Effect of *Curcuma longa* rhizome extract and curcumin against laboratory-reared *Aedes Aegypti* (Diptera: Culicidae) larvae: Alterations of cell ultrastructure and immunoreactivity of octopamine and tyramine in the midgut

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Abstract

Plant bioactive compounds, as an alternative insecticide, play an essential role in mosquito control. The purpose of this study was to evaluate the effect of *Curcuma longa* rhizome extract and curcumin against *Aedes aegypti* larvae, focusing on changes in the cell ultrastructure and immunoreactivity of octopamine (OA) and tyramine (TA) in the midgut. The larval bioassay was used following WHO protocols. *Ae. Aegypti* 3rd - 4th instars larvae collected from the laboratory were exposed to both different concentrations of *C. longa* rhizome extract and curcumin. Ultrastructural changes in the midgut cells were tested by transmission electron microscope (TEM). OA and TA in the midgut were detected by the immunohistochemical method. At 24 h, curcumin killed 100% of *Ae. Aegypti* larvae at a concentration of 4 ppm. The LC50 values of curcumin and the rhizome extract were 1.522 and 4.074 ppm respectively. Curcumin caused ultrastructural changes in the larval midgut; damaged cells, epithelial cells microvilli, cell membranes, nucleus, mitochondria, and other cell organelles. Curcumin and the rhizome extract decreased the immunoreactivity of OA and TA in the larval midgut. Curcumin showed significant larvicidal activity against *Ae. aegypti* larvae mediated by damaged cells and decreased immunoreactivity of OA and TA in the midgut.

Keywords: Plasmodia is, early childhood, Nigeria

Introduction

*Aedes aegypti*, known as a dengue virus vector, transmits mosquito-borne diseases such as yellow fever, dengue fever, chikungunya, and Zika fever [1, 2]. Dengue hemorrhagic fever (DHF) caused by the dengue virus, as a dangerous arbovirus, is still a global health problem. According to the WHO, the actual number of dengue cases are underreported and 3.9 billion people, representing half of the global population, are estimated at risk of infection [3-5]. In addition, the ZIKA virus caused newborn microcephaly and Guillain-Bareé syndrome is still a public health problem in Brazil and around the world [6].

Synthetic insecticides are frequently used to control mosquitoes leading to negative impacts on the environment, human health, and non-target organisms, and many mosquito species develop into to be insecticide-resistant mosquitoes [7, 8]. Recently, *Ae. Aegypti* has been reported that it developed into to be a resistant mosquito to synthetic insecticides in many DHF endemic countries including Indonesia [9, 10]. Thus, alternative insecticides, natural products, are needed to combat insecticide-resistant mosquitoes.

Previous studies reported that natural products have a wide variety of bioactive compounds such as alkaloids, flavonoids, phenol, and terpenoids [11-13]. These bioactive compounds can kill various stages of the mosquito such as egg, larval, pupal, and adult stages. Additionally, advantages of the plant bioactive compound are that they do not pollute the environment, are not toxic to non-target organisms, and prevent mosquitoes to be resistant to insecticides [14]. Curcumin, a polyphenolic yellow compound, is the main compound of turmeric rhizome
2.3. Collection of *Ae. Aegypti* 3rd – 4th instars larvae

*Ae. Aegypti* eggs were obtained from the entomology laboratory of the Department of Parasitology, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, West Java, Indonesia. The present study used egg hatching protocols as previously described. Briefly, a cup (500 ml) was filled with 375 mL water and added 5 ml liver powder slurry. A egging paper containing approximately 300-1000 embryos was cut and put into the cup. After 1-2 days, larvae were transferred with a pipette to a larger rearing pan. Only 3rd – 4th instars larvae were used for the larval bioassay [19]. Hatching of *Ae. Aegypti* eggs was conducted in the entomology laboratory of the Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia.

2.4. Larval Bioassay

A larval bioassay was conducted as described previously by the WHO [20]. The bioassay used 3rd - 4th instars larvae of *Ae. Aegypti*. A total of 25 larvae were introduced into 250 ml of curcumin with concentrations of 0.25, 0.5, 1, and 4 ppm in a 300 mL paper cup. Replication of the larval bioassay was done 4 times. For *C. longa* rhizome extract (0.25, 0.5, 1, and 4 ppm), *Ae. Aegypti* larval bioassay was carried out in the same way as curcumin. In the control group, 25 *Ae. Aegypti* larvae were put into a paper cup (200 ml in volume) containing tap water. The larval bioassay was conducted within 24 h only.

2.5. Transmission Electron Microscopy (TEM)

The present study used only *Ae. Aegypti* larvae exposed to curcumin to examine TEM. The samples were processed according to Ma et al. [18] with a slight modification of the fixation liquid. The whole bodies of the treated *Ae. Aegypti* larvae were pre-fixed in 2.5% glutaraldehyde at 4°C for a minimum of 2 days and then washed three times with cacodylate buffer for 15 min each time. The samples were fixed in 2% osmium tetroxide and 2.5% K3Fe (CN)6 in the buffer for 2 h, and then rinsed in cacodylate buffer as described in the previous step. The samples were then dehydrated in an ethanol series in ascending order (30%, 50%, 70%, 80%, 90%, and 100%) for 30 min each. The samples were embedded in Spurr resin. The prepared samples were cut using an ultramicrotome (Leica UC6, Wetzlar, Germany) and observed using TEM (JEOL JEM 1010, Japan).

2.6. OA and TA immunohistochemical staining

An immunohistochemical (IHC) technique was conducted as previously described by Ramos-Vara et al. [21]. The IHC staining procedure was performed using diagnostic system kits (Abnova, PAB14697 and Cloud-Clone Corp., PAG048GE01). Briefly, deparaffinization was carried out using xylene 1 and xylene 2 (5 min each), and rehydration were carried out with 100%, 96%, and 80% alcohol followed by rinsing with distilled water. Next, endogenous peroxidase was quenched with 0.3% H2O2 in methanol followed by a tap water wash. The sections were then heated with Tris-EDTA buffer (pH 9.0) antigen retrieval using Retrieval Generation 1 (RG1, BIO GEAR, BGRG-0118) for 15 min, chilled at room temperature.

The rhizome extract solution was made with 4 concentrations, 0.25, 0.5, 1, and 4 ppm.
temperature (15 min), and embedded in PBS solution (3 min). Then, nonspecific binding sites were blocked with a background blocker for 5 min. For trypan blue, the sections were incubated with the primary antibody Polyclonal Antibody (Abnova, PAB14697) at 1:1000 overnight at 4EC. For octopamine, the sections were incubated with the primary antibody Polyclonal Antibody to Octopamine (Cloud-Clone Corp., PAG048Ge01) at 1:50 overnight at 4EC and then washed with PBS solution. The sections were then incubated with the secondary antibody PolyVue Plus Mouse/Rabbit Enhancer (ten minutes) at room temperature and washed with PBS solution. Next, the sections were incubated with PolyVue Plus HRP Label (10 min) at room temperature and washed with PBS solution. The sections were treated with chromogen substrate and one drop of DAB mixed with 1 mL of DAB buffer, washed with distilled water, treated with hematoxylin, washed with distilled water (3 min), and then treated with one drop of bluing reagent (10 sec). Next, the sections were dehydrated with 80%, 96%, and 100% alcohol and treated with xylene 1 and xylene 2 for clearing. Finally, the sections were washed with distilled water (3 min), and then treated with one drop of bluing reagent (10 sec). Next, the sections were incubated with PolyVue Enhancer (ten minutes) at room temperature and washed with PBS solution. The sections were then incubated with the primary antibody anti-Octopamine (Abnova, PAB14697) at 1:50 overnight at 4EC and then washed with PBS solution. The sections were then incubated with the secondary antibody PolyVue Plus Mouse/Rabbit Enhancer (ten minutes) at room temperature and washed with PBS solution. The sections were then incubated with the secondary antibody PolyVue Plus HRP Label (10 min) at room temperature and washed with PBS solution. The sections were treated with chromogen substrate and one drop of DAB mixed with 1 mL of DAB buffer, washed with distilled water, treated with hematoxylin, washed with distilled water (3 min), and then treated with one drop of bluing reagent (10 sec). Next, the sections were dehydrated with 80%, 96%, and 100% alcohol and treated with xylene 1 and xylene 2 for clearing. Finally, the sections were embedded in Entellan (Merck, 1.07961.0500) under glass coverslips.

2.7. Data analysis

Data analysis used statistical package for social sciences (SPSS) ver.26. Data on the mortality rate of the dead larvae of *Ae. Aegypti* exposed to curcumin and *C. longa* rhizome extract were tested for normal distribution (Shapiro-Wilk test). Data with normal distribution were tested by analysis of variance (one-way ANOVA test), while data with non-normal distribution were tested by the Kruskal-Wallis H test.

### Table 1: The mortality rate of *Ae. Aegypti* larvae after treatment with curcumin and *C. longa* rhizome extract.

| Treatment         | Concentration (ppm) | n  | Mortality rate (24 h) Dead larvae | %   |
|-------------------|---------------------|----|----------------------------------|-----|
| Curcumin *        | 0.25                | 100| 3                                | 3   |
|                   | 0.5                 | 100| 10                               | 10  |
|                   | 1                   | 100| 30                               | 30  |
| C. longa rhizome  | 4                   | 100| 100                              | 100 |
| Extract **        | 0.25                | 100| 0                                | 0   |
|                   | 0.5                 | 100| 3                                | 3   |
|                   | 1                   | 100| 8                                | 8   |
|                   | 4                   | 100| 13                               | 13  |

*one-way ANOVA test, p-value = 0.000

**Kruskal-Wallis H test, p-value= 0.000

The LC\(_{50}\) and LC\(_{90}\) values of curcumin were 1.522 ppm (95% CI = 1,121 - 1,994 ppm) and 4.074 ppm (95% CI = 2,949 – 7,110 ppm) respectively. However, the LC\(_{50}\) and LC\(_{90}\) values of the rhizome extract were higher than curcumin. The LC\(_{50}\) and LC\(_{90}\) values of the rhizome extract were 48.512 ppm (95% CI = 10.415 – 994.100 ppm) and 1028.575 ppm (95% CI = 64.920-4102.918 ppm) respectively (Table 2 and Table 3).

### Table 2: The LC\(_{50}\) value of curcumin and *C. longa* rhizome extract.

| Treatment           | LC\(_{50}\) (ppm) | 95% CI          | Parameter estimates | Chi-square test |
|---------------------|-------------------|-----------------|---------------------|-----------------|
| Curcumin            | 1,522             | 1,121 - 1,994   | 0,335 8,959 0      | 26,976 13 0,013 |
| C. longa rhizome    | 48,512            | 40,415 – 994,100| 0,345 2.8 0,005    | 19,801 13 0,1   |

Figure 1 shows the relationship between curcumin concentration and the mortality rate of *Ae. aegypti* observed at 24 h after treatment. Based on the probit regression analysis, a linear R\(^2\) value of 0.863 was obtained with a p-value of 0.000 which indicated that there was a strong and positive (+) correlation between the concentration of curcumin and the mortality rate of larvae after 24 h of treatment. In this study, the probit regression model showed that the independent...
variable curcumin concentration was strongly correlated with the mortality rate of *Ae. aegypti* larvae.

![Figure 1: The relationship between curcumin concentration and mortality rate of *Ae. aegypti* larvae. Log of Cum Con = curcumin concentration.](image)

Figure 2 shows the relationship between the rhizome extract concentrations of *C. longa* with larval mortality of *Ae. aegypti* observed at 24 h after treatment. Based on the probit regression test, the value of $R^2$ is obtained linearly by 0.043 with a p-value of 0.005 which indicates that there is a positive (+) correlation between *C. longa* crude extract concentration with larval mortality after 24 h treatment. The probit regression model of *C. longa* rhizome extract showed that the independent variable concentration of rhizome extract *C. longa* was weakly correlated with the mortality rate of *Ae. aegypti*.

![Figure 2: The relationship between *C. longa* rhizome extract concentration and mortality rate of *Ae. aegypti* larvae. Log of Crude_Con = *C. longa* rhizome extract.](image)

The present study found that the neurotransmitters, OA and TA, were detectable in the control and treatment groups in the midgut of *Ae. aegypti* larvae. These neurotransmitters were detected in the midgut epithelial cell membrane, microvilli, peritrophic membrane, and food bolus (Table 4). The control group showed strong immunoreactivity for OA and TA as seen in Figure 3. OA showed dark brown spots in the cell of the epithelial layer, while TA showed light brown spots in the cell of the epithelial layer. However, in the curcumin group, both OA and TA were difficult to detect in the midgut of *Ae. aegypti* larvae. The results of the immunohistochemical examination showed a fairly low immunoreactivity in epithelial cells. In contrast to the food bolus, these neurotransmitters can still be detected (Table 4).
To reduce the incidence of dengue cases, the control of *Ae. aegypti* for a long period time. [27,28] Hamid et al. [10] reported *Ae. aegypti* was associated with mutations in the *V1016G* gene. In Nepal, *F1534C* and *V1016G* genes were found in *Ae. aegypti* which is resistant to pyrethroids (d-allethrin), in New Guinea, *F1534C* and *1016I* genes, and Peru *kdr* gene was found, Burkina Faso (West Africa) found the *F1534C* and *1016I* *kdr* genes, and Peru found the *V1016I* and *F1534C* genes [27,28]. This study proved that at 24 h, curcumin with a concentration of 4 ppm showed 100% of the mortality rate of *Ae. aegypti* larvae, while at a concentration of 0.25 – 3 ppm the mortality rate was 3.0% – 30.0%. In contrast to the turmeric rhizome extract of *C. longa*, the mortality rate ranged from 0% to 13%. Thus, curcumin showed higher larvicidal activity against *Ae. aegypti* larvae compared with the rhizome extract of *C. longa*. Mezzacappo et al. [29] reported that curcumin showed 71.3% of the mortality rate for *Ae. Aegypti* larvae. The findings of this study are under the research of de Souza et al. [13].

**Table 4:** The immunoreactivity of OA and TA in the midgut of *Ae. Aegypti* larvae.

| Treatment                  | Octopamine (OA) | Tyramine (TA) |
|----------------------------|-----------------|---------------|
|                            | CE | Mv | PM | FB | CE | Mv | PM | FB |
| Control                    | ++ | ++ | ++ | +++| +++| ++ | ++ | +++|
| Curcumin                   | +  | -  | +  | +  | +  | -  | +  | +  |
| *C. longa* rhizome extract | +  | -  | +  | +  | +  | -  | +  | +  |

**CE**= cell of the epithelial layer, **Mv**= microvilli, **PM**= peritrophic membrane, **FB**= food bolus. +++= strong, ++= moderate, + = weak, negatif (-) = no

TEM observations showed that curcumin damaged midgut epithelial cells of *Ae. Aegypti* larvae. This is indicated by the shape of the midgut epithelial cells *Ae. aegypti* irregular. The TEM image also does not show organelles such as mitochondria which can be seen in normal midgut epithelial cells. The endoplasmic reticulum is not as clearly visible as in normal cells. The microvilli of the midgut epithelial cells appear to be atrophic. The morphology of the cell nucleus appears irregular with the lysis of the nuclear wall. Chromatin threads are irregular in shape. In addition, the daughter nucleus (nucleolus) could not be seen clearly. The cell membrane of the epithelium is irregular (Figure 4).

**Figure 4:** Changes in the ultrastructural epithelial cell of the midgut larvae of *Ae. Aegypti* after treatment with curcumin. **N** = nucleus, **Mv** = microvilli.

### 4. Discussion

To reduce the incidence of dengue cases, the control of *Ae. aegypti* is the best method at present. [26] Control of *Ae. aegypti* using synthetic insecticides from the pyrethroid and organophosphate groups is a very common method in various countries. However, the use of these insecticides causes various problems such as environmental pollution and insecticide-resistant *Ae. aegypti* due to the frequent use of insecticides for a long period time. [27,28] Hamid et al. [10] reported *Ae. aegypti* have developed into resistance to pyrethroid insecticides in Jakarta. Insecticide-resistant *Ae. Aegypti* was associated with mutations in the *V1016G* gene. In Nepal, F1534C and V1016G genes were found in *Ae. aegypti* which is resistant to pyrethroids (d-allethrin), in New Mexico the F1534C gene was found, Burkina Faso (West Africa) found the F1534C and 1016I *kdr* genes, and Peru found the V1016I and F1534C genes [27,28]. This study proved that at 24 h, curcumin with a concentration of 4 ppm showed 100% of the mortality rate of *Ae. aegypti* larvae, while at a concentration of 0.25 – 3 ppm the mortality rate was 3.0% – 30.0%. In contrast to the turmeric rhizome extract of *C. longa*, the mortality rate ranged from 0% to 13%. Thus, curcumin showed higher larvicidal activity against *Ae. Aegypti* larvae compared with the rhizome extract of *C. longa*. Mezzacappo et al. [29] reported that curcumin showed 71.3% of the mortality rate for *Ae. Aegypti* larvae. The findings of this study are under the research of de Souza et al. [13].

**Fig 3:** OA and TA were detected in the midgut of *Ae. Aegypti* larvae. The control group (A) and the curcumin group (B).

**Table 4:** The immunoreactivity of OA and TA in the midgut of *Ae. Aegypti* larvae.

| Treatment                  | Octopamine (OA) | Tyramine (TA) |
|----------------------------|-----------------|---------------|
|                            | CE | Mv | PM | FB | CE | Mv | PM | FB |
| Control                    | ++ | ++ | ++ | +++| +++| ++ | ++ | +++|
| Curcumin                   | +  | -  | +  | +  | +  | -  | +  | +  |
| *C. longa* rhizome extract | +  | -  | +  | +  | +  | -  | +  | +  |

**CE**= cell of the epithelial layer, **Mv**= microvilli, **PM**= peritrophic membrane, **FB**= food bolus. +++= strong, ++= moderate, + = weak, negatif (-) = no
investigated further. The results of the probit regression analysis obtained the value of R² of 0.863 with p-value = 0.000 and p < 0.05. This means that there is a strong and positive (+) correlation between the concentration of curcumin (independent variable) with larval mortality rate (dependent variable). Additionally, the correlation between the independent and dependent variables is significant. Explanation, in short, the probit regression correlation between the independent and dependent variables (extract concentration) with the dependent variable is significant. Explanation, in short, the probit regression correlation between the independent and dependent variable mortality rate (dependent variable). Additionally, the correlation between independent and dependent variables will also increase. Statistically, the results of this study are significant which means that the treatment with concentrations of curcumin (0.25 - 4 ppm) has been proven to kill the larvae of *Ae. aegypti*. The present study showed that curcumin damaged the midgut epithelial cells of *Ae. aegypti* larvae observed by TEM (Figure 4). The shape of the midgut epithelial cells *Ae. aegypti* was irregular. Cell organelles such as mitochondria cannot be found in the midgut epithelial cells. The endoplasmic reticulum is not as clearly visible as in normal cells. The microvilli of the midgut epithelial cells appear to be atrophic. The morphology of the cell nucleus appears irregular with the lysis of the nuclear wall. In addition, the daughter nucleus (nucleolus) could not be seen clearly. The cell membrane of the epithelium is irregular (Figure 4). The findings supported a previous report who reported synergistic effects of botanical curcumin-induced programmed cell death on the management of *Spodoptera litura* Fabricius with avermectin [33].

The mechanisms of the damaged midgut of *Ae. Aegypti* larvae are mediated by oxidative stress. Zhang et al. [28] reported damage to the midgut epithelium of *Ae. aegypti* due to oxidative stress caused by α-terthienyl substances. As a result, there is damage to the nuclear membrane, condensed nuclear chromatin, and some of the chromatin is on the periphery and around the nuclear membrane. In addition, nuclear pyknosis was also found which is a characteristic of cells undergoing apoptosis and necrosis. [31] Other researchers, Wang et al. [33] in China reported that honokiol extract from the seeds of *Magnolia denudate* can damage the nuclear membrane of the midgut epithelial cells in *Ae. aegypti* and also found destroyed cellular components of the midgut and various organelle debris in the cytoplasm. Additionally, reactive oxygen species (ROS) can destroy the midgut of *Ae.aegypti* larvae. Kovacic et al. [16] revealed that the phenol group of curcumin plays an important role in inducing ROS. Curcumin is a bioactive compound that has a phenyl group such as capsaicin and gingerol compounds. These bioactive substances cause an increase in electron transfer ability. It has an important role in physiological responses, but in excessive amounts, it can result in a redox cycle. The redox cycle causes oxidative stress resulting in damage to cells. Zhang et al. [34] revealed that mitochondria are the main source of ROS and the main target of ROS attack. Increased ROS will induce a permeability transition that will damage the mitochondrial structure. This damage will cause mitochondrial dysfunction which can lead to mitochondrial destruction and release more ROS into the cytoplasm. Additionally, mitochondrial damage releases several apoptotic factors into the cytoplasm that cause programmed cell death. At low concentrations, various DNA strands that encode the process of apoptosis were found. However, after being given higher concentrations, it was found that apoptosis was inhibited and ATG gene expression peaked, indicating that autophagy was induced. Previous studies have shown that autophagy is required in the early stages of cell necrosis, and the process contributes to cell destruction during necrosis and supports the spread of necrotic cells. The findings are in line with electron microscopy findings that show cell destruction. Therefore, it can be concluded that at high ROS concentrations, autophagy is induced which results in inhibition of apoptosis, organelle damage, promotion of necrosis, and accelerated cell death. High levels of ROS also result in the accumulation of damaged mitochondria. In addition, large amounts of Ca²⁺ are excreted into the cytoplasm and cause cell necrosis.[31] Curcumin also has an effect on the octopaminergic system in *Ae. Aegypti* larvae. OA is a protein found in high concentrations in various insect tissues with TA as its precursor which is the main constituent of the octopaminergic system. OA acts as a neurotransmitter, neurohormone, and neuromodulator in insects.[17] The octopaminergic system in insects that play a role in various physiological activities of insects is often used as a target for insecticides with a minimum non-target effect. [36] In this study, the administration of curcumin decreased the immunoreactivity of OA and TA in the midgut larvae of *Ae. aegypti* due to oxidative stress induced by these substances. In the control group, *Ae. aegypti* found stronger immunoreactivity to OA and TA compared to the treatment group with curcumin and the rhizome extract. These findings suggest that curcumin targets OA and TA in the midgut larvae of *Ae. aegypti*. Mossa et al. [37] explained that essential oils can interfere with various physiological processes of insects by disrupting the insect's nervous system. Plant essential oils are known to interfere with GABA chloride channels thereby inducing toxicity and alterations in insect physiology. In addition, essential oils also have neurotoxic mechanisms by inhibiting acetylcholinesterase (ACHE) or by blocking OA receptors. [38] Jankowska et al. [39] in Poland revealed that the insect octopaminergic system is one of the potential targets for the larvicidal activity of plant essential oil extracts such as cinnamic alcohol. OA has receptors located on cell membranes and belongs to the family of G protein-coupled receptors which together with their ligands are involved in the control of intracellular calcium concentrations and inactivation or inhibition of adenylate cyclase. [37] Therefore, curcumin can exert a similar effect by targeting OA and TA in the midgut of *Ae. aegypti*. The role of curcumin in the treatment of several diseases in humans. Vollono et al. [40] reported that curcumin is effective in treating various skin diseases such as atopic dermatitis, skin cancer, and skin aging. Tomeh et al. [41] revealed that curcumin also has anti-cancer activity. It was recently reported that curcumin in combination with nanoparticles can be used to treat various disorders of the central nervous system such as Alzheimer's disease, Huntington's disease, multiple sclerosis, epilepsy, and Amyotrophic Lateral Sclerosis [41]. Thus, in the future, it is hoped that curcumin can be used as a new drug to cure various diseases in humans. This study has limitations, namely only *Ae. Aegypti* larvae were studied while female mosquitoes of *Ae. Aegypti* could
not cover in the study. Ae. Aegypti female mosquitoes are very important to transmit the dengue virus and to support curcumin that has a potential alternative insecticide. It is necessary to examine the activity of detoxifying enzymes and antioxidants carried out in research to explain the mechanism of larvicidal compounds in more detailed curcumin. In addition, the study employed the reference of Fiaz et al. [42] who reported that the larvae of Ae. Aegypti as the control group showed no changes in the ultrastructural midgut examined by the TEM method.

5. Conclusion
Curcumin showed higher larvicidal activity against Ae. Aegypti larvae compared to the rhizome extract of C. longa. Based on the TEM findings, curcumin damaged the epithelial cells of the midgut; the membrane of the cell and nucleus ruptured, damaged microvilli and unclear other cell organelles. Both the rhizome extract of C. longa and curcumin caused decreased immunoreactivity of OA and TA in the midgut. Our results suggested that curcumin showed a potential alternative insecticide to control the Ae. aegypti population.

6. Conflict of interest
The author declares that there is no conflict of interest.

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