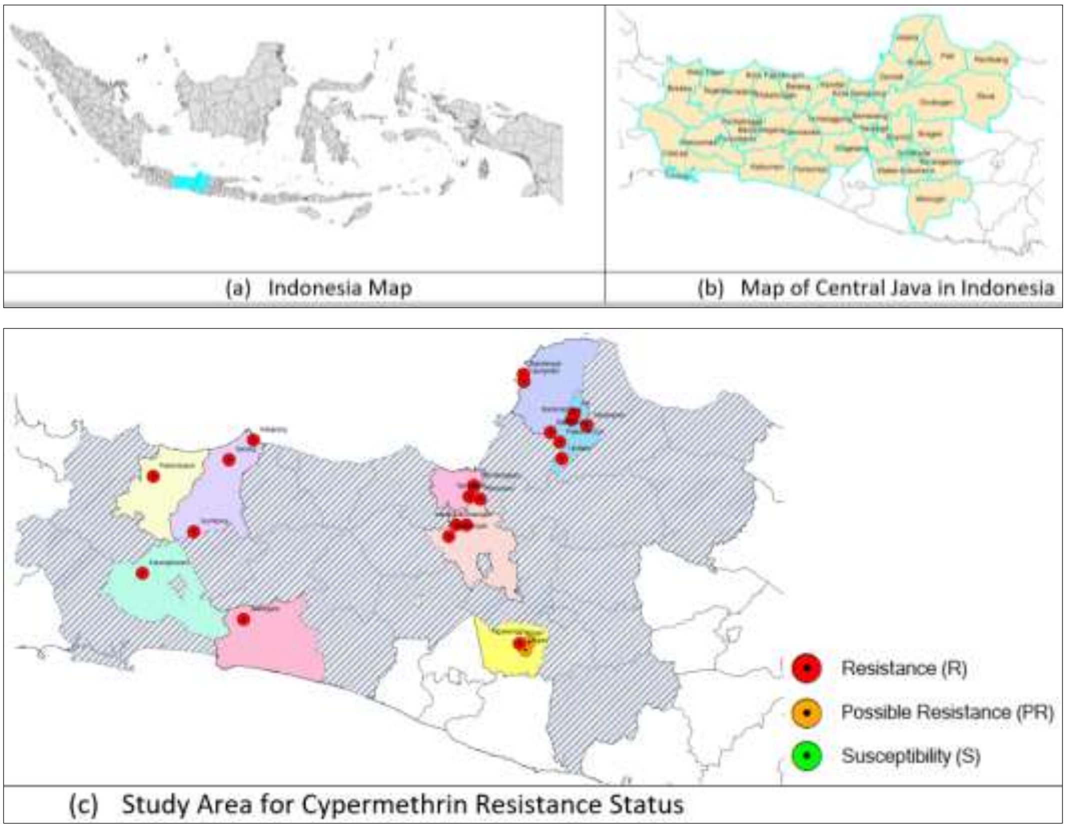


Fig 4: Study areas and risk categories for dengue fever transmission

In addition to mosquito density, the mosquito resistance status is also very important to analyze as a risk factor for dengue fever caused by *Aedes aegypti* and *Aedes albopictus* mosquitoes. From the study obtained on the resistance status of the insecticide groups of pyrethroid (Cypermethrin) and organophosphate (malathion and temephos), these are Semarang Regency, Pemalang Regency, Tegal Regency, Kudus Regency, Semarang City, Jepara Regency, Klaten Regency, and Banyumas Regency. Each type of insecticide has its resistance status. The type of insecticide cypermethrin has a resistance status (Table 1), namely all areas are resistant and only the Klaten district (Ngawonggo village and Kajen village) is in the Possible resistance category. The types of malathion insecticides in the organophosphate group vary greatly from susceptibility (Tolerant) to resistance. However, the Semarang district (Karangjati village and Gebungan village) and the Semarang city (Rowosari village) are tolerant to the insecticide malathion. In the insecticide section, the organophosphate group temephos, all areas experienced resistance except Pangkah village, Tegal district, which was in the Possible Resistance category.

The data obtained show that almost all areas in Central Java are resistant to malathion, temephos, and cypermethrin. Only a few areas are tolerant to malathion insecticides. As a view, it can be seen in Figure 3 as the study area.



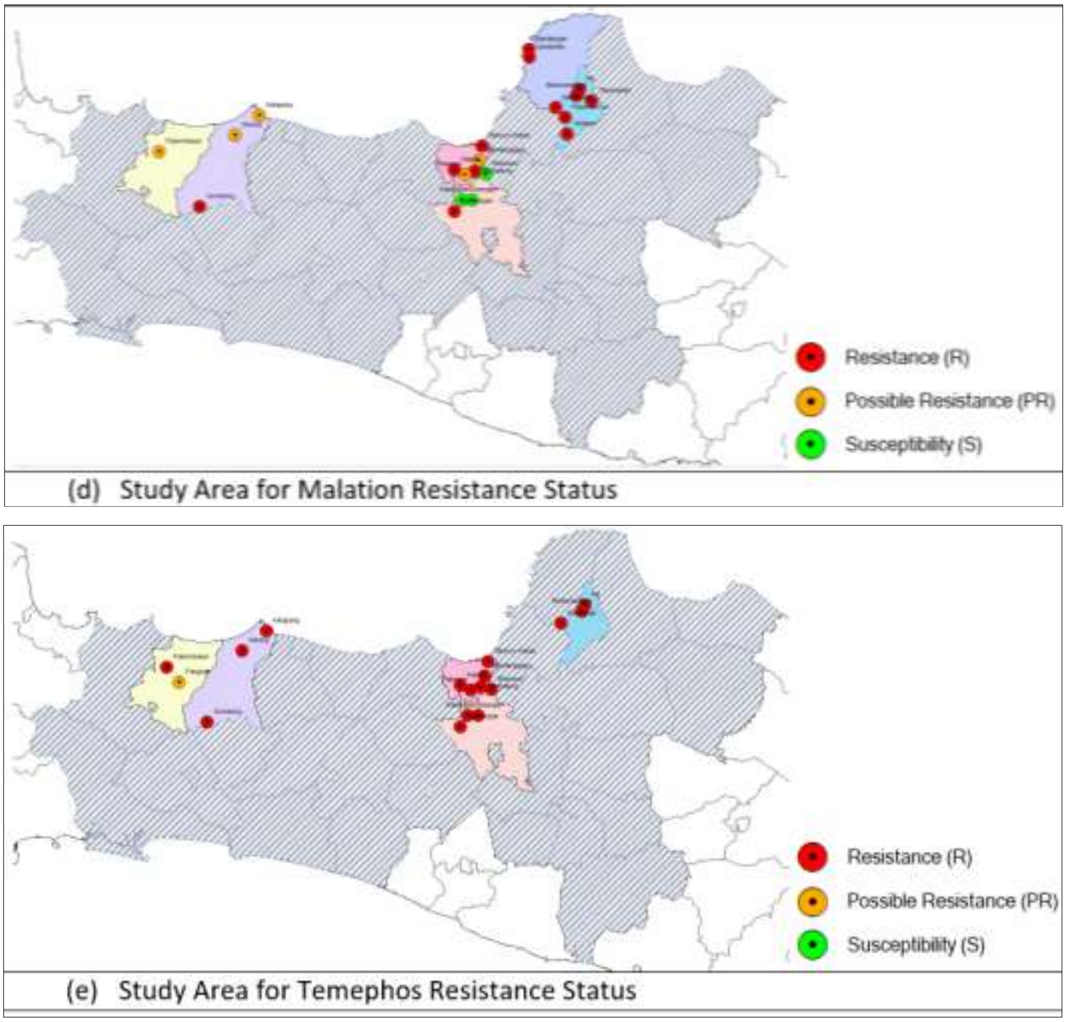


Fig 5: Resistance status study area based on cypermethrin, malathion, and temephos

In the study area for this resistance status (Figure 4), it can be seen that cypermethrin has 23 regional points from 7 districts/cities, malathion has 21 regional points from 5 districts/cities, and temephos has 17 regional points from 5 districts/cities. The resistance status to insecticides is cypermethrin (Resistance 91.3%; possible resistance 8.7%; susceptibility 0%), malathion (resistance 57.14%; possible resistance 28.57%; susceptibility 14.29%) and temephos (resistance 94.12%; possible resistance 5.88%; susceptibility 0%). The results of this study show that almost all areas are becoming resistant and are already resistant to these three insecticides, and only three areas are susceptible (tolerant), namely to malathion.

This indicates that the use of insecticides in Central Java has been used too frequently, so mosquitoes have become resistant to the insecticides cypermethrin, malathion, and temephos. The occurrence of resistant mosquitoes is also not far from community behavior, where people assume that if there is a case of dengue fever, fogging is carried out immediately. This must follow existing procedures and criteria to carry out fogging. In addition to that, there are still people who ignore instructions for the correct use of insecticides, which can cause mosquito resistance to insecticides [31]. Prolonged use of insecticides will also have the effect of causing mosquito resistance, so that mosquitoes experience gene mutations to adapt to the genetic conditions in the mosquito's body.

The breeding sites, the density of the mosquitoes and the resistance status have a major influence on the transmission of dengue fever. The potential for mosquito breeding sites to grow and develop can influence the risk of dengue fever transmission, which is also supported by the HI, CI and BI in the area. In addition, continuous or prolonged use of insecticides causes mosquitoes to become resistant so that the spread of dengue fever can continue. All of these incidents require increased movement to implement mosquito nest eradication programs, one of which is the 3MPlus program. This program will reduce the number of cases and deaths from dengue fever if the community can optimize it.

Conclusion

In this study, it can be concluded that the breeding sites with the greatest potential for mosquito breeding are tanks for bathrooms, buckets, and toilet tubs. Mosquito density based on HI, CI, and BI has a potential risk of transmitting dengue fever in the moderate to high category. The resistance status of mosquitoes to the insecticides cypermethrin, malathion, and temephos is in the resistant category, only a few in the Semarang area are in the susceptibility to malathion category. The suggestion from this study is that natural preventive measures are needed to reduce the increase in resistance, and it is necessary to increase public awareness in implementing the 3M (Drain, Cover, and Recycle) program regularly.

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