IN VITRO LARVICIDAL ACTIVITY OF NAPHTHOQUINONES AGAINST DENGUE VECTOR Aedes aegypti (Linnaeus, 1762) (Diptera: Culicidae).

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ABSTRACT
Napthoquinones are the class of organic compounds. Present research work includes to study of larvicidal activity against Aedes aegypti by several synthetic Napthoquinones namely Lawsone, Juglone and Plumbagin were measured, with significant results. A. aegypti is a vector parasite of the Dengue. In the present work we evaluated the potential of some Napthoquinones like Plumbagan, Juglone and Lawsone as larvicide. Larvicidal achieving 100 % larval mortality in 48 hours when tested in the concentration of 5 µg/ml of Plumbagan. LC₅₀ were found 7 µg/mL; 4 µg/mL and 1µg/mL for lawsone, Juglone and Plumbagan respectively. The high larvicidal activity of Plumbagan rather than Juglone and Lawsone. Therefore, this research works effective potential of above napthoquinones against vector responsible for diseases of public health importance.

KEY WORDS: Aedes aegypti, dengue, Diptera: Culicidae, high fever, Juglone, larvicidal, Lawsone, Napthoquinones, Plumbagin, skin rash.

INTRODUCTION
Dengue
Dengue fever is a painful, debilitating mosquito-borne disease caused by any one of four closely related dengue viruses. It is considered as a serious public health problem in the world, mainly in tropical countries where the favorable environmental conditions are responsible for the proliferation of vectors A. aegypti (WHO 2006, 2009). Dengue has emerged as a worldwide problem only since the 1950s. Although dengue rarely occurs in the continental United States, it is endemic in Puerto Rico and in many popular tourist destinations in Latin America, Southeast Asia and the Pacific islands (CDC, 2014). Dengue is transmitted by the bite of a mosquito infected with one of the four dengue virus serotypes. It is a febrile illness that affects infants, young children and adults with symptoms appearing 3-14 days after the infective bite. Dengue is not transmitted directly from person-to-person and symptoms range from mild fever, to incapacitating high fever, with severe headache, pain behind the eyes, muscle and joint pain, and rash on the skin. There is no vaccine or any specific medicine to treat dengue. Severe dengue (also known as dengue hemorrhagic fever) is characterized by fever, abdominal pain, persistent vomiting, bleeding and breathing difficulty and is a potentially lethal complication, affecting mainly children. Early clinical diagnosis and careful clinical management by trained physicians and nurses increase survival of patients (WHO, 2014).

Periodic treatment with chemical insecticides and synthetic pyrethroids are done in breeding sits. Among the arbovirus in India, distribution of all the dengue virus type is continuously expanding. Remarkably the reemergence of Chikungunya virus (CHIK) since 2005 is posing an additional concurrent diseases burden in the country including the Maharashtra. Both these virus are born by the mosquito A. aegypti (L) (Diptera: Culicidae) (Fulmali et al., 2008; Kumar et al., 2008). Sukumar et al. (1991) listed 346 species for 276 genera and 99 families which have been tested against mosquitoes for various effects such as toxicity, growth inhibition, ovipositional determinacy and repellent. Treatment of acute dengue is supportive, using either oral or intravenous rehydration for mild or moderate disease, and intravenous fluids and blood transfusion for more severe cases. The number of cases of dengue fever has increased dramatically since the 1960s, with between 50 and 528 million people infected yearly (Bhatt et al., 2013; Whitehorn and Farrar, 2010). In this work we evaluate the larvicidal activity of synthetic Napthoquinones on vector parasite of the Dengue, A. aegypti Research efforts to prevent and treat dengue include various means of vector control (WHO, 2009) vaccine development, and antiviral drugs.

With regards to vector control, a number of novel methods have been used to reduce mosquito numbers with some success including the placement of the guppy (Poecilia reticulata) or copepods in standing water to eat the mosquito larvae. (WHO, 2009). In 2005, Pérez-Sacau et al., (2005), observed the Antiplasmodial activity of napthoquinones related to lapachol and beta-lapachone. Da Silva et al. (2009), studied Antitumoral, antileishmanial and antimalarial activity of pentacyclic 1,4-naphthoquinone derivatives.

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Napthoquinones are a group of highly reactive organic chemical species that interact with biological systems to promote inflammatory, anti-inflammatory (Poul et al., 1999), antihelminthic (Dama and Jadhav, 1997), Biological activity of Napthoquinones (Ferreira et al., 2010), antimicrobial (Dama et al., 1998), phytonematicidal (Dama et al., 1999; Dama, 2002) and anticancer actions (Da Rocha et al., 2011), antimumor (Eyong et al., 2008; Francisco et al. 2010), West Nile virus vector Culex pipiens (Michaelakis et al., 2009), and to induce toxicities. Da Costa et al. observed the Synthetic 1,4-Pyran Napthoquinones are Potent Inhibitors of Dengue Virus Replication in 2013.

![Diagram of Napthoquinones](image)

**Figure 1. Napthoquinones**

a) Lawsone (2-Hydroxy-1,4-napthoquinone)

b) Juglone (5-Hydroxy-1,4-napthoquinone)

c) Plumbagin (5-Hydroxy-2-Methyl-1,4-napthoquinone)

a) Lawsone

Molecular Formula: C_{10}H_{6}O_{3}, Average mass: 174.153 Da, Monoisotopic mass: 174.031693 Da, Systematic name: 2-Hydroxy-1,4-napthoquinone Chemspider (2014).

b) Juglone

Juglone, also called 5-hydroxy-1,4-naphthalenedione (IUPAC) or 5-hydroxynaphthoquinone, is an organic compound with the molecular formula C_{10}H_{6}O_{3}. In the food industry, juglone is also known as C.I. Natural Brown 7 and C.I. 75500. Other names in industry are Nucin, Regianin, NCI 2323, and Oil Red BS. Systematic name: 5-Hydroxy-1,4-napthoquinone (Wikipedia, 2014).

c) Plumbagin

Plumbagin or 5-hydroxy-2-methyl-1,4-napthoquinone is an organic compound with the chemical formula C_{11}H_{8}O_{3}. It is regarded as a toxin and it is genotoxic and mutagenic. Plumbagin is a yellow dye, formally derived from napthoquinone Systematic name: 5-Hydroxy-2-Methyl-1,4-napthoquinone (Wikipedia, 2014).

Present study we evaluated the potential of commercial synthetic Napthoquinones (Sigma-Aldrich, St. Louis, USA) namely, Lawsone (Figure 1a), Juglone (Figure 1b) and Plumbagin (figure 1c) used for present study for potentials of larvicidal activity on A. aegypti.
MATERIALS AND METHODS

Compounds
Naphthoquinones namely, Lawsone Juglone and Plumbagin, are commercial synthetic materials (Sigma-Aldrich, St. Louis, USA) used for present study for potentials of larvicidal activity on *A. aegypti*.

Mosquito Larva Identification
A small amount of water with a mosquito larvae was drop in a slide to be able to view the specimen under the compound microscope. The target larva in this study was the third instar larva of dengue carrying mosquito *A. aegypti*. *A. aegypti* larvae can be distinguished from any other mosquito larvae since it normally has a single hair, three branch hair tufts on each side of the air tube. When the hair tuft has two or more branches, all the branches arise from the same socket. Other species have two or more hairs, branches and hair tufts on each side of the air tube (pedro, 2014).

Rearing of larvae
Larvae were reared in plastic and enamel trays in tap water. They were maintained at 28–30°C, 75–85% relative humidity under 14:10 light and dark photo period cycle. The larvae were fed with fresh food consisting of a mixture of Biscuit Petit Regal-dried yeast.

Bioassays and Larval Mortality
The larvicidal properties of three Naphthoquinones were evaluated under laboratory conditions against the larvae of the mosquito species. The mosquito strains of *A. aegypti*, evaluated in the present study, are collected from Solapur District, Maharashtra state, India, very much affected by dengue in starting months of winter season (October and November 2014). Larvae were reared (Pelah et al., 2002) and third instars larvae were selected for bioassay. Larvae were transferred into the test solution with pasture pipette (10 larvae/solution). Batches of 10 third-instars larvae of *A. aegypti* were placed in a small plastic container with 50 ml dechlorinated water and lay in the netted area in the Laboratory room at 28–30°C. As a solvent, DMSO is used to soluble the extract in test water.

Appropriate mixture of dimethylsulphoxide (DMSO) and dechlorinated water the test compounds were dissolved and the resulting solution diluted with dechlorinated water to give a final concentration of DMSO of 1 %. An appropriate volume of this test solution was added to a test tube containing twenty larvae of *A. aegypti* in sufficient dechlorinated water (containing 1 % DMSO) in order to give a final volume of 1 ml. The test tubes were incubated in darkness at 28 – 30°C for 24 h and 48 h. For the control group, the mosquito larvae were exposed to DMSO with used experiment set concentration, since it is the solvent used in the dissolution of Naphthoquinonic compounds. The larval mortality was observed under the stereo zoom Microscope. Mortality (Trevan, 1927) at which 50% of the test population were dies (LC$_{50}$), was determined. (Finney, 1971; Jawale et al., 2010). The treatments were replicated three times, and each replicate set contained one control. Mortality of Naphthoquinones were determined after 24 hr and 48 hr exposure at 28°C and the percentage mortality was computed following the protocol of WHO (1981). The calculations were done with the help of Social science statistical online calculator designed by Jeremy Stangroom (2004).

**Formula for Percentage of Mortality**

\[
\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100
\]

Data from all replicates should be pooled for analysis. Standard deviation or confidence intervals of the means of LC$_{50}$ values are calculated and recorded on a form.

RESULTS AND DISCUSSION
In this report, we evaluated the effect of several naphthoquinones against 3rd instar larvae of *A. aegypti*, the vector of dengue. The assayed compounds belong to the general class of naphthoquinones.
Table 1. Mean and Percentage Mortality of *Aedes aegypti* mosquito larvae in the Control and Experimental Group after 24 hours exposure of Napthoquinones.

<table>
<thead>
<tr>
<th>Napthoquinones</th>
<th>Concentration [µg/ml]</th>
<th>Mean Mortality</th>
<th>Mean percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lawsone</td>
<td>1</td>
<td>1</td>
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<td>Juglone</td>
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<td>50</td>
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<tr>
<td>Plumbagin</td>
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<td>5</td>
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<td>80</td>
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</table>

Figure 2. Percentage Mortality of *Aedes aegypti* larvae treated with Napthoquinone compounds like A. Lawsone B. Juglone C. Plumbagin with control and experimental group after 24 hours.
Calculations

The r Value and P-Value of the Mortality of *A. aegypti* larvae treated with Napthoquinone compounds

1. Experimental group after 24 hours- Treated with Lawsone

\[ r = 0.9487 \]

The value of \( r \) is 0.9487. This is a strong positive correlation, which means that high X variable scores go with high Y variable scores (and vice versa).
The value of \( r^2 \), the coefficient of determination, is 0.9.
The P-Value is < 0.00001. The result is significant at \( p < 0.05 \).

2. Experimental group after 24 hours- Treated with Juglone

\[ r = 0.9701 \]

The value of \( r \) is 0.9701. This is a strong positive correlation, which means that high X variable scores go with high Y variable scores (and vice versa).
The value of \( r^2 \), the coefficient of determination, is 0.9411.
The P-Value is < 0.00001. The result is significant at \( p < 0.05 \).

3. Experimental group after 24 hours- Treated with Plumbagin

\[ r = 0.9623 \]

The value of \( r \) is 0.9623. This is a strong positive correlation, which means that high X variable scores go with high Y variable scores (and vice versa).
The value of \( r^2 \), the coefficient of determination, is 0.926.
The P-Value is < 0.00001. The result is significant at \( p < 0.05 \).

Table 2. Mean and Percentage Mortality of *Aedes aegypti* mosquito larvae in the Control and Experimental Group after 48 hours exposure of Napthoquinones.

<table>
<thead>
<tr>
<th>Napthoquinones</th>
<th>Concentration</th>
<th>Mean Mortality</th>
<th>Mean percentage</th>
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<td>100</td>
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</table>
Figure 2. Percentage Mortality of *Aedes aegypti* larvae treated with Napthoquinone compounds like A. Lawsone  B. Juglone  C. Plumbagin with control and experimental group after 48 hours.

**The r Value and P-Value of the Mortality of *A. aegypti* larvae treated with Napthoquinone compounds**

1. **Experimental group after 48 hours- Treated with Lawsone**
   \[ r = 0.8839 \]
   The value of \( r \) is 0.8839. This is a strong positive correlation, which means that high X variable scores go with high Y variable scores (and vice versa).
   The value of \( r^2 \), the coefficient of determination, is 0.7813.
   The P-Value is < 0.00001. The result is significant at \( p < 0.05 \).

2. **Experimental group after 48 hours- Treated with Juglone**
   \[ r = 0.9764 \]
   The value of \( r \) is 0.9764. This is a strong positive correlation, which means that high X variable scores go with high Y variable scores (and vice versa).
   The value of \( r^2 \), the coefficient of determination, is 0.9534.
   The P-Value is < 0.00001. The result is significant at \( p < 0.05 \).

3. **Experimental group after 48 hours- Treated with Plumbagin**
   \[ r = 0.9487 \]
   The value of \( r \) is 0.9487. This is a strong positive correlation, which means that high X variable scores go with high Y variable scores (and vice versa).
   The value of \( r^2 \), the coefficient of determination, is 0.9.
   The P-Value is < 0.00001. The result is significant at \( p < 0.05 \).
Lawsone, Juglone and Plumbagin were employed, LC\textsubscript{50} values < 5 ppm for A. aegypti larvae were indicative of promising active synthetic compounds. Larvicidal achieving 100 % larval mortality in 48 hours when tested in the concentration of 5 µg/ml of Plumbagin. LC\textsubscript{50} were found 7 µg/mL; 4 µg/mL and 1µg/mL for lawsone, Juglone and Plumbagin respectively. The high larvicidal activity of Plumbagin rather than Juglone and Lawsone. The observed data compiled with the results obtained from Ribeiro et al, 2009, Activities of naphthoquinones against Aedes aegypti (Linnaeus, 1762) (Diptera: Culicidae), vector of dengue. The larvicidal activity on Aedes species by synthetic naphthaquinones were done very less. Thus to establish relevance of these Naphthaquinonic compounds with the mosquitocidal activity, will be further evaluate their potential to broaden use and their possible toxic effect upon the other organism. Results indicate that Naphthaquinones, compared with other natural compounds with larvicidal activity, are very toxic against mosquito larvae. The present in vitro results reinforce the potential use of substituted hydroxyquinones and derivatives as very promising larvicidal drugs and suggest a continuing study within this class of compounds, with the aim of designing new products with better properties. Further studies on the insecticidal mode of action, their effects on non-target organisms and the environment, and formulations for improving the insecticidal potency and stability are needed for their practical use as a naturally or hemisynthetic occurring mosquito larval control agent, in search for an efficient bio control agent against larvae of the mosquito A. aegypti.

REFERENCES


