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Temephos resistance in *Aedes aegypti* at Dumai Seaport: implications for vector control

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Abstract

Purpose: This study aimed to assess the resistance status of *Aedes aegypti* (*Ae. aegypti*) to temephos at Dumai International Seaport, Indonesia, and to identify the biochemical mechanisms underlying this resistance to support more effective vector control strategies. **Methods:** An experimental study was conducted using larval bioassays at four concentrations of temephos (0.005–0.625 mg/L) to determine susceptibility levels in field-collected mosquito populations from operational and industrial zones. A laboratory strain was used as a control. Additionally, biochemical assays were performed to measure α-esterase and monooxygenase activity, which are potential indicators of metabolic resistance. **Results:** Larval bioassays revealed reduced susceptibility to temephos in both operational and industrial populations compared to the laboratory strain. The resistance ratios were 9.75 and 11.75, respectively, indicating moderate to high resistance. Biochemical analysis showed significantly increased α-esterase activity in both field populations ($p<0.000$), while the seaport population also exhibited elevated monooxygenase activity ($p<0.020$), suggesting enzyme-mediated resistance mechanisms. **Conclusion:** The presence of temephos resistance in *Ae. aegypti* at Dumai Seaport underscores the need to revise existing insecticide use practices. Integrating biochemical surveillance and diverse control measures within the framework of integrated vector management is essential to ensure the sustainability and effectiveness of vector control programs.

Keywords: *Aedes aegypti*; seaport; temephos resistance; vector control

INTRODUCTION

Arboviral infections such as dengue, Zika, chikungunya, and yellow fever remain major global public health threats, collectively responsible for over 700,000 deaths annually. Dengue alone causes more than 390 million cases each year, with the majority occurring in Asia and Latin America [1]. The rapid pace of urbanization, increasing global travel, and intensified trade activities have amplified human–vector contact, significantly altering patterns of virus transmission [2]. Among the primary vectors, *Aedes aegypti (Ae. aegypti)* and *Aedes Albopictus (Ae. albopictus)* are recognized as the most invasive mosquito species, mainly due to their adaptability and global spread. Their widespread distribution has been facilitated by maritime transportation routes, especially since the opening of the Suez Canal in 1869, making international ports key points for vector introduction and arbovirus transmission [3–5].

Dumai International Seaport, located in Riau Province, Indonesia, serves as a strategic international

gateway for trade, particularly the export of crude palm oil (CPO), with over 4.75 million metric tons exported in 2019. The port also recorded over 495,000 passengers and 9,646 vessel movements in 2022, illustrating high levels of human mobility and commercial activity. These conditions make Dumai a high-risk site for the introduction and transmission of arboviruses. Moreover, Indonesia contributes to 40% of all chikungunya cases reported among international travelers, reinforcing the urgent need for effective vector surveillance and control at critical entry points such as seaports [6].

Vector control, primarily through insecticide use, is crucial for preventing the spread of arboviruses at seaports [7–9]. At Dumai Seaport, organophosphate insecticides, such as temephos, have been used since 2013. However, the extensive use of these insecticides has led to insecticide resistance, threatening the effectiveness of control strategies [10,11]. While resistance of *Ae. aegypti* populations to temephos and other insecticides has been documented in various regions of Indonesia, research on resistance at seaports, where human activity and trade converge, remains limited [12,13].

A significant gap in current research is the lack of studies focusing on the biochemical mechanisms underlying insecticide resistance at seaports. Although insecticide resistance in *Ae. aegypti* has been reported in other regions of Indonesia, there is a lack of studies examining the enzymatic pathways (e.g., monooxygenases, esterases) that contribute to resistance in critical sites like Dumai Seaport. Understanding these biochemical mechanisms is essential for developing more targeted and effective vector control measures.

This study aims to fill this gap by assessing the temephos resistance status of *Ae. aegypti* populations at Dumai Seaport and investigating the enzymatic mechanisms that underlie this resistance. By exploring both resistance patterns and enzyme activity, this study will provide valuable insights to improve vector control strategies at international seaports, which are critical points for arbovirus transmission.

METHODS

This study employed an experimental design to assess the susceptibility and resistance of *Ae. aegypti* larvae to temephos. The design involved controlled larval bioassays to measure mortality at various temephos concentrations, as well as biochemical assays to evaluate enzyme activity associated with insecticide resistance. This study was conducted at Dumai International Seaport in Riau Province, Indonesia, focusing on two distinct zones. The operational zone includes passenger terminals and administrative offices, where the Port Health Authority manages the use of insecticides. In contrast, the industrial zone is characterized by oleochemical facilities and frequent insecticide application by private pest control services, driven by higher pest pressure. Dumai itself is a major industrial port city located in northern Sumatra, approximately 200 kilometers north of Pekanbaru, covering an area of 2,065.59 square kilometers. The city serves as a key hub for several industries, including palm oil, petroleum, and logistics.

Mosquito collection and rearing

Aedes spp egg sample collections in the field were carried out using ovitraps from January to February 2024, following the sampling protocols of the Ministry of Health [14]. Ovitraps were installed both inside and outside buildings, as well as in potential breeding sites such as vegetation. Specifically, two ovitraps were placed at each residential building and six at office and industrial sites. These ovitraps were positioned in sheltered locations and collected after five days [15]. An *Ae. aegypti* susceptible laboratory strain was used in this study. The rearing of late third and/or early fourth larval instars of F1 generation of field collected *Ae. aegypti* populations from Dumai International Seaport were maintained following the methodology by Perez, et al [16].

Larval bioassay

Larval bioassays were used to test the susceptibility of *Ae. aegypti* to temephos following standard protocols [14]. Each test included four replicates for each concentration and two control cups, totaling 120 larvae. Temephos was tested at four concentrations: 0.005, 0.025, 0.125, and 0.625 mg/L. In each cup, 20 larvae at the late third or early fourth instar stage were placed in 249 mL of tap water mixed with 1 mL of temephos solution. Larval mortality was recorded 24 hours after.

Biochemical assays

Biochemical tests were conducted to determine whether insecticide resistance in *Ae. aegypti* from Dumai International Seaport was related to increased enzyme activity. The enzyme levels of a field strain were compared to those of a susceptible laboratory strain using modified CDC methods [17]. Individual fourth instar larvae were tested for nonspecific esterase and mixed-function oxidase (MFO) activity. A total of 24 larvae from each group were homogenized in phosphate-buffered saline (PBS), centrifuged, and the supernatants were used in the assays. Esterase

activity was measured at 450 nm, while MFO activity was measured at 620 nm after incubation with specific reagents. The results were used to compare enzymatic activity between field and laboratory strains.

The susceptibility of *Ae. aegypti* was determined based on mortality rates, with 98-100% indicating susceptibility, 90-97% indicating tolerance or intermediate resistance, and less than 90% indicating resistance. LC50 and LC95 values were calculated using probit analysis via SPSS software. Resistance ratios (RR), defined as the LC of the field population divided by that of the susceptible laboratory population, were used to assess resistance intensity. Populations with an RR of less than 5 were considered susceptible, those with an RR between 5 and 10 exhibited moderate resistance, and populations with an RR of 10 or greater were classified as highly resistant.

The susceptibility status of mosquitoes, based on α-esterase and monooxygenase activity, was determined by the absorbance value (AV). Box plots were used to compare the field strain to the reference laboratory strain visually. Data were analyzed to compare enzyme activity between field-collected larvae and the laboratory strain. Statistical significance was determined using t-tests, depending on data distribution.

RESULTS

Table 1 illustrates the mortality response of *Ae. aegypti* larvae from different populations—operational, industrial, and laboratory—across increasing concentrations of temephos. The laboratory strain consistently demonstrated high susceptibility, achieving near-complete mortality even at low concentrations. In contrast, both field populations exhibited substantially lower mortality at the same doses, indicating reduced sensitivity. As the concentration of temephos increased, mortality in field populations gradually improved, but complete lethality was only achieved at the highest dose tested. These results suggest the presence of tolerance or resistance in the operational and industrial mosquito populations, with a stronger resistance pattern observed in the industrial zone.

Table 2 presents the lethal concentration values (LC_{50} and LC_{90}) and resistance ratios of *Ae. aegypti* larvae from different populations in response to teme-

Table 1. Mortality rate of *Ae. aegypti* larvae at various temephos concentrations

Population	Mortality (%)			
	0,005	0,025	0,125	0,625
Operational	3,75	25	91,25	100
Industries	11,25	36,25	95	100
Laboratories	95	98,75	100	100

Table 2. Lethal concentration (LC) and resistance status of *Ae. aegypti* larvae

Population	LC_{50} (mg/L)	LC_{90} (mg/L)	95%CI	RR_{50}	Status
Seaport	0,039	0,120	0,028-0,050	9,75	Tolerance
Industries	0,047	0,103	0,036-0,059	11,75	Resistance
Laboratory	0,004	0,013	-	1,0	Susceptible

-phos exposure. The laboratory strain showed the lowest LC values, confirming its susceptibility and serving as a reliable control. In contrast, the field populations required higher concentrations to achieve similar mortality, with the industrial population showing the highest resistance ratio, followed by the operational population. These differences indicate varying levels of resistance, with the industrial zone exhibiting high resistance and the operational zone showing moderate resistance. The elevated LC values and resistance ratios among field populations reflect the diminished effectiveness of temephos, likely due to prolonged and repeated exposure in these environments.

Figure 1 compares the enzymatic activity of α-esterase and monooxygenase in *Ae. aegypti* populations from Dumai International Seaport. The results show that both the operational and industrial field populations had significantly higher α-esterase activity compared to the susceptible laboratory strain, suggesting a strong involvement of metabolic detoxification in resistance. Additionally, the seaport (operational) population exhibited elevated monooxygenase activity, while the industrial population showed a less pronounced increase. These enzymatic patterns support the hypothesis that resistance to temephos in field populations is at least partially mediated by enhanced metabolic enzyme activity, particularly through elevated α-esterase and, in the case of the seaport population, monooxygenase as well.

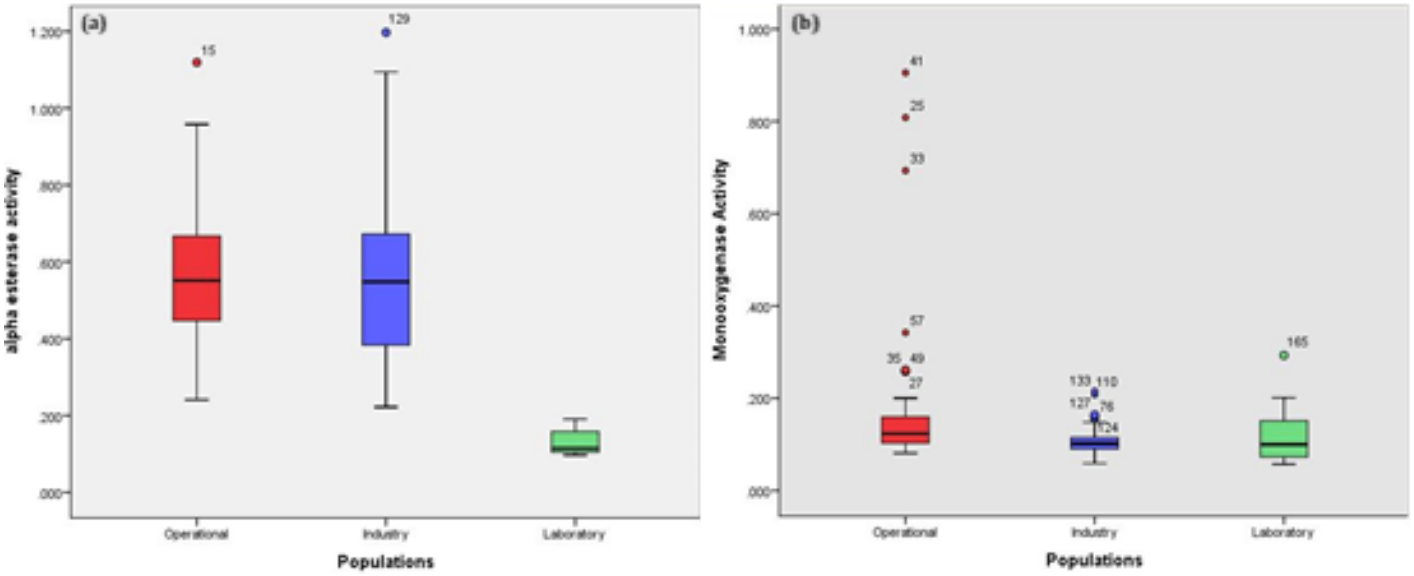

Figure 1. Alpha esterase and monooxygenase for *Ae. aegypti* from Dumai International Seaport (n=72 pes assay)

DISCUSSION

The discovery of *Ae. aegypti* resistance to temephos at Dumai Port has significant implications for public health and vector control strategies. The observed resistance, likely due to over a decade of temephos application, suggests that its efficacy as a primary larvicide is diminishing. This reinforces the urgent need for public health programs to reevaluate and diversify control approaches through Integrated Vector Management (IVM), which incorporates routine resistance monitoring and the use of combined strategies [18,19]. Our findings are consistent with global evidence that prolonged use of a single insecticide class contributes to resistance development [10,11].

Biochemical assays revealed elevated α-esterase activity, supporting prior associations between metabolic enzyme elevation and resistance to organophosphates and pyrethroids, as reported in regions such as Guerrero, Mexico, and Southeast Asia [20,21]. These results highlight the importance of incorporating biochemical surveillance into resistance monitoring programs. However, biochemical data alone are insufficient. Genetic analysis targeting key resistance-related mutations—such as those in the Vgsc and Ace-1 genes—is needed to confirm underlying mechanisms and predict the spread of resistance [22,23].

From a public health perspective, insecticide resistance is more than a technical hurdle; it increases the risk of uncontrolled dengue outbreaks, extends epidemic durations, and escalates healthcare and eco-nomic burdens [24]. Additionally, intersectoral oordination, especially with the agricultural sector, is essential to slow resistance selection driven by non-public health insecticide use. While this study offers valuable insights into temephos resistance, its focus on a single port and a single larvicide limits generalizability. Future research should expand geographically and include multiple insecticide classes to capture broader resistance patterns. A holistic framework that integrates entomological surveillance, resistance genetics, community engagement, and informed policy is crucial for ensuring the sustainability and effectiveness of vector control efforts in Indonesia.

CONCLUSION

This study demonstrates that *Ae. aegypti* exhibits significant resistance to temephos at Dumai Port, highlighting the urgent need to reassess and diversify current insecticide strategies. The findings emphasize the importance of integrating alternative insecticides and adopting Integrated Vector Management (IVM), which should include continuous resistance monitoring and the use of diverse control methods. The observed diminished efficacy of temephos due to its prolonged use underlines the necessity for adaptive management strategies. Moreover, incorporating biochemical assays into resistance surveillance will provide deeper insights into the underlying mechanisms of resistance, helping to develop more precise and effective control approaches. To ensure sustainable and effective vector

control, ongoing research and dynamic management practices remain essential.

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