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Original Article

Morphological Changes of *Aedes aegypti* Larvae After Exposure to 0.02 ppm Temefos

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Abstract

Introduction: *Ae. aegypti* is the primary vector of arbovirus diseases such as Dengue Fever (DHF), Zika, and Chikungunya. The use of the larvicide temephos remains the primary vector control strategy. Still, the emergence of resistance in several regions requires an assessment of its effectiveness and impact on larval morphology.

Objective: To determine the morphology of *Ae. aegypti* larvae after exposure to temephos at a concentration of 0.02 ppm.

Methods: Larval sampling was conducted in three dengue-endemic areas: Semarang City, Jepara Regency, and Brebes Regency. The specimens used for observation were third-instar larvae. Post-exposure to temephos at a concentration of 0.02 ppm was repeated three times, with 20 larvae exposed at each replication. Morphological observations were made on larvae that died after 24 hours of holding using a stereomicroscope with 10x and 40x objective lenses.

Results: All larvae died, placing them in the susceptible category. Morphological changes were found throughout the larvae's bodies. The head showed darkening, shrinkage, and loss of the visual margin of the eyes. The thorax appeared smaller, with the boundaries between segments disappearing and black spots appearing. The abdomen darkened, especially in segments 4–5. The body stiffened and became curved. The siphon and anal segments showed swelling and black spots, indicating tissue damage and an immune response in the form of melanization.

Conclusion: Temephos at 0.02 ppm was still highly effective against *Ae. aegypti* larvae and caused morphological disturbances reflecting physiological stress and immune response activation.

Keywords: *Ae. aegypti*, Temephos, Larval morphology, Insecticide susceptibility

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Introduction

Arbovirus diseases are a global public health problem, with 215 countries potentially affected by exposure to the *Ae. aegypti* mosquito (1,2). The *Ae. aegypti* mosquito can transmit the dengue virus, which causes dengue hemorrhagic fever (3). This mosquito is also the main vector for several other diseases, such as Rift Valley Fever (RVF), Chikungunya, Zika, and Yellow Fever (2,4). Tropical and subtropical climates are endemic areas with a tendency for increasing infection severity (5).

Every year, the number of global dengue cases continues to increase, especially in the Western Pacific (6), and several other regions such as Oceania, East Asia, and Southeast Asia (7). The increase in dengue cases in the world in 2023 was 5 million cases, with a death toll of more than 5,000 people. Southeast Asia and the Western Pacific are endemic areas (8). Dengue fever in Indonesia is a major health problem (9), which has increased since 1968. The number of dengue cases in 2023 was 114,720 sufferers, with a death toll of 894 people. The distribution of dengue fever continues to expand in Indonesia with an Incidence Rate (IR) higher than the control target in 27 provinces during 2013-2023 (10). The dengue IR in Central Java Province in 2023 was reported at 17.7‰. The highest figure was in Kudus Regency at 44.0 and the lowest at 2.1‰ in Temanggung Regency (11).

Vector control is the only method of preventing dengue fever because vaccination is not yet effective, and there is no antiviral treatment (12,13). The increasing trend of dengue fever cases has triggered public attention to eradicate mosquitoes using various methods. One of these is the use of larvicides in mosquito breeding areas (14) and adulticides (15,16). Larvicides are used to eradicate larvae and pupae before they develop into adult mosquitoes (17). Temephos is one of the active ingredients of organophosphate larvicides that is most widely used worldwide (18). The standard use of temephos has been set at 0.02 ppm, but in reality, temephos use in the community reaches 1% (19).

Excessive use of chemical insecticides will accelerate the development of resistance in mosquito vectors (20,21). Peru has experienced temephos resistance. Resistance in Peru has reached very high levels in several locations, including the Amazon Madre de Dios, due to the use of temephos for more than 25 years (22). Indonesia has also experienced

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temephos resistance in several areas, namely Padang, Riau, Kuningan, and Denpasar. The *Ae. aegypti* larval population in Indonesia has become resistant to diagnostic doses of temephos due to prolonged use (23–25).

Vector resistance is a problem in controlling dengue fever (26). Resistant insects will produce resistant offspring, which will gradually increase the number of resistant vectors in the population (27). Resistance mechanisms include mutations at the target site, metabolic detoxification, reduced insecticide penetration through the mosquito cuticle, and changes in mosquito behavior (28). Temefos has a toxic effect on insects. One of its toxic effects is inhibition of the acetylcholinesterase (AChE) enzyme (29). Inhibited AChE causes an accumulation of acetylcholine at the nerve endings, resulting in paralysis and cell death (30).

Organophosphate insecticides are easily absorbed and fat-soluble, so they are easily absorbed by the insect cuticle (31). Temephos, which is lipophilic, works as a stomach poison by penetrating the mouth or cuticle and entering the midgut cells. Theoretically, changes in the morphology of larvae exposed to temephos show damage to the head, cuticle surface, digestive and respiratory tracts, including the siphon, as well as detached or damaged setae (32,33). Information on the effects of temephos exposure at certain concentrations on the morphology of *Ae. aegypti* larvae are still limited.

Method

Larvae Material Sites

Larval sampling was conducted in three dengue-endemic areas: Semarang City, Jepara Regency, and Brebes Regency (Figure 1). Larval specimens were obtained through a rearing process at the Entomology Laboratory, Faculty of Public Health, Muhammadiyah University of Semarang, by hatching eggs from adult mosquitoes until they reached the third instar.

Larvae Rearing

Field-caught larvae were raised in the Entomology Laboratory. They were placed in trays filled with distilled water and sufficient food. Larvae were kept in a room at 30°C and 70% humidity. Larval development was monitored daily. When the pupal stage developed, they were transferred to cups and kept in mosquito net cages. When adult mosquitoes began to emerge from the pupae, the cages were supplemented with 10% sugar water to replace the food source for mosquitoes. An ovitrap was placed in the cages to provide egg-laying media for female mosquitoes. Once a large number of mosquito eggs were visible, the ovistrips were removed and dried at room temperature. This process continued until a sufficient number of mosquito eggs were collected for testing.

Preparation of Test Larvae

Ovistrips containing mosquito eggs were immersed in a tray filled with distilled water. Observations were made daily to ensure larvae had hatched from the eggs. Observations continued until the larvae reached the third instar stage. Third-instar larvae were transferred to a bowl for larvicide testing.

Larvicide Testing

The WHO recommends using temephos at 0.02 ppm

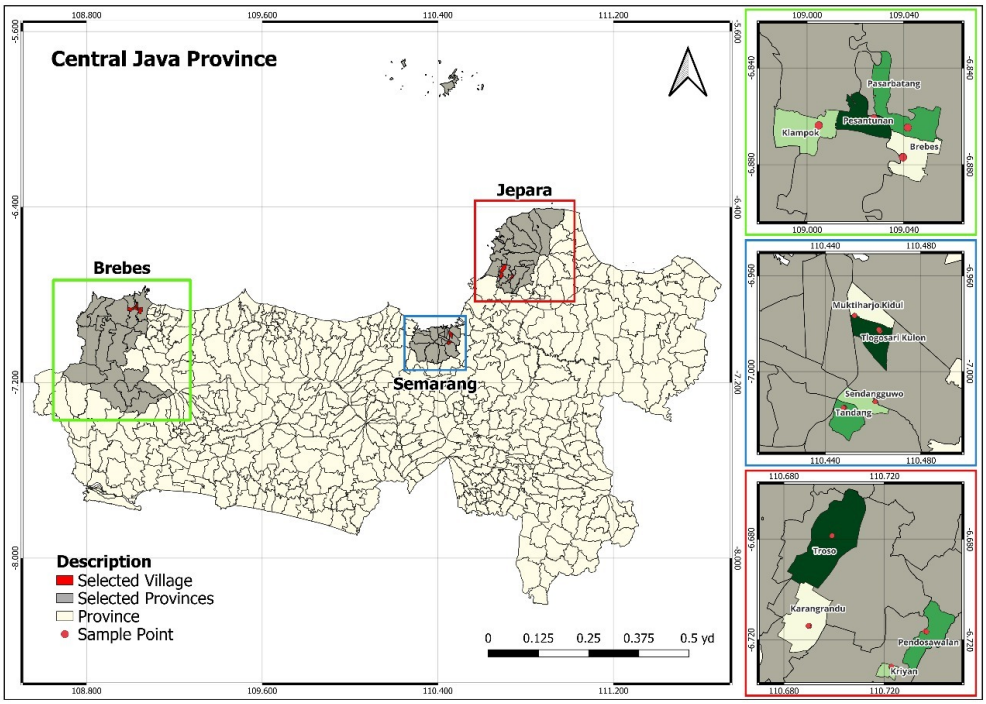


Figure 1. Research site

to test larvicide effectiveness (34). Larvae from each region were tested three times, with 20 third-instar *Ae. aegypti* larvae in each repetition. Larvae in the test dish were exposed to 100 ml of temephos at 0.02 ppm. After 24 hours of contact, larval mortality was counted. Dead larvae were defined as those that ceased to move after contact. Mortality 98%-100% indicates that larvae were still susceptible to temephos at 0.02 ppm.

Identification of Larval Damage

Morphological examination was performed using a stereomicroscope at 10×to 40×magnification. Larvae that died after 24 hours of exposure were separated and morphologically observed using a stereomicroscope. The observation process was carried out by placing the larvae on a glass slide using a pipette. Morphological examination was observed at each repetition. The morphological parameters observed included the structure of the head, thorax, abdomen, siphon, and anal segment.

Results

Ae. aegypti larvae from 12 locations demonstrated high susceptibility to temephos at a concentration of 0.02 ppm, with a mortality rate reaching 100%. All specimens were classified as susceptible and exhibited significant morphological changes throughout their bodies. Morphological changes occurred in all tested larval specimens.

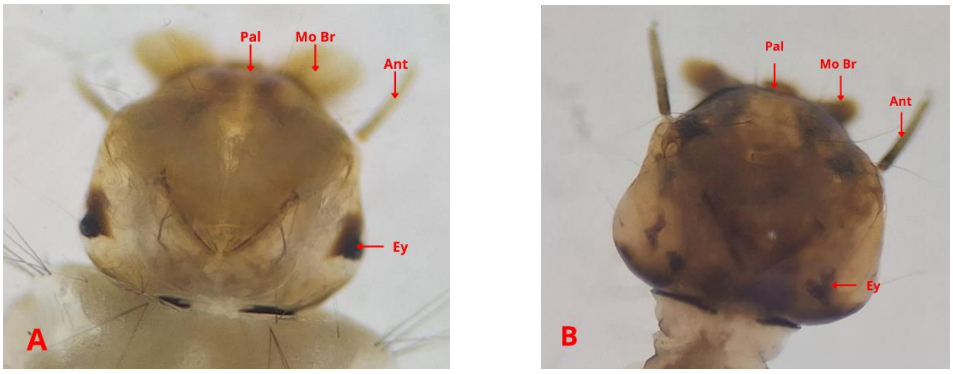
Morphological Changes in Ae. Aegypti Larvae

Head

Control larvae showed no morphological changes in the head. The palate, mouth brush, antennae, and eye structures appeared intact and normal (Figure 2A). Larvae exposed to 0.02 ppm temephos exhibited consistent morphological changes across all replicates. The main change observed was darkening of the head area, which appeared darker or blackish than normal. This darkening spread to other parts of the head, including the mouth brush, palate, and antennae. The larval eye structures appeared to merge with the surrounding tissue, losing their distinct visual boundaries. The larval head also appeared to have shrunk in size and lost its structural shape (Figure 2B).

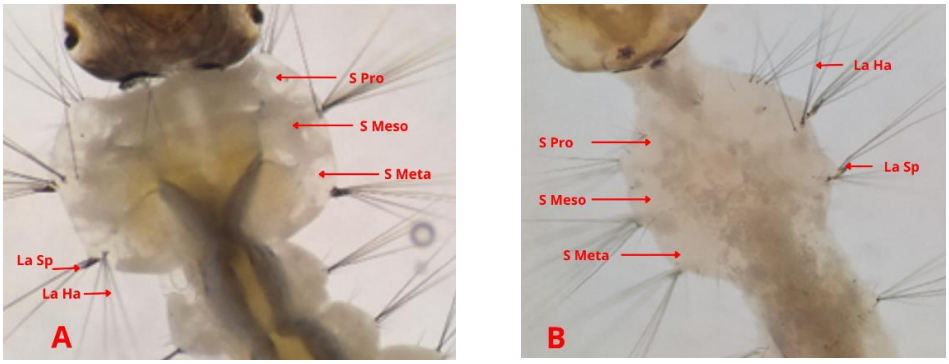
Thorax

The thorax morphology of control larvae consisted of prothorax, mesothorax, and metathorax segments, with clearly visible intersegment boundaries and erect lateral hairs and spines (Figure 3A). Post temefos-exposure showed clear and consistent changes in thoracic morphology across all replicates. The boundaries between thoracic segments were less distinct compared



Pal = Palatum, Mo Br= Mouth Brush, Ant= Antena, Ey= Ey

Figure 2. Head of a normal *Ae. Aegypti* larvae (A) and exposed to 0.02 ppm temephos for 24 hours (B)



S Pro= Segmen Prothoraks, S Meso= Segmen Mesothoraks, S Meta= Segmen Metathoraks, La Sp= Lateral Spine, La Ha= Lateral Hair

Figure 3. Thorax of normal *Ae. Aegypti* larvae (A) and exposed to 0.02 ppm temephos for 24 hours

to control larvae. The thoracic surface darkened in color, accompanied by black spots scattered throughout the thorax. Another change was a size reduction. The lateral hairs and spines on each thoracic segment remained erect, resembling those of normal larvae (Figure 3B).

Abdomen

The abdomen of control larvae showed clearly visible segments 1-8, with lateral hairs standing upright on each segment (Figure 4A). Larvae exposed to temephos exhibited consistent morphological changes across all replicates. The abdominal surface of the larvae appeared to darken throughout, with black spots appearing in several areas. The most extreme changes were observed in abdominal segments 4 and 5, which were darker than the rest of the body, particularly in the posterior midgut. Other morphological changes included a less flexible, stiffer, and curved body structure. The lateral hairs (setae) scattered along the abdominal segments showed no morphological changes, remaining upright as in normal larvae (Figure 4B).

Siphon and Anal Segment

Control larvae exhibited intact siphon structures and anal segments with anal papillae, as well as visible pecten and comb scales (Figure 5A). Larvae exposed to temephos exhibited consistent morphological changes across all replicates. The siphon structure darkened in color compared to normal conditions. The siphon appeared swollen, shortened, and stiffened. The anal papilla also underwent similar changes, with scattered black spots and swelling. The pecten and comb scales showed no morphological changes, remaining upright as in normal larvae (Figure 5B).

Discussion

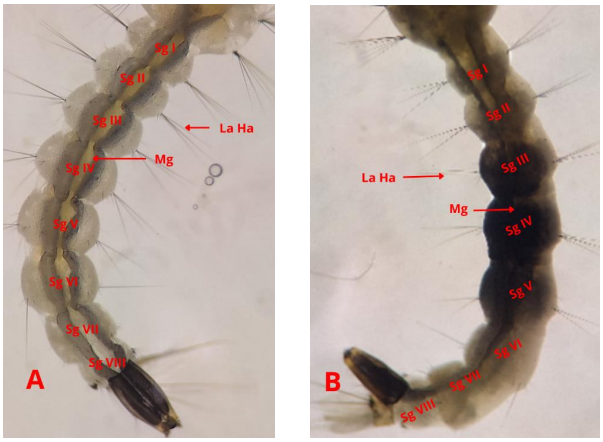
After exposure to 0.02 ppm temephos, *Ae. aegypti* larvae showed a 100% mortality rate. Deaths occurred at all observation points spread across Brebes Regency, Semarang City, and Jepara Regency. The 100% larval mortality rate indicates that the tested larval population is still in the highly susceptible category to 0.02 ppm temephos (35).

The 100% larval mortality rate demonstrates that temephos remains a highly effective insecticide for controlling the Aedes vector. Temephos is an organophosphate insecticide that works by inhibiting the enzyme acetylcholinesterase. Inhibition of the enzyme acetylcholinesterase causes the accumulation of signaling substances in the nerves and disrupts nerve function, ultimately leading to nerve failure, seizures, and even death in larvae (30).

Temephos exposure in *Ae. aegypti* larvae occur not only through cuticle penetration, but also through filter feeding, which utilizes lateral palatal brushes to generate and direct water currents and fine particles toward the mouth (36). After the insecticide compound enters the digestive tract, temephos is absorbed by the epithelial cells of the midgut, which is physiologically the main organ for digestion, nutrient absorption, and distribution of chemical molecules into the insect's hemolymph (37). Temephos, as a stomach poison, destroys midgut epithelial cells, causing cytoplasmic vacuolization and loss of microvilli, which disrupt nutrient absorption and ionic homeostasis (32).

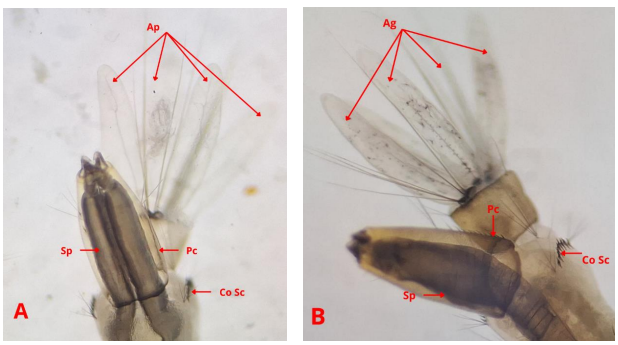
Morphological damage to the larvae's head, thorax, abdominal segments, siphon, and anal segments indicates physiological stress on the tissues. This confirms that temephos not only acts neurotoxically but can also trigger oxidative stress that impacts cell structure (32). At the cellular level, temephos works by inhibiting the enzyme acetylcholinesterase (AChE), which causes the accumulation of acetylcholine in nerve synapses, triggering muscle spasms, paralysis, and neuromuscular disorders (38).

Damage also impacts the larval respiratory system, such as the siphon, inhibiting oxygen diffusion and causing systemic hypoxia. In response to hypoxia, larvae activate the Hypoxia-Inducible Factor (HIF-1α) signaling pathway, a key transcriptional regulator in metabolic



Gambar. La Ha= Lateral Hair, Mg= Mid-gut, Sg I-VIII= Segmen I-VIII

Figure 4. Normal abdomen of *Ae. aegypti* larvae (A) and exposed to 0.02 ppm temephos for 24 hours (B)



Sp= Siphon, Pc= Pecten, Co Sc= Comb Scale, Ap= Anal Papillae

Figure 5. Siphon and anal segment of normal *Ae. aegypti* larvae (A) and those exposed to 0.02 ppm temephos for 24 hours (B)

adaptation to low-oxygen environments (39). Prolonged hypoxia can disrupt mitochondrial function, particularly in the inefficient electron transport chain. This condition leads to the accumulation of Reactive Oxygen Species (ROS) such as superoxide and hydrogen peroxide. When the amount of ROS exceeds the capacity of the larval antioxidant system, oxidative stress occurs, causing damage to membrane lipids, proteins, and DNA, and exacerbating cellular dysfunction (40).

Larvae activate the phenoloxidase enzyme as a defense mechanism. Activation of the phenoloxidase enzyme, which catalyzes melanin formation, triggers the melanization process, manifested by a darker abdominal color (41). Synergism between nervous system, metabolic, respiratory, and immune system disorders synergistically causes death at toxic temephos concentrations. Bioassay tests with larval mortality rates reaching nearly 100% indicate that larvae are still highly susceptible to temephos (42). The consistency of mortality and morphological damage across locations shows the absence of adaptive variation or resistance to temephos. These results support the use of temephos as an effective larvicide in controlling the *Ae. aegypti* vector.

Head Morphological Changes

The head of *Ae. aegypti* larvae contain central nervous systems, such as the supraesophageal ganglion and antennal lobe, which are responsible for sensory signal processing and initial motor control (43,44). Temephos, which inhibits the AChE enzyme, causes the accumulation of acetylcholine in the synaptic cleft and disrupts nerve signal transmission (45). This disruption triggers dysfunction in the larva's central and peripheral nervous systems, including the antennal structure, palate, and mouth brush, resulting in impaired sensory and motor function (46). This dysfunction also affects the antennal lobe, which processes olfactory signals, disrupting food orientation and responsive behavior to the environment. Disruption of neurotransmission in the subesophageal ganglion, which regulates mouth brush movement, leads to decreased efficiency of food and water particle uptake (43,44).

Temephos exposure causes consistent morphological changes in head structures across replicates. The primary change observed was darkening of the head tissue, including the mouth brush, palate, and antennae, indicating activation of a melanization response as a defense against tissue damage or physiological stress. This response involves the prophenoloxidase (pro-PO) pathway, which activates the active enzyme phenoloxidase, which catalyzes the formation of melanin pigment in damaged tissue (46). This enzyme converts tyrosine to dopaquinone, which then polymerizes to form melanin. This pigment accumulates in areas of tissue damage as part of the insect's immune response (47). However, no specific reports have been found describing head darkening due to melanization in *Ae. aegypti*.

Another morphological change is in the size of the larva's head. The head appears to shrink, and the eye structure merges with the surrounding tissue, indicating tissue damage resulting in loss of visual clarity. This is related to the neurotoxic effect of temephos, which works by inhibiting the enzyme acetylcholinesterase, thereby disrupting the transmission of nerve signals important for sensory and motor functions (48). This disruption impacts the larva's metabolic pathways, including oxidative phosphorylation, carbon metabolism, and amino acid biosynthesis, which are important pathways in energy production and the synthesis of cellular components. When these pathways are disrupted, cells experience a lack of energy, decreased protein synthesis, and impaired cell division. The impact is the excessive use of energy reserves to maintain essential functions, and tissue growth is hampered (49). This condition causes a shrinkage in the larva's body size, including the head, supported by reports of tissue damage such as wrinkles on the surface of the head cuticle and tissue damage, exposing the inner muscles (50). Loss of visual boundaries in the eyes has also been reported as a result of histological damage in larvae exposed to neem oil-based niosome compounds (51).

Thoracic Morphology Changes

The thorax of *Ae. aegypti* larvae include part of the ventral nerve cord (VNC), which is composed of thoracic ganglia and functions in controlling motor activity through longitudinal and transverse muscle contractions (52). Temephos exposure inhibits the enzyme acetylcholinesterase, causing impaired nerve impulse transmission, uncontrolled muscle contractions, and paralysis (45). Neuropeptides such as cardio-acceleratory peptide (CAPA) and allatotropin, expressed in the ventral nerve cord and thoracic ganglia of insects, regulate visceral muscle contraction, ionic fluid balance, and energy metabolism in insects, although specific data on *Ae. aegypti* larvae are limited (53). Nervous system disorders due to temephos exposure, including AChE inhibition and disruption of ionic homeostasis, are suspected to disrupt neurosecretory processes such as neuropeptide production or release. This can indirectly affect the metabolic balance and physiology of larvae (48). Morphologically, temephos exposure in *Ae. aegypti* larvae cause shrinkage, loss of prothorax, mesothorax, and metathorax segment boundaries, and the appearance of black spots on the thoracic surface. These changes are associated with disruption of the peripheral and motor nervous systems, particularly the VNC, which regulates segmental muscle function. This mechanism is similar to neuromuscular toxicity observed in mammals, where exposure to organophosphates that inhibit AChE

causes muscle necrosis and impaired neuromuscular coordination (54,55). Specific data on *Ae. aegypti* larvae are not yet available; it is suspected that temephos exposure can cause neuromuscular dysfunction, including disruption of segmental muscle tone and morphological changes caused by abnormal muscle contractions.

Oxidative stress can also exacerbate cellular structural damage due to an imbalance of reactive oxygen species, with the destruction of proteins, lipids, and DNA (56). This damage can also activate the insect's immune pathway through the melanization process (57,58). Metabolic disorders cause damage to thoracic morphology due to decreased protein synthesis and utilization of energy reserves, shown in studies using the natural insecticide honokiol (59) and the synthetic pesticide imidacloprid (60).

Abdominal Morphological Changes

The posterior abdominal segment of *Ae. aegypti* larvae, with their VNC, play a role in integrating the abdominal nervous system and the main digestive system, namely the mesenteron or midgut (52,61). The posterior mesenteron in segments 4 and 5 is responsible for digestion and nutrient absorption, and the primary site of damage from temephos exposure (32,61). Abdominal nervous system dysfunction induced by the accumulation of acetylcholine, resulting from AChE inhibition (45), can disrupt neuromuscular coordination, including visceral movements such as gut motility. This activity is controlled by branches of the VNC that extend to the posterior abdominal segments (52).

Temephos exposure is known to cause disorganization of midgut epithelial cells, loss of microvilli, and damage to cell membranes and cell nuclei (32). The formation of reactive oxygen species (ROS) causes oxidative stress that can damage cellular structures through lipid peroxidation, producing toxic compounds such as lipid hydroperoxide (LOOH) and trans-4-hydroxy-2-nonenal (HNE) (62). This damage triggers the activation of the phenoloxidase pathway and the release of melanin in the damaged area, mediated by peroxinectin in the adhesion and encapsulation processes (58). Darkening in segments 4 and 5 is an indicator of an immune response, melanization activated by the prophenoloxidase system as a form of defense against tissue damage (58,63).

These metabolic disturbances and oxidative stress also have the potential to disrupt cellular respiration, which can trigger local hypoxia. This is characterized by activation of the transcription factor HIF-1α and mitochondrial damage (64–66), although this has not been widely reported in the abdominal segment specifically.

Siphon and Anal Segment Morphological Changes

The siphon in Aedes larvae functions as the primary respiratory organ, connected to the posterior spiracles and internal tracheal system. Air from the environment is channeled through the spiracles into the tracheal system to support respiration (67). The neuromuscular structure of larvae is highly susceptible to disruption caused by exposure to organophosphate insecticides such as temephos. The toxic mechanism involves inhibition of the enzyme acetylcholinesterase (AChE), causing accumulation of acetylcholine in synapses, leading to excessive muscle stimulation, repetitive contractions, and muscle cell damage or death (45). Consequently, larvae exhibit stiffness in the siphon, tissue swelling, and darkening of the anal papillae as indicators of morphological damage. These changes have also been reported in larvae exposed to Lansium domesticum extract, which exhibit similar morphological changes (68).

There are no specific reports regarding tracheal structural disruption due to temephos exposure. Dysfunction in the larval respiratory system can generally reduce the efficiency of gas exchange. This condition has the potential to cause local hypoxia, which is responded to by the activation of HIF-1α and the oxidative stress metabolic pathway, as observed in other insects (64,65). The anal papilla also plays an important role in larval osmoregulation, particularly in ion transport and xenobiotic detoxification (69). Temephos exposure can damage the anal papilla tissue, which impacts the ionic balance and osmotic pressure of the larvae (62). As part of the immune response, activation of the phenoloxidase pathway triggers the melanization process characterized by the appearance of black spots on the anal segment, as a form of defense against tissue damage and oxidative stress (70-73).

Conclusion

Ae. aegypti larvae from Brebes Regency, Jepara Regency, and Semarang City were still highly susceptible to temephos at a concentration of 0.02 ppm. Morphological changes in larvae exposed to temephos included darkening of the head, shrinkage in size, and loss of visual boundaries of the eyes, a shrunken thorax, disappearance of segment boundaries and the appearance of black spots, a darkened abdomen, especially in segments 4–5, stiffness and a curved body posture, and swelling and black spots on the siphon and anal segments. These findings indicate structural and physiological disorders reflecting tissue damage and activation of the larval immune response due to insecticide exposure.

Recommendations

Temefos is still highly recommended as an effective vector control insecticide at the three research sites. Susceptibility monitoring status should continue to be carried out to detect the potential emergence of resistance in the future.

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Methodology: Sayono Sayono, Didik Sumanto, Risyandi Anwar.
Project administration: Maulidya Endah Dwi Cahyani.
Resources: Risyandi Anwar.
Software: Sayono Sayono.
Supervision: Didik Sumanto.
Validation: Didik Sumanto.
Visualization: Maulidya Endah Dwi Cahyani.
Writing–original draft: Didik Sumanto.
Writing–review & editing: Didik Sumanto.

Competing Interests
The authors declare that there is no conflict of interest.

Ethical Approval
Ethical acceptance was taken from the Health Research Ethics Commission of the Faculty of Public Health, Universitas Muhammadiyah Semarang, No. 0038/KEPK-FKM/UNIMUS/2025.

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