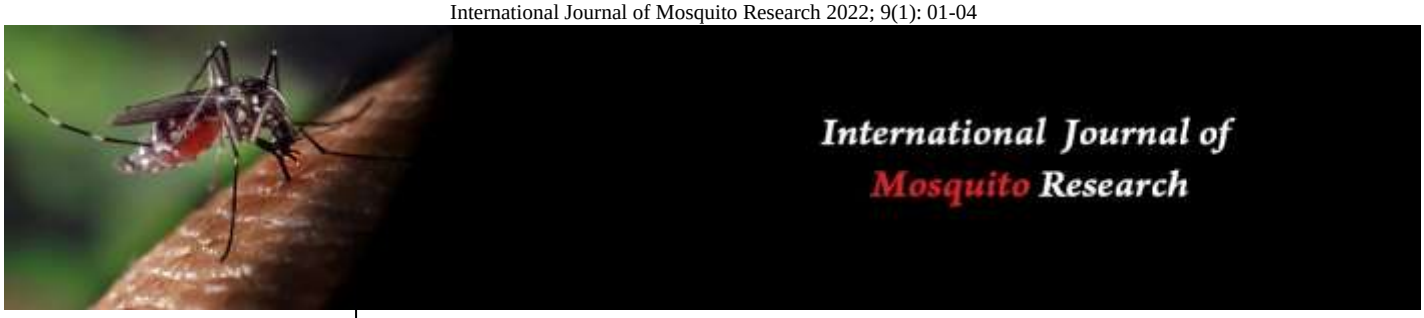


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# Larvicidal and pupicidal effect of Methyl triphenylacetate on larvae of *Aedes aegypti* (Linnaeus, 1762)

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**Abstract**
Synthetic insecticides are the main method of controlling vectors, but they haven't worked very well because the bugs have become resistant to them. Based on this, insecticides made from plants seem like a good choice for both man and the environment. Thus, the goal of the present work was to evaluate the effect of methyl triphenyl acetate (1-Propanone, 3-(2-hydroxyphenyl)-1,3-diphenyl)on 4$^{th}$ instar larvae of *Aedes aegypti* by larvicidal, pupicidal and histopathological assays. The larvae and pupae were exposed to methyl triphenylacetate (MTA) at different concentrations viz. 3, 6, 9, 12 and 15 ppm for 72 hours. The larval $LC_{50}$ and $LC_{90}$ mortality rate was 4.68 ppm while $LC_{90}$ values were 12.483 ppm; the pupa $LC_{50}$ and $LC_{90}$ mortality was 5.182 ppm, and $LC_{90}$ value was 13.180 ppm MTA, respectively. Further, the histopathological analysis revealed that MTA treated larvae showed damaged midgut than control. The present findings presented that the MTA possess suitable insecticidal property against *Aedes aegypti.*

**Keywords:** Methyl triphenyl acetate, *Aedes aegypti,* Larvicidal, pupicidal and histology

## 1. Introduction
Mosquitoes are vectors for many life-threatening diseases [1]. Mosquito species are found all over the world. One of them is *Aedes aegypti*. It spreads diseases like dengue and Zika and causes many people to die and have serious health problems all over the world [2]. Despite the fact that these viral diseases can be very dangerous, there aren't any good treatments or ways to stop them from spreading. The main thing that has been done to get rid of these diseases has been to get rid of the vectors that spread them [3]. Due to their small size (like small pools and puddles), researchers have been looking for drugs to control mosquito populations. Mosquitoes in both their embryonic and larval stages have also been a top target [4].
Currently, the majority of larvicides used in vector management are synthetic insecticides, including as growth inhibitors such as diflubenzuron and methoprene. However, due to widespread resistance among mosquitoes and the detrimental effect they have on non-target creatures and the environment, bio-larvicides have become the preferred alternative [5]. Additionally, the widespread use of insecticides has resulted in the establishment of insecticide-resistant vector populations, most notably *Aedes aegypti*. As a result, it is critical to develop natural insecticides with a novel mode of action for vector control [4, 6].
Since ancient times, active toxic compounds derived from plant secondary metabolites have been used as an alternate mosquito control approach. These are non-toxic, readily available at reasonable rates, biodegradable, and exhibit broad-spectrum activity against a variety of vector mosquito species [7]. The methyl triphenyl acetate (1-Propanone, 3-(2-hydroxyphenyl)-1,3-diphenyl) is a secondary-metabolite in *Artemisia argyi,* which is showing insecticidal activity against *cabbage aphid*s. The compound had been previously reported in ethanolic extract of the *A. argyi* that had been previously reported [8]. In our previous *in silico* study, we confirmed that 1-propanone, 3-(2-hydroxyphenyl)-1,3-diphenyl act as sterol carrier protein inhibitors [9]. Thus, the incumbent work seeks to identify the insecticidal phytocompound among the 21 phytocompounds selected by conducting in silico docking experiments and analysing their inhibitory activities for the sterol carrier protein (AeSCP-2). Among all the compounds evaluated against carrier sterol protein (PDB ID: 1PZ4), methyl triphenylacetate had the lowest docking energy, i.e., -13.7362 Kcal/Mol.

Thus, methyl triphenyl acetate had the lowest binding energy score of all the substances. It interacted with the active site region via one hydrogen bond, one pi-anion, and nine pi-alkyl interactions. The goal of this study was to see how methyl triphenyl acetate affected the larvae of the *Aedes aegypti* mosquito.

## 2. Materials and methods

### 2.1 Chemicals

Methyl triphenylacetate was purchased from ALFA Chemistry (Smithtown Avenue, Ronkonkoma, USA). All other chemicals, as well as the reagents used, were of analytical grade and were purchased from Merck, Himedia, Mumbai, India.

### 2.2 Maintenance of Eggs and Larvae

*Aedes aegypti* eggs were collected from VCRC (Vector Control Research Centre) at Puducherry and reared at the Department of Zoology, Vector Control Laboratory, Annamalai University. The eggs were then incubated in seasoned water for 24 hours before hatching. The hatching process was initiated with 0.2 g of larval feeding (food ratio; 3:1 of yeast and biscuit). The eggs were kept at room temperature (28+2) $^0$C, 70-85% relative humidity, and a photo cycle of 14h light, 10h dark [10].

### 2.3 Larvicidal and pupicidal activity

The larvicidal activity was carried out in accordance with World Health Organization norms, with certain changes [11]. A stock solution with an initial concentration of 10000 ppm was created. Various concentrations of the chemical were generated in triplicate from the stock solution, namely 3, 6, 9, 12 and 15 ppm with distilled water. For the larvicidal assay, a total of 25 early IV instars (in a 250-ml cup with 200 ml of water) were utilised for each concentration, with five replicates maintained for each concentration. During the exposure period, the larvae were not given any food. At varying exposure times of 72 hours, the % mortality was computed. All of the mosquito larvae that were afflicted were deemed dead. As a control, the same concentration of solvents (distilled water) that was used to make the stock solution was employed. All bioassays were carried out at room temperature (28+2)$^0$C. The mortality of each larvae was measured after 72 hours of exposure to the drug (Methyl triphenyl acetate).

### 2.4 Histopathology

They were put in a solution of formaldehyde and kept there for 24 hours at room temperature. The larvae sample was then dehydrated in a series of different alcohols before it was put into paraffin. To get a midgut section, the larvae were cut with a microtome at a thickness of 4 m. A light microscope was used to look at sections that had been stained with HE. Use a light microscope with a 40X magnification to check the glass slides to see if they were out of place [12].

### 2.5 Statistical Analysis

The following statistical methods were utilised in this study: The Arithmetic Mean was used to determine the average number of dead mosquito larvae, the significant difference in mosquito larval mortality between the control and experimental groups, and the Probit Analysis was used to calculate the $LC_{50}$ and $LC_{90}$ values for the phytocompound on *Aedes aegypti* mosquito larvae after 72 hours of treatment [13].

## 3. Results

### 3.1 Effect of Methyl triphenylacetate on larvae of *Aedes aegypti*

The larvae of *Ae.aegypti* was exposed to MTA at different concentrations viz. 3, 6, 9, 12 and 15 ppm for 72 hours to evaluate the larvicidal property of MTA (Table 1). The larval mortality was 32.8, 53.2, 72.8, 92.7 and 100 per cent in 3, 6, 9, 12 and 15 ppm of MTA, respectively. The highest larvae mortality was 100 per cent, and the lowest larvae mortality was 32.8 per cent at a concentration of 3 ppm of MTA, respectively. The $LC_{50}$ value of larval mortality was 4.68 ppm, while $LC_{90}$ values were 12.483 ppm MTA. When the concentration of MTA increased, the larvae mortality also significantly increased.

### 3.2 Pupicidal activity of Methyltriphenyl acetate against *Ae. Aegypti*

The mortality results of pupae of *Ae. aegypti* at different concentrations (3, 6, 9, 12 and 15 ppm) of MTA for 24 hours in table 1.The mortality value was 29.6, 48.6. 69.3, 86.8 and 97.5 per cent at a concentration of 3, 6, 9, 12 and 15 ppm MTA, respectively. The MTA $LC_{50}$ value was 5.182 ppm, and the $LC_{90}$ value was 13.180 ppm for pupa of *Ae. aegypti.* The pupa mortality was notably increased when the concentration of MTA increased.

**Table 1:** Effect of methyl triphenylacetate (MTA) on larvae and pupa of *Aedes aegypti*

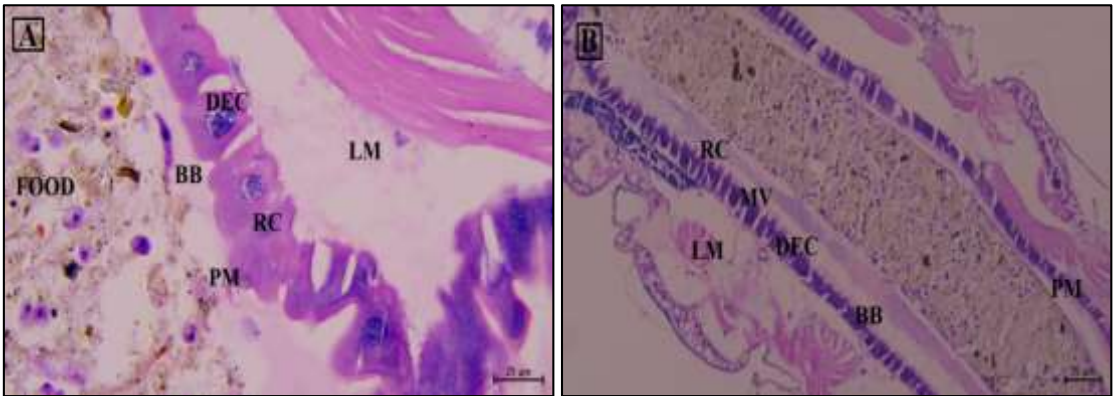
| Organism | Concentration (ppm) | Mortality of larvae (%) | $LC_{50}$ (µg/mL) (LCL-UCL) | $LC_{90}$ (µg/mL) (LCL-UCL) | Regression Equation | $x^2$ df=3 |
|---|---|---|---|---|---|---|
| **Larvae** | 3 | 32.8 ± 0.8$^a$ | 4.68 (3.436–6.394) | 12.483 (9.151-17.028) | y = 3.0558x + 2.951 | 0.527 |
| | 6 | 53.2 ± 1.2$^b$ | | | | |
| | 9 | 72.8 ± 1.8$^c$ | | | | |
| | 12 | 92.7 ± 2.0$^d$ | | | | |
| | 15 | 100.0 ± 0.0$^e$ | | | | |
| **Pupa** | 3 | 29.6 ± 0.4$^a$ | 5.182 (3.901-6.882) | 13.180 (9.923-17.506) | y = 3.3749x + 2.612 | 0.383 |
| | 6 | 48.6 ± 0.8$^b$ | | | | |
| | 9 | 69.3 ± 1.6$^c$ | | | | |
| | 12 | 86.8 ± 1.2$^d$ | | | | |
| | 15 | 97.5 ± 2.2$^e$ | | | | |

**Control:** Nil mortality; $LC_{50}$: Lethal concentration that kills 50% of the exposed larvae or pupa; $LC_{90}$: Concentration that kills 90% of the exposed larvae or pupa; LCL: Lower concentration limit; UCL: Upper concentration limit; $x^2$: Chi-square value; df: degrees of freedom.

### 3.3 Histology Examination

The histological study was carried out to compare the MTA

action mechanism in *Aedes aegypti*. This was accomplished through comparative histological studies of larvae in control and treated forms. The histology study showed damaged internal parts when compared to the control. Generally, the *Aedes aegypti* larva midgut wall comprises five layers, namely innermost columnar epithelial layers, Basement membrane, Circular muscle layer, Longitudinal muscle layer, and Outermost peritoneal membrane. The histological study showed significant damages caused in innermost columnar brush-border epithelial cells and microvilli of digestive epithelial cells. Besides, the treated midgut of the per trophic membrane and nucleus was also deformed (Figure 1).




Control Treated

**Fig 1:** Longitudinal sections of the Control (A) and Treated (B) midgut of fourth instars of *Aedes agypti* larvae. MV- Microvilli, DEC – Digestive epithelial cells, LM – Longitudinal Muscles, PM – Peritrophic Membrane, BB – Brush Border.

## 4. Discussion

Continuous application of synthetic insecticides results in vector species developing resistance, bio magnification of hazardous compounds throughout the food chain, and detrimental consequences on the ecosystem and non-targeted creatures, including human health [14]. Secondary metabolites are produced by plants as defence measures against predators. This trait demonstrates the critical function of natural insecticides in vector control, and their use is a great alternative to synthetic insecticides [15]. The current findings indicate that the MTA exhibited significant larvicidal and pupicidal activity when applied to the midgut of *Aedes aegypti*.

The greatest effect on mosquito populations would occur when they are densely packed, immovable, and easily accessible. This emphasis is on habitat management as well as control of the mosquitoes' immature stages, such as egg, larva, and pupa, prior to their emergence as adults [16]. This strategy maximises the efficiency of the pesticides administered and limits the use of insecticides on a large scale. Larvicides are used to kill larvae in breeding habitats before they mature into adult mosquitoes and disperse. Larvicide treatment of nesting habitats contributes to the reduction of adult mosquito populations in surrounding areas [17, 18]. In the present investigation, larvae and pupa were exposed to MTA at different concentrations for 72 hrs. The $LC_{50}$ value of larvae and pupa mortality was 4.68/5.182 ppm, while $LC_{90}$ values were 12.483/13.180 ppm MTA, respectively. This result showed that MTA might independently contribute to larvae mortality and delayed growth. Our earlier report confirmed these findings. Due to the immobilisation of mosquito metamorphosis, we confirmed that MTA could impede sterol carrier protein synthesis [8]. According to, the larval mortality (LC50 and LC90) values of naringenin-treated groups were 3. 537 and 9.940 ppm against *Aedes aegypti*, respectively, and 3.537 and 9.940 ppm for Cu. Quinquefasciatus [19]. Numerous plant pesticides target the mosquito larvae's midgut, and several can impair larval development into the adult stage, even at sublethal concentrations [20]. The metamorphosis of *Ae. aegypti* larvae entails extensive changes to the insect's body, including a remodelled midgut in which larval digestive cells are completely replaced [21]. MTA-treated larvae exhibited midgut damage in the innermost columnar brush-border epithelial cells and the digestive epithelial cells' microvilli.

When mosquito larvae are exposed to insecticides, some alterations in midgut morphology occur, such as destructive brush border and degenerative digestive cells, as previously observed [22]. The present histopathological examination displays the damages mentioned above in the midgut of larvae, showing that MTA possesses insecticidal property against mosquito larvae. The current result was reinforced by our previous report [9]. We reported that MTA inhibits the sterol carrier protein, which is mainly secreted from the midgut of larvae. These findings were backed up by who found that when a dengue vector was exposed to naringenin, morphological alterations in the midgut occurred [19]. such as destructive brush border, degenerative digestive cells, degenerative basal membrane, degenerated digestive cells, cellular vacuolization, degeneration in the peritrophic membrane, distribution of food bolus, vacuolated intestinal epithelial and smaller fat bodies compare to control.

## 5. Conclusion

Thus, according to the present findings, we can conclude that MTA, a plant-derived phytocompound, have strong insecticidal properties, and it can be used as a key lead compound to eradicate *Ae. aegypti*. Hence, it can be an excellent compound to control other mosquito populations. However, further examinations are warranted to understand their long-term effects as well as its potential in the field conditions and its impact on the ecosystem.

## 6. Potential conflict of interest

The authors declare that they have no conflicts of interest in connection with this work.

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