Jurnal Kesehatan Lingkungan
Journal of Environmental Health
Journal Home Page: https://e-journal.unair.ac.id/JKL
Vol. 16 No. 1
DOI: 10.20473/jkl.v16i1.2024.10-17
ISSN: 1829 - 7285 | E-ISSN: 2040 - 881X

ORIGINAL RESEARCH Open Access

# ENTOMOLOGICAL SURVEY AND TEMEPHOS SUSCEPTIBILITY OF AEDES, CULEX AND ANOPHELES IN NAENA MUKTIPURA MIMIKA PAPUA

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***Article Info***
Submitted : 1 June 2023
In reviewed : 19 September 2023
Accepted : 19 January 2024
Available Online : 31 January 2024

***Keywords :*** *Aedes, Anopheles, Culex, Papua, Susceptibility*

***Published by*** *Faculty of Public Health Universitas Airlangga*

***Abstract***

***Introduction:*** *Arthropod-borne diseases are infectious diseases mediated by mosquitoes that have incurred a considerable health problem. The high prevalence of this disease (56.66%) in some rural areas, like Papua, is attributable to some environmental factors such as abundant breeding sites and favorable climatic conditions, which contribute to a high rate of disease transmission.* ***Methods:*** *This experimental research aims to determine the breeding site, mosquitos' diversity, and susceptibility to temephos insecticide through an entomological survey to find out the breeding site of the larvae. Mosquito larvae from positive breeding sites were captured and identified in the laboratory. Temephos susceptibility tests were conducted on each species at 0.02 ppm for Aedes and Culex, and 0.25 ppm for Anopheles.* ***Results and Discussion:*** *Entomology survey in Naena Muktipura village, Papua, revealed that 5 of 27 stagnant water bodies harbored mosquito breeding activity. The survey identified Aedes, Culex, and Anopheles larvae, with Culex and Anopheles preferring dirty stagnant water covered with leaves, while Aedes albopictus prefers sunlit puddles. All three species exhibited susceptibility to temephos.* ***Conclusion:*** *This study found that all three Diptera genera in Naena Muktipura village, despite their varied breeding site preferences, were susceptible to temephos.*

## INTRODUCTION

Arthropod borne diseases are infectious diseases that are mediated by mosquitoes. Mosquitoes act as the main vectors for numerous infectious diseases, including malaria, dengue fever, chikungunya, filariasis, Japanese encephalitis, zika and others (1). Several of these diseases, like malaria, dengue fever, filariasis, chikungunya, and Japanese encephalitis, are endemic in Indonesia and exhibit varying degrees of intensity across some areas in the archipelago. Papua, situated in the easternmost areas of Indonesia, stands out as a hotspot for several of these diseases, including malaria, filariasis, and dengue fever. Although the morbidity of these diseases has notably dwindled, the province's high transmission potential due to suitable mosquito breeding grounds continues to be a major public health concern (2).

Malaria, dengue fever, and lymphatic filariasis are endemic diseases in Papua. Ministry of Health data indicate that 90% of malaria cases occur in Papua (2). Dengue fever and lymphatic filariasis persistently affect specific endemic areas within the province. These diseases are transmitted by three mosquito genera: *Anopheles*, *Culex*, and *Aedes*. Papua implements standard prevention measures with insecticides like organophosphates and pyrethroids (3). Despite these interventions, the diseases remain persistent (4). Environmental factors contributing to persistent larval habitats and malaria cases require further investigation (5).

Mosquitoes, notorious vectors of arthropod-borne diseases from the diptera order, pose a major public health threat in Indonesia. The country's hot and humid

***Cite this as :***
Lusiyana N. Entomological Survey and Temephos Susceptibility of *Aedes*, *Culex* and *Anopheles* in Naena Muktipura Mimika Papua. *Jurnal Kesehatan Lingkungan*. 2024;16(1):10-17. https://doi.org/10.20473/jkl.v16i1.2024.10-17

environment provides ideal conditions for mosquito growth and survival, particularly during their aquatic stage (6-7). This stage requires specific habitat features, including suitable water sources with specific organic and inorganic components, depending on the breeding site type. Understanding the location and characteristics of these breeding sites forms the foundation for developing effective mosquito control strategies (8).

Understanding the diverse breeding site preferences of different mosquito species is crucial for effective mosquito control in Indonesia. The three main mosquito genera responsible for most arthropod-borne diseases there are *Aedes*, *Culex*, and *Anopheles*. Each genus exhibits distinct breeding preferences and biological characteristics, even within individual species (4,9). The variations in breeding preferences determine the most effective mosquito control strategy. Notably, some species like *Culex fatigans* and *Aedes aegypti* flourish in urban settings due to their preference for stagnant water in drains and containers, while others such as *Aedes albopictus*, *Anopheles sp., Mansonia*, and *Armigeres* are more prevalent in rural areas. Mosquito distribution across urban and rural environments further determines the types of breeding sites encountered, highlighting the need for differenr control strategies. One of the government's disease prevention efforts focuses on controlling mosquitoes as disease vectors by using insecticides. Another approach involves deploying various chemical and biological insecticides (10–14). While common insecticides can reduce mosquito populations, including both larval and adult stages (11-12), their effectiveness is challenged by the emergence of resistance. Temephos, an organophosphate larvicide considered safe for other organisms when used in zstandard doses, is one example of an insecticide facing resistance challenges (11,13-14). Several studies across Indonesia have documented resistance to various insecticides (11,13-14). On this basis, this study investigates breeding site characteristics, mosquito genera, and temephos susceptibility in Naena Muktipura, Mimika Papua.

## METHODS

### Research Design

This study combined an entomological field survey and a laboratory susceptibility test in a two-stage design. The survey involved identification of larval habitats and mosquito genus or species, while the laboratory test assessed the susceptibility of mosquito larvae to temephos insecticide. Both stages were conducted simultaneously for efficient data collection. Ethical approval for this research was obtained from the Health Research Ethics Commission of the Faculty of Medicine at Universitas Islam Indonesia (Number 33/Ka.Kom.Et/70/KE/V/2023).

### Research Sites and Entomological Surveys

This research was conducted in early May 2023 in Naena Muktipura village, the largest village in the Iwaka sub-district of Mimika, Papua. Located 30 km from Mimika downtown, Naena Muktipura is surrounded by forests of shrubs and sago trees and interspersed with swamps. The Mimika region is characterized by a predominantly rainy climate with a brief dry season, with the early rainy season occurring from May to December and the dry season from January to April (15).

Entomological surveys for larvae were conducted around houses and yards near residents in the study area. Employing a single larvae collection technique, all potential outdoor breeding sites were inspected near houses and yards. Each container with larvae was recorded, and the genus or species were identified. Larvae were collected using long-sleeved dippers and pipettes, particularly for reaching puddles away from the edge. Larvae were sampled following WHO standard dipping methods (16). The steps involved identifying the active breeding surface, performing ten dips per habitat with a 45-degree angle scoop, withdrawing quickly after filling the dipper ¾ full, pausing for 2-3 minutes between dips, clearing vegetation if necessary and waiting for 3-5 minutes, using deep full-dipper scoops for large larvae and maximizing water collection for small larvae, counting each larva in each dipper, and transferring them to labeled containers using pipettes for laboratory testing.

The larvae from each collection site were recorded and brought to the laboratory of the Naena Muktipura Village Auxiliary Health Center for morphological identification using a binocular microscope. Mosquito genus and species identification was conducted utilizing species identification keys (17) by an entomological analyst.

### Breeding Site Characterization

Every puddle was checked to investigate the presence of the larvae. The larval breeding site was characterized based on the breeding site type, number of positive breeding site, location of the breeding site, and the type of the mosquitoes (18).

### Population and Sample Test

This study targeted the mosquito species identified in Naena Muktipura village, Mimika, Papua. All identified mosquito species were included in the subsequent susceptibility test. The susceptibility test involved four replicates for each mosquito species, using 20 larvae per replicate for a total of 80 larvae per species. Standard temephos doses set by the World Health Organization (WHO) were used for the test (19).

### Susceptibility Test

The susceptibility test employed glass beakers, micropipettes, stir bars, Pasteur pipettes, acetone, temephos, and chlorine-free rainwater. It was conducted at the Naena Muktipura primary healthcare center (or laboratory if applicable). Larval susceptibility tests for all identified mosquito species followed the WHO protocol (19) using established concentrations: 0.02 ppm for *Aedes* and *Culex* larvae and 0.25 ppm for *Anopheles* larvae using chlorine-free rainfed water free of chlorine (19). Third-instar larvae, deprived of food for 24 hours, were used for the test.

Initially, 1 ml of temephos solution was pipetted into a glass beaker and diluted with chlorine-free rainwater to achieve a final concentration of 0.02 ppm, followed by mixing for 15-30 minutes to ensure homogeneity. Twenty larvae from each mosquito species were then transferred to separate containers containing 100 ml of this temephos solution (or chlorine-free rainwater for the negative control) and exposed for 24 hours, with four replicates for each species. Temperature (°C) and relative humidity (%) were recorded throughout the test using a thermometer and hygrometer. Each test with a different mosquito species group included a negative control, as is standard practice. The negative control consisted of larvae exposed to acetone (19).

### Data Analysis

The type, abundance, and presence of larval breeding sites were presented in tables and percentages. Larval susceptibility to temephos was assessed after 24 hours. Larval mortality was counted in each species, with dead larvae defined as those remaining immobile at the bottom of the glass beaker for 1 minute after gentle stimulation with a probe. Mortality rate was calculated as the percentage of dead larvae compared to the initial number of test larvae. Larval susceptibility was categorized as susceptible, moderately susceptible, or resistant based on mortality rates. Complete susceptibility was defined as 100% mortality, while mortality between 90-97% indicated moderate susceptibility, and mortality below 90% suggested low susceptibility or potential resistance to temephos insecticide (20).

## RESULTS

### Entomological Survey

Entomological surveys in this study involved identifying both breeding sites and the genus or species of mosquitoes present. Surveys around residents' homes and yards were conducted to identify potential breeding sites. Naena Muktipura Village consisted of semi-permanent houses (e.g., wooden dwellings) with brick bases and walls partially constructed with wood. Houses stood approximately 50-100 meters apart, separated by ditches. Uneven land contours created depressions that potentially formed puddles during the rainy season. However, the entomological survey conducted in May, coinciding with the end of the dry season in Mimika, identified numerous dried-up puddles, likely impacting the overall breeding site distribution and mosquito population observed.

Observations were made on 27 pools of water outside the house as documented in Table 1. However, some potential breeding sites were dry and lacked mosquito larvae, particularly in sun-exposed yards In contrast, some larvae-positive breeding sites found in yards were covered by shrubs, bushes, and trees, preventing direct sunlight exposure (Figure 1).

**Table 1. Breeding Site Type and the Presence of Larvae in Naena Muktipura Village**

| Types of Breeding Site | Amount | The Presence of Larvae | | Genus of Mosquito |
|---|---|---|---|---|
| | | Positive | Negative | |
| **Natural** | | | | |
| Water stream | 1 | 0 | 1 | |
| **Human made** | | | | |
| Abandoned well | 1 | 1 | 0 | *Anopheles sp* *Culex sp* |
| Water tower | 1 | 0 | 1 | |
| Drainage | 7 | 1 | 6 | *Culex sp* |
| Abandoned water drums | 3 | 0 | 3 | |
| Unused bucket | 3 | 1 | 2 | *Aedes sp** |
| Pool | 3 | 0 | 3 | |
| Unused tires | 3 | 3 | 0 | *Aedes sp** |
| Puddle of water | 5 | 1 | 4 | *Culex sp* |
| **Total** | **27** | **7** | **20** | |

*Aedes albopictus

**Figure 1. Breeding Sites for *Anopheles sp* and *Culex sp***

The entomology survey found mosquito larvae in 7 out of 27 identified breeding sites. One positive breeding site, a former dug well, contained two mosquito genera: *Culex* and *Anopheles*. This site was shaded by bushes and trees and its water was stagnant, green algae-coated, and debris-filled, as shown in Figure 1. Other breeding sites included abandoned household appliances and unused cans.

Mosquito larvae from each pool of water were identified and classified into three genera: *Anopheles*, *Culex*, and *Aedes*, as detailed in Table 1. *Culex* and *Anopheles* larvae typically preferred breeding in dirty, leaf-covered water, with *Anopheles* often found in well water (e.g., groundwater or rainwater pool) without a cement layer. *Aedes* larvae were the most prevalent, and due to their distinct 8$^{th}$ abdominal segment, species identification was possible. This revealed the larvae to be *Aedes albopictus*. *Aedes albopictus* larvae were found in unused tire containers and buckets outside the house, potentially exposed to direct sunlight. This differed from *Aedes aegypti*, which favors indoor breeding sites.

### Susceptibility Test

All containers with mosquito larvae breeding sites were included in the susceptibility test. Each container contained at least 80 third-instar mosquito larvae. All larvae in each type of stagnant water exhibited 100% mortality after the susceptibility test using temephos insecticide. The results confirmed susceptibility to temephos for all three mosquito species: *Aedes, Culex*, and *Anopheles*, as shown in Table 2. The susceptibility test was conducted at room temperature (24-27°C) and humidity (72-80%).

**Table 2. Status of Mosquito Susceptibility Based on the Type of Species and Positive Larvae Containers**

| Type of Puddle | Mosquito Species | Percentage of Larvae Mortality | Vulnerability State |
|---|---|---|---|
| Abandoned well | *Anopheles sp* | 100 % | Susceptible |
| Abandoned well | *Culex sp* | 100 % | Susceptible |
| Unused tires 1 | *Aedes sp* | 100 % | Susceptible |
| Unused tires 2 | *Aedes sp* | 100 % | Susceptible |
| Unused tires 3 | *Aedes sp* | 100 % | Susceptible |
| Unused bucket | *Aedes sp* | 100 % | Susceptible |
| Drainage | *Culex sp* | 100 % | Susceptible |
| Puddle of water | *Culex sp* | 100 % | Susceptible |

## DISCUSSION

The Naena Muktipura Village entomological survey identified three mosquito species: *Anopheles sp, Aedes sp*, and *Culex sp*. This study revealed the prevalence of *Culex sp*, found in 3 breeding sites. While previous research (21) describes *Culex sp.* preferring turbid water, often found in urban areas like ditches and polluted waterways (14), our findings highlight their adaptability to rural environments (22), which made it clear that the habitat of *Culex sp* mosquitoes varies greatly (23). *Culex sp* breeding sites in urban areas were in the form of stagnant dirty water, such as ditches, waste disposal of bathing water, and rivers full of garbage (14). Contrary to urban *Culex sp.* habitats, we observed them breeding primarily in rural Naena Muktipura in ditches filled with water hyacinth, dug wells in fields, and abandoned wells in yards covered with shrubs and moss. This aligns with previous reports (22) identifying similar breeding sites like puddles, ditches, and shrubs in rural areas. Notably, the water in these *Culex* breeding sites was often polluted with organic compounds and complex mixtures (24).

Numerous studies have identified various factors influencing larval mosquito presence in breeding sites, including pH, water temperature, dissolved oxygen, turbidity, soluble solids, salinity, resistance, and nutrition (19-21). High dry-season temperatures might reduce breeding site availability, as observed in this study (25), and supported by other research (26). This could explain some of the negative breeding sites found during this dry-season study. Further, conducting this study in the dry season likely contributed to some negative breeding site results. Notably, another study demonstrated a correlation between the presence of larval breeding sites and malaria cases (27). Furthermore, this study revealed a striking fact: 26 out of 27 breeding sites were human-made, aligning with previous research showing that human-constructed larval habitats often outnumber natural ones (28). This predominance of human-made breeding sites emphasizes the critical role of human involvement in mosquito control efforts, as highlighted by earlier research (29). Increasing public awareness and participation in breeding site control is crucial to reduce the prevalence of mosquito-borne diseases.

The results of this study confirmed that *Culex sp* larvae were 100% susceptible to temephos insecticide, which was similar with the *Culex sp* larvae in Makassar (14). The widespread and diverse habitats of *Culex sp.* larvae in stagnant water likely contribute to the lack of routine larvicide use, despite laboratory studies demonstrating the effectiveness of biological insecticides for their control (10). In contrast, urban settings often rely on spray-type household insecticides to control adult mosquitoes, which have been reported to induce *Culex sp.* resistance due to rapid or massive use (11).

This study also found another mosquito species belonging to the genus *Aedes*. Morphological examination under laboratory conditions confirmed the species to be *Aedes albopictus* based on the presence of a diagnostic spike on the 8th abdominal segment. This identification was further supported by the characteristic outdoor location and breeding site water types, as reported in previous studies (15,17). Furthermore, *Aedes albopictus*, like *Aedes aegypti*, can be found in clear standing water, although its preferred breeding locations differ (20).

Our findings reveal that breeding sites identified as *Aedes* were primarily found in unused buckets and tires, which are common outdoor containers for stagnant water. This observation aligns with another study (30) showing *Aedes albopictus* breeding in similar outdoor locations. This contrasts with *Aedes aegypti*, which thrives in indoor containers like buckets, dispensers, and bird feeders (30) Furthermore, as confirmed by other studies breeding sites for *Aedes sp.* species commonly occur closest to residential areas (31). Additionally, these *Aedes* breeding sites tend to be shady and open. (15,17). Interestingly, *Aedes albopictus* larvae in this study showed susceptibility to temephos insecticide. This may be attributed to the fact that the *Aedes albopictus* breeding places are outside the home, which makes residents ignorant on the application of insecticides.

This study identified only one out of 27 breeding sites containing *Anopheles* larvae. This appears to contradict evidence pointing to Papua as the highest endemic area of malaria in Indonesia. This low detection rate might be due to the timing of the study, April to May, when Mimika experiences lower rainfall compared to other months. Consequently, fewer stagnant water breeding sites suitable for *Anopheles* larvae might be available during this period. Thus, investigating *Anopheles* breeding site characteristics across different seasons might provide valuable insights into their seasonal dynamics.

In this study, *Anopheles sp.* larvae were only found in one location: an abandoned dug well in a resident's yard, sheltered by shrubs and a mossy water surface, adjacent to a forest edge. This finding aligns with previous research showing increased malaria risk near edge of forests or stagnant water bodies like swamps (32). Similarly, in Keerom Papua, environments featuring shrubs and stagnant water near houses were identified as major malaria risk factors, and residential proximity to forests was also linked to increased malaria risk (33). The existence of forests near residential areas also poses a risk of transmitting malaria (34). The risk of malaria transmission is related to the presence of *Anopheles sp*. Mosquitoes (35). The presence of *Anopheles sp.* mosquitoes facilitates malaria transmission around residents' home. Therefore, mosquito control interventions targeting breeding sites are essential for malaria prevention. Given the lack of species-level identification in this study, further research is needed to determine the *Anopheles* species present, as different species exhibit diverse habitat preferences (35).

Previous studies of *Anopheles* mosquitoes in the Papua region identified *Anopheles* species such as *Anopheles farauti*, *Anopheles koliensis*, *Anopheles punctulatus* and *Anopheles bancrofti* (34). In contrast to Papua, the breeding sites for *Anopheles sp* in different areas vary substantially. For example, Java Island's breeding sites, such as puddles on riverbanks, rice fields, irrigation canals, and cow hoof prints, host different *Anopheles* species like *Anopheles vagus*, *Anopheles maculatus* and *Anopheles aconitus* (36). Suitable breeding sites are crucial for mosquito egg-laying (oviposition) and depend on climate conditions, human activities, and local geography climate conditions, human activities, and local geograpy. These can comprise diverse materials, be natural or artificial, exposed to sunlight or not, permanent or temporary, and vary in size (21-22).

Temephos in *Anopheles* larvae was confirmed to be susceptible as evidenced by100% mortality, thereby demonstrating its potential as a larvicide against *Anopheles sp*. While traditional *Anopheles* control primarily targets adults through methods like pyrethroid insecticides applied via indoor residual spraying (IRS), peridomestic spraying, and long-lasting insecticide-treated nets (LLINs) (37) temephos offers a valuable tool for tackling the mosquito at its aquatic breeding stage. Even though susceptibility is good, mosquito-borne diseases were still high. Vector control using larvicides needs to be improved, such as through correct usage of targeted larviciding (38). Larviciding should be based on routine entomological surveys and evaluated routinely.

Tested mosquito populations in Naena Muktipura Village were susceptible to the temephos organophosphate insecticide. Mosquito susceptibility in any location depends on various factors, including perceived ease of application, previous resistance status, and government policy. This study found all tested mosquito genera (*Anopheles, Culex*, and *Aedes*) susceptible to temephos, significantly exceeding the 22% susceptibility reported in previous studies for the district (37). Therefore, temephos insecticide remains a viable control option for *Aedes sp*, *Culex sp*, and *Anopheles sp*. larvae in Mimika Papua.

Furthermore, we could improve this research by checking the breeding site characteristics, including pH, water temperature, water body type, substrate types, predators, vegetation, salinity, and dissolved oxygen. These parameters can then be used to analyze the relationship between physicochemical parameters and mosquito presence in their breeding sites. The present study has limitations in assessing other potential breeding sites, but future studies exploring sites inside houses and near the forest could expand our understanding. Comparing breeding sites between rainy and dry seasons would also be valuable. Continued species identification and studies on vectorial capacity are crucial for identifying potential vectors of endemic diseases.

## ACKNOWLEDGMENTS

I would like to express my utmost gratitude to the Environmental Health Officers at the Limau Asri and Health Centers, and to the residents of Naena Muktipura Village for their invaluable contributions to this research. I also extend my sincere thanks to the Research and Community Service Unit of the Faculty of Medicine, Universitas Islam Indonesia for funding this research.

## CONCLUSION

The present study has shown that artificial containers are more prevalent mosquito breeding sites than natural ones. Mosquito breeding sites in Naena Muktipura vary in characteristics depending on the mosquito species. All three larvae of the order Diptera in *Aedes*, *Culex*, and *Anopheles* from Naena Muktipura village are susceptible to temephos insecticide. Continuous collaboration between relevant agencies and residents is crucial for controlling mosquitoes around homes and preventing mosquito-borne disease transmission.

## REFERENCES

1. World Health Organization. Vector-borne Diseases. Geneva: World Health Organization; 2020. https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases
2. Ministry of Health of Republic Indonesia. Health Profile of Indonesia 2021. Jakarta: Ministry of Health of Republic Indonesia; 2021. https://www.kemkes.go.id/id/profil-kesehatan-indonesia-2021
3. Ariati J, Perwitasari D, Marina R, Shinta S, Lasut D, Nusa R, et al. Status Kerentanan *Aedes Aegypti* terhadap Insektisida Golongan Organofosfat dan Piretroid di Indonesia. *J Ekol Kesehat*. 2019;17(3):135–145. https://doi.org/10.22435/jek.17.3.847.135-145
4. Fadilah I, Djaafara BA, Lestari KD, Fajariyani SB, Sunandar E, Makamur BG, et al. Quantifying Spatial Heterogeneity of Malaria in the Endemic Papua Region of Indonesia: Analysis of Epidemiological Surveillance Data. *Lancet Reg Heal*. 2022;5(100051):1-15. https://doi.org/10.1016/j.lansea.2022.100051
5. Hasyim H, Ihram MA, Fakhriyatiningrum, Misnaniarti, Idris H, Libertyet IA, et al. Environmental Determinants and Risk Behaviour in the Case of Indigenous Malaria in Muara Enim Regency, Indonesia: a Casecontrol Design. *PLoS One*. 2023;18(1):1–10. http://dx.doi.org/10.1371/journal.pone.0289354
6. Novianto D, Hadi UK, Soviana S, Supriyono, Darusman HS, et al. Species Diversity and Breeding Site of Mosquito Larvae (*Diptera: Culicidae*) in Macaca Fascicularis Breeding Area. *IOP Conf Ser Earth Environ Sci*. 2021;948(1):012039. https://iopscience.iop.org/article/10.1088/1755-1315/948/1/012039
7. Wigati RA, Maksud M. Diversity of Mosquito Larvae Breeding Sites in Several Sampling Locations in Donggala Regency, Central Sulawesi, Indonesia. *In: Proceedings of the International Conference and the 10th Congress of the Entomological Society of Indonesia (ICCESI 2019)*. 2020;8(1):259-265. https://doi.org/10.2991/absr.k.200513.044
8. Ali EOM, Babalghith AO, Bahathig AOS, Toulah FHS, Bafaraj TG, Al-Mahmoudi SMY, et al. Prevalence of Larval Breeding Sites and Seasonal Variations of *Aedes aegypti* Mosquitoes (*Diptera: Culicidae*) in Makkah Al-Mokarramah, Saudi Arabia. *Int J Environ Res Public Health*. 2021;18(14):7368. https://www.mdpi.com/1660-4601/18/14/7368
9. Manzoor F, Shabbir R, Sana M, Nazir S, Aslam M. Original Article Determination of Species Composition of Mosquitoes in Lahore, Pakistan. *J Arthropord-Borne Dis*. 2020;14(1):106–115. https://doi.org/10.18502%2Fjad.v14i1.2717
10. Hitipeuw D. Efikasi Larvasida *Bacillus thuringiensis var. israelensis* terhadap Larva *Aedes*, *Anopheles*, dan *Culex*. *Higeia*. 2023;7(2):66286. https://doi.org/10.15294/higeia.v7i2.66286
11. Sunbanu AH, Almet J, Wuri DA. Uji Resistensi Nyamuk *Culex sp*. terhadap Insektisida Golongan Piretroid (permethrin 0,25 %) di Kota Kupang. *J Vet Nusant*. 2021;4(1):1–10. https://doi.org/10.35508/jvn.v4i1.6037
12. Ayu K, Aris L. Eksplorasi Insektisida Jenis *Cypermethrin* 0,05% Pada Kasus Demam Berdarah Dengue di Kabupaten Kudus Tahun 2017. *Kesehat Lingkung Masy.* 2018;37(3):339–346. https://doi.org/10.31983/keslingmas.v37i3.3898
13. Suparyati S. Uji Daya Bunuh Abate Berdasarkan Dosis dan Waktu terhadap Kematian Larva Nyamuk *Aedes sp* dan *Culex sp*. *Pena J Ilmu Pengetah dan Teknol*.2020;34(2):1193.http://dx.doi.org/10.31941/jurnalpena.v34i2.1193
14. Ramly WL, Ahmad H, Saleh M. Resistance Test Of Synthetic Larvicides with Dose Comparison to *Culex Sp* . Larvae in The Paccerakkang Village, Biringkanaya District, Makassar City. *Higiene*. 2022;8(2):91–96. http://journal.uin-alauddin.ac.id/index.php/higiene/article/view/30897
15. National Center Mimika Regency of Statistics. West Mimika District in Numbers. Mimika: National Center Mimika Regency of Statistics; 2023. https://mimikakab.bps.go.id/

16. World Health Organization. Guidelines for Entomological Surveillance of Malaria Vectors in Sri Lanka. Sri Lanka: Who Global Malaria Programme; 2009. http://amc.health.gov.lk/images/Publication%20Repository/SOP/Revised_Guidelines_for_Entomological_surveillance.pdf
17. Snell AE. Identification Keys to Larval and Adult Female Mosquitoes (*Diptera: Culicidae*) of New Zealand. *New Zeal J Zool*. 2005;32(2):99–110. https://doi.org/10.1080/03014223.2005.9518401
18. Doosti S, Yaghoobi EMR, Sedaghat MM, et al. Larval Habitats Characteristics of Culicinae Subfamily in the South of Iran. *Nusant Biosci*. 2021;13(2):194–201. https://doi.org/10.13057/nusbiosci/n130209
19. World Health Organization. Instruction for Determining the Susceptibility or Resistance of Mosquito Larvae to Insecticide. Geneva: World Health Organization; 1981. https://iris.who.int/bitstream/handle/10665/69615/WHO_VBC_81.807_eng.pdf?sequence=1&isAllowed=y.
20. Subahar R, Aulia AP, Yulhasri Y, Felim RR, Susanto L, Winita R, et al. Assessment of Susceptible *Culex quinquefasciatus* Larvae in Indonesia to Different Insecticides Through Metabolic Enzymes and the Histopathological Midgut. *Heliyon*. 2022;8(12):e12234. https://doi.org/10.1016/j.heliyon.2022.e12234
21. Oktafian M, Siwiendrayanti A. Karakteristik Tempat Perindukan Nyamuk *Culex sp*. di Sekitar Tempat Tinggal Penderita Filariasis Limfatik di Kabupaten Brebes Tahun 2020. *Indones J Public Heal Nutr*. 2021;1(1):101–113. https://journal.unnes.ac.id/sju/index.php/IJPHN/article/view/45337
22. Fadhilla SN, Siwiendrayanti A. Spatial Analysis of Environmental Factors with the Existence of Filariasis Vectors in Brebes Regency. *J Public Heal Trop Coast Reg*. 2021;4(2):53–62. https://ejournal2.undip.ac.id/index.php/jphtr/article/view/10791
23. Purwatiningsih, Oktarianti R, Setiawan R, Agustin WT, Mursyidah A, et al. Keanekaragaman Jenis Nyamuk yang Berpotensi sebagai Vektor Penyakit (*Diptera: Culicidae*) di Taman Nasional Baluran, Indonesia. *Al-Kauniyah J Biol.* 2021;14(2):184–194. http://dx.doi.org/10.15408/kauniyah.v14i2.12918
24. Huzortey AA, Kudom AA, Mensah BA, Ntiri BS, Anderson B, Akyea A, et al. Water Quality Assessment in Mosquito Breeding Habitats Based on Dissolved Organic Matter and Chlorophyll Measurements by Laser-Induced Fluorescence Spectroscopy. *PLoS One*. 2022;17(7):1–14. http://dx.doi.org/10.1371/journal.pone.0252248
25. Tuno N, Farjana T, Uchida Y, Iyori M, Yoshida S. Effects of Temperature and Nutrition during the Larval Period on Life History Traits in an Invasive Malaria Vector *Anopheles stephensi*. *Insects*. 2023;14(543):1-10. https://doi.org/10.3390/insects14060543
26. Hinne IA, Attah SK, Mensah BA, Forson AO, Afrane YA. Larval Habitat Diversity and Anopheles Mosquito Species Distribution in Different Ecological Zones in Ghana. *Parasites and Vectors*. 2021;14(1):1–14. https://doi.org/10.1186/s13071-021-04701-w
27. Ndiaye A, Niang EHA, Diène AN, Nourdine MA, Sarr PC, Konaté L, et al. Mapping the Breeding Sites of Anopheles Gambiae S. L. in Areas Of Residual Malaria Transmission in Central Western Senegal. Carvalho LH, editor. *PLoS One*. 2020;15(12):e0236607. https://dx.plos.org/10.1371/journal.pone.0236607
28. Forson AO, Hinne IA, Sraku IK, Afrane YA. Larval Habitat Stability and Productivity in Two Sites in Southern Ghana. *Malar J*. 2023;22(74):1-13. https://doi.org/10.1186/s12936-023-04498-2
29. Abdelwahab SI, Elhassan IM, Albasheer O, Taha MME, Ali NA, Al-Jabiri YS, et al. Knowledge, Attitudes, and Practices (KAP) During the Malaria Elimination Phase: a Household-Based Cross-Sectional Survey. *Medicine*. 2023;102(22):E33793. https://doi.org/10.1097/MD.0000000000033793
30. Pramadani AT, Hadi UK, Satrija F. Habitat *Aedes aegypti* dan *Aedes albopictus* sebagai Vektor Potensial Demam Berdarah Dengue di Kecamatan Ranomeeto Barat, Provinsi Sulawesi Tenggara. *ASPIRATOR.* 2020;12(2):123–136. https://doi.org/10.22435/asp.v12i2.3269
31. Manik JR, Luma D, Kutani LF. Karakteristik Habitat Perkembangbiakan *Aedes aegypti* di Desa Gosoma, Halmahera Utara, Indonesia. *Biosf J Biol dan Pendidik Biol*. 2020;5(1):31–36. https://doi.org/10.23969/biosfer.v5i1.2385
32. Hastuty HSB, Setyowati DW. Distribusi Spasial Penderita Malaria di Distrik Sentani Timur Kabupaten Jayapura. *Sebatik*. 2021;25(1):68–73. https://doi.org/10.46984/sebatik.v25i1.1295
33. Manangsang F, Ganing A, Purba ER V, Rumaseb E, Sarwandhamana RJ, et al. Analisis Faktor Risiko Lingkungan terhadap Kejadian Malaria di Kabupaten Kerom Provinsi Papua Analysis of Environmental Risk Factors Against Malaria Events in Kerom Province District Papua. *J Hosp Adm*. 2021;4(2):37–42. http://dx.doi.org/10.21927/ijhaa.2021.4(2).37-42
34. Setiyaningsih R, Yanti S AO, Lasmiati L, Mujiyono M, Prihatin MT, Widiarti, W et al. Keanekaragaman Anopheles dalam Ekosistem Hutan dan Resiko Terjadinya Penularan Malaria di Beberapa Provinsi di Indonesia. *Media Penelit dan Pengemb Kesehat*. 2019;29(3):243–254. https://doi.org/10.22435/mpk.v29i3.1460
35. Mahdalena V, Wurisastuti T. Gambaran Distribusi Spesies Anopheles dan Perannya sebagai Vektor Malaria di Provinsi Nusa Tenggara Timur, Papua dan Papua Barat. *Spirakel*. 2021;12(1):46–59. https://doi.org/10.22435/spirakel.v12i1.3441
36. Mulyawati A, Sukesi TW, Mulasari SA, Setiawan YD, Yuliani Y, Patmasari Y et al. Analisis Situasi Luas Wilayah Reseptif Malaria di Kabupaten Gunungkidul Daerah Istimewa Yogyakarta Tahun 2021. *Sanitasi J Kesehat Lingkung.* 2022;15(1):47–60. http://journalsanitasi.keslingjogja.net/index.php/sanitasi/article/view/27/51
37. Ariati J, Perwitasari D, Marina R, Shinta S, Lasut D, Nusa R, et al. Status Kerentanan *Aedes aegypti* terhadap Insektisida Golongan Organofosfat

dan Piretroid di Indonesia. *J Ekol Kesehat*. 2019;17(3):135–145. https://doi.org/10.22435/jek.17.3.847.135-145

38. Loonen JACM, Dery DB, Musaka BZ, Bandibanone JB, Bousema T, Lenthe MV, et al. Identification of Main Malaria Vectors and Their Insecticide Resistance Profile in Internally Displaced and Indigenous Communities in Eastern Democratic Republic of the Congo (DRC). *Malar J*. 2020;19(1):1–10. https://doi.org/10.1186/s12936-020-03497-x