Larvicidal and adulticidal activities of castor oil against the dengue vector, *Aedes aegypti*

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**Abstract.** Plants contain numerous bioactive compounds that can be used to develop environmentally-safe insect control agents. Castor oil is a vegetable oil derived from the seeds of castor bean and is widely used as an industrial lubricant and medicinal purgative. In search of an alternative natural insecticide, the objective of this study was to evaluate the larvicidal and adulticidal activities of castor oil against the important dengue vector, *Ae. aegypti*. Larvicidal and adulticidal bioassays were conducted following the World Health Organization methods. Larvicidal activity was observed at castor oil concentrations of 10, 25, 50, 75, and 100 ppm; larval mortality was checked after 48 h of exposure and the lethal concentration (LC) at LC50 and LC 90 were 51.38 and 116.26 ppm, respectively. Adulticidal activity was determined by topical application at the concentrations of 1, 5, 10, 15, and 20 µg/mg on female mosquitoes and the mortality was checked after 24 h of exposure. The effective adulticidal activity was apparent with the LD50 and LD 90 values of 6.03 and 25.07 µg/mg against female mosquitoes. The results indicated that castor oil has potential in the practical control of both immature and adult stages of the mosquito vector.

**INTRODUCTION**

Dengue fever is a serious public health problem, especially in tropical and subtropical regions. In 2015, the World Health Organization (WHO) reported that the incidence of dengue throughout the world had grown gradually. There are an estimated 390 million cases of dengue infection each year, with risk of dengue virus transmission in 128 countries (WHO, 2015). In the absence of specific therapeutics, controlling and preventing the disease transmission is mainly focused on the mosquito vector, *Aedes aegypti*. Application of chemical insecticides is highly successful in reducing mosquito densities, but poses risks and hazards to humans, animals, non-target organisms and the environment (Abe et al., 2014). Moreover, the resistance of mosquitoes to chemical insecticides has been increasingly reported (Ponlawat et al., 2005; Aponte et al., 2013). In view of environmental pollution and development of insect resistance to synthetic insecticides, there is an urgent need to promote environment-friendly natural mosquito control products. In general, the natural pesticides contain the chemical compounds that possess wide ranges of biological activities and are safe to humans, the environment, and other non-target organisms. Several natural compounds have been used as model for commercial insecticides, such as synthetic pyrethroids (Mann & Kaufman, 2012). Furthermore, numerous studies have shown that several
plant species have biological activity against insects, both insecticidal and repellency activities (Barbosa et al., 2014; Haldar et al., 2014). *Ricinus communis*, also known as castor bean or castor oil plant, is a member of family Euphorbiaceae, which is an ubiquitous plant around the world (Scarpa & Guerci, 1982). The methanolic seed extract of *R. communis* was screened for macrofilaricidal activity against adult cattle filarial worm, *Setaria digitata* (Nisha et al., 2007). The toxicities of methanol, ethyl acetate, chloroform, and petroleum ether extract of the leaf of *R. communis* were tested against the third-instar larvae of *Musca domestica* (Singh & Kaur, 2016). The crude methanolic leaf extracts of *R. communis* had a moderate acaricidal and insecticidal activities against *Haemaphysalis bispinosa* and *Hippobosca maculate* (Zahir et al., 2010a).

Castor oil was obtained by pressing the seeds of *R. communis*. The effect of castor oil on the development, oviposition, and adult emergence of *Callosobruchus maculatus*, *C. phaseoli* and *C. chinensis* were evaluated (Pacheco et al., 1995; Ahmed et al., 1999). Fenigstein et al. (2001) demonstrated that castor oil had antifeedant, oviposition deterrent, and adulticidal activities against the sweet potato whitefly, *Bemisia tabaci*. The leaves and oil of *R. communis* had an adulticidal activity against termites (Sharma et al., 1991). Mushobozy et al. (2009) reported that castor oil displayed the insecticidal activity against Mexican bean weevil, *Zabrotes subfasciatus*. However, very few studies have been conducted to investigate the insecticidal properties of castor oil on mosquito vectors. Therefore, the aim of this study was to evaluate the larvicidal and adulticidal activities of castor oil against *Ae. aegypti* under laboratory condition.

**MATERIALS AND METHODS**

**Mosquito rearing**

The *Ae. aegypti* female mosquitoes were obtained from the Insecticide Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand. Eggs were allowed to hatch for 3 h to obtain synchronized larvae. After hatching, 200 larvae were transferred to individual plastic trays containing dechlorinate water and kept at room temperature (27 ± 2 °C). The larvae were fed daily on Fish Food Sticks (Kyorin Co., Ltd, Japan). When the larvae reached the pupal stage, they were transferred from the plastic trays into plastic cup containing dechlorinate water, and placed into a screen cage. Cotton soaked in 10% sucrose solution was provided for emerge adults. Mosquitoes 5-7 d after emergences were starved for 12 h before feeding. Each time, 100 mosquitoes per cup were fed on blood using artificial membrane feeding. Three days after blood meal, the gravid mosquitoes were allowed to lay their eggs in an oviposition cup filled with dechlorinate water, the inside of which contained a cone-shaped filter paper (Whatman No. 1). After oviposition, the filter papers were removed and dried at room temperature. For long-term storage, the filter papers were stored in plastic zip-lock bags at room temperature.

**Larvicidal bioassay**

Castor oil was purchased from Sigma-Aldrich (St. Louis, MO). The stock solutions of castor oil for larvicidal bioassay were prepared by dissolving in absolute ethanol (Merck, Germany). Larvicidal activity was determined following the WHO protocol (WHO, 2005) with slight modification. The larvicidal activity was determined at castor oil concentrations of 10, 25, 50, 75, and 100 ppm (0.01, 0.025, 0.05, 0.1 ml/L, respectively). Batches of 20 synchronized third-instar larvae were exposed in 200 ml of dechlorinated water treated with the desired concentrations. Five replicate sets were tested simultaneously with a total of 100 larvae for each concentration. Solutions containing dechlorinated water and ethanol without castor oil served as control, while the untreated solution was dechlorinated water only. Larval mortality was checked at 48 h after exposure. The 95% confidence intervals (CI) of the lethal dosage of 50%, 90%, and 95% (LC50, LC90, and LC95, respectively)
were calculated by using probit analysis program (Finney, 1971).

**Adul ticidal bioassay**

The stock solutions for adulticidal bioassay were prepared by dissolving castor oil in acetone (Scharlau, Spain). The adulticidal activity was determined by topical application to adult female mosquitoes following the WHO guideline (WHO, 2006) with minor modification. From the stock solution, five different test concentrations including 1, 5, 10, 15, and 20 µg/mg female mosquitoes were prepared. Non-blood fed females (2-5 d old) were anæsthetized at a temperature of -20 °C for 30 s, and placed on a cold plate. A volume of 0.5 µl of the castor oil solution was dropped onto the pronotum of the immobilized mosquitoes using a hand micro-applicator (Burkard Manufacturing Co. Ltd., England), with two batches of 25 female mosquitoes for each concentration. Controls were treated with 0.5 µl of pure acetone alone, while anæsthetized mosquitoes with no treatment served as untreated. After the application, all mosquito groups were maintained at 27 ± 2 °C, and cotton soaked in 10% sucrose solution was provided for feeding. Mortality was recorded after 24 h of exposure. The experiment was replicated 3 times. The 95% confidence intervals (CI) of the lethal dosage of 50%, 90%, and 95% (LD$_{50}$, LD$_{90}$ and LD$_{95}$, respectively) were calculated by probit analysis program (Finney, 1971).

### RESULTS AND DISCUSSION

The percentage mortality of third-instar larvae to various concentrations of castor oil was dose dependent, with higher concentrations cause in higher larval mortality. Castor oil started to exhibit the larvicidal activity at a concentration of 10 ppm with 1% larval mortality. Among the five concentrations tested, the highest dosage (100 ppm) was the most effective against third-instar larvae, with mortality of 85.33%. In 48 h, treated larvae showed nervous movement, followed by falling to the bottom of test cups, subsequently convulsions and death. The LC$_{50}$ and LC$_{90}$ values of castor oil were 51.38 and 116.26. The mortalities in both control and untreated groups were less than 5% for all experiments (Table 1).

Several plant oils are readily commercial available in many regions around the world and have strong toxic effect against medically important mosquito vectors (Khater & Shalaby, 2008; Maguranyi et al., 2009). Some natural plant products such as neem oil and rotenone have been successfully exploited as organic insecticides and are commercially available (Duke et al., 2010). The well-known castor oil is a unique and useful vegetable oil with many applications. Numerous biological activities of castor oil have been reported, including anti-oxidant, anti-nociceptive, anti-asthmatic, anti-fertility, anti-microbial, molluscicidal, insecticidal, and larvicidal activities (Jena & Gupta, 2012).

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**Table 1. Larvicidal activity of different concentrations of castor oil against third-instar *Ae. aegypti* larvae after 48 h**

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>% Mortality (Mean±SE)</th>
<th>Larvicidal activity (95% C.L., ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LC$_{50}$</td>
</tr>
<tr>
<td>10</td>
<td>1.00±0.58</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>11.67±4.63</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>53.00±2.89</td>
<td>51.38</td>
</tr>
<tr>
<td>75</td>
<td>73.33±10.20</td>
<td>(47.94-53.10)</td>
</tr>
<tr>
<td>100</td>
<td>85.33±4.26</td>
<td>116.26</td>
</tr>
<tr>
<td>Control</td>
<td>0.33±0.33</td>
<td>(103.70-123.41)</td>
</tr>
<tr>
<td>Untreated</td>
<td>1.00±1.00</td>
<td>(127.89-158.18)</td>
</tr>
</tbody>
</table>

SE = standard error.
Earlier authors reported that castor oil exhibited a strong larvicidal activity against third-instar larvae of diamondback moth, *Plutella xylostella* with 100% mortality. The treated larvae died shortly after getting in contact with or ingesting the castor oil. Dead larvae were characterized by burned cuticle in both ingestion and contact topical application tests (Kodjo et al., 2011). Similarly, castor oil showed an excellent larvicidal effect against late third and early fourth-instar larvae of *Ae. aegypti* with the LC$_{50}$ and LC$_{90}$ values of 0.016 and 0.082 µl/ml after 48 h of exposure, respectively (Candido et al., 2013). Batabyal et al. (2009) evaluated the lethal toxicity of carbon tetrachloride seed extracts of *R. communis* against *Culex quinquefasciatus* larvae with LC$_{50}$ values of 144.11 and 92.44 ppm after 24 and 48 h of exposure. Borah et al. (2012) reported that the LC$_{50}$ and LC$_{90}$ values of petroleum ether seed extract were 80.83, 424.50 ppm and 113.22 and 601.11 ppm against fourth-instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. The aqueous leaf extracts of *R. communis* proved to be toxic killing 100% of the larvae of *Ae. aegypti* at the concentration of 1,000 ppm after 24 h of exposure (Kumar et al., 2012). Elimam et al. (2009) reported the effect of aqueous leaf extracts of *R. communis* against second, third, and fourth-instar larvae of *An. arabiensis* and *Cx. quinquefasciatus* with LC$_{50}$ values of 403.65, 445.66, 498.88 ppm and 1091.44, 1364.58, 1445.44 ppm, respectively. In addition, it has been reported that leaf methanol extract of *R. communis* showed potent antiparasitic activity against adult *Paramphistomum cervi* larvae of *Rhipicephalus (Boophilus) microplus*, larvae of *An. subpictus* and *Cx. tritaeniorhynchus* (Zahir et al., 2009).

In our study, the treated larvae with castor oil (which commonly derived from seed extracted) showed nervous movement, fell to the bottom of test cups, convulsed and died. The ethanolic-water extract from leaves of *R. communis* showed the effects of disturbance *An. stephensi* larvae like curling up, vigorous body movement, and discoloration (Senthilkumar et al., 2009). Similar observation was obtained with larvicidal efficacy of *Ipomoea cairica* extract against *Ae. aegypti* and *Ae. albopictus* larvae. The exposed larvae showed restless movement for some time, followed by sluggishness, convulsions, paralysis at the bottom of test cups, and death (Ahbi Rami et al., 2014). The signs of intoxication may be the characterization of neurotoxicity. As has been reported earlier, leaves and pericarp extract of *R. communis* were shown to possess the neurological disorders in livestock (Tokarnia et al., 2002). Ricinine, an alkaloid isolated from fruit pericarp of *R. communis* also showed central nervous system stimulant effects when administered to adult male mice (Ferraz et al., 1999).

The biological activity of castor oil may be due to the chemical constituents that affect the metabolic activities of *Ae. aegypti* larvae. Studies of the various solvents extraction prepared from the different parts of *R. communis* have been reported the numerous bioactive phytochemical constituents like alkaloids, anthrocyanins, flavonoids, phenolics, tannins, terpenoids, etc (Alugah & Ibraheem, 2014). The synergistic activity of the mixture of bioactive components present in the acetone seed extracts of *R. communis* had an excellent larvicidal activity against *An. stephensi, Cx. quinquefasciatus, and Ae. albopictus* (Mandal, 2010). The flavonoids isolated from aqueous leaf extract of *R. communis* showed excellent insecticidal, ovicidal and oviposition deterrent activities against stored grain pest, *C. chinensis* (Upasani et al., 2003). Epichatechin, the flavonoid isolated from *R. communis*, has demonstrated considerable as anthelminthic activity against sheep fluke, *P. cervi* (Zahir et al., 2012). On the other hand, the compounds other than flavonoid probably also play a role in their mode of action. Johnson (2007) reported that ricinoleic acid was the main fatty acid component of castor oil, following by oleic acid, linoleic acid, palmitic acid, stearic acid, and dihydrostearic acid. No studies have reported on the effects of ricinoleic acid on mosquitoes. However, the insecticidal effect of ricinoleic acid ester on the tick, *R. sanguineus* was reported. The constituent made oocytes of exposed ticks...
non-viable and preventing them reaching advanced stages of maturation (Arnosti et al., 2011). Although ricinoleic acid affects the vitellogenesis of tick, its action on mosquitoes remains unclear. Therefore, the effectiveness of ricinoleic acid on mosquito development requires investigation.

In the adulticidal bioassay, the percentage mortality of *Ae. aegypti* female mosquitoes in response to the concentration of castor oil was dose dependent. According to the increasing concentrations used, the mortality values increased from 13.33 to 93.33%. The highest percentage of *Ae. aegypti* mosquito died when treated at dosage of 20 µg/mg, with adult mortality of 93.33%. The LD$_{50}$ and LD$_{90}$ of castor oil were 6.03 and 25.07 µg/mg, respectively. No mortality in both control and untreated groups were observed (Table 2).

The effects of *R. communis* leaf with acetone extracts on *An. stephensi* showed adult emergence inhibition activity with EI$_{50}$ value of 139.39 µg/ml, whereas the chloroform extract gave highest adulticidal activity with the LD$_{50}$ values of 163.11 µg/ml, respectively (Zahir et al., 2010b). The dose-dependent mortality was observed for hexane extract of *Curcuma aromatica* against *Ae. aegypti*, with LD$_{50}$ value of 1.60 µg/mg (Choochote et al., 2005). The toxicity of ethanolic extract of the whole plant of *Piper longum*, *P. ribesoides*, and *P. samentosum* against *Ae. aegypti* was showed dose-dependent mortality and the susceptibility varied with the plant species. The highest adulticidal activity for *P. samentosum*, followed by *P. ribesoides* and *P. longum*, with the LD$_{50}$ values of 0.14, 0.15, and 0.26 µg/mg female, respectively (Choochote et al., 2006). The hexane, ethyl acetate, and methanol leaf extract of *Ageratum houstonianum* showed adulticidal activity against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*, and the mortality rates were increasing with higher concentrations for all mosquitoes. Treated *An. stephensi* and *Cx. quinquefasciatus* adults were more susceptible to methanol extract, with LD$_{50}$ values of 0.12 µg/mg female; whereas *Ae. aegypti* adults were more susceptible to ethyl acetate and hexane extracts, with LD$_{50}$ of 0.10 µg/mg female (Ravindran et al., 2012).

The results of the present study shown that treated adults at the concentrations of 15 and 20 µg/mg could not rest on the wall of the tested cup, followed by fell down and then died. Similarly, castor oil possesses remarkable insecticidal activity against *C. maculatus*. The dead adults showed signs of toxic effects such as rapid immobilization, flexing their legs, and clinging to the container surface (Haghtalab et al., 2009). Govindarajan & Sivakumar (2014) reported that crude extract of *Erythrina indica* showed dose-dependent action: at higher concentration, treated adults were restless with abnormal movement and then died. The highest adulticidal activity of *E. indica* was for methanol extract against *An. stephensi*,

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**Table 2. Adulticidal activity of different concentrations of castor oil against adult *Ae. aegypti* female after 24 h**

<table>
<thead>
<tr>
<th>Concentration (µg/mg female)</th>
<th>% Mortality (Mean±SE)</th>
<th>Adulticidal activity (95% C.I., µg/mg female)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LD$_{50}$</td>
</tr>
<tr>
<td>1</td>
<td>13.33±1.33</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>21.33±1.33</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>62.67±7.05</td>
<td>6.03</td>
</tr>
<tr>
<td>15</td>
<td>86.67±2.67 (2.43-14.90)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>93.33±2.67</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0±0.00</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>0±0.00</td>
<td></td>
</tr>
</tbody>
</table>

SE = standard error.
Ae. aegypti, and Cx. quinquefasciatus. The mode of action of castor oil is yet to be confirmed. The high mortality rate of treated adults obtained in the present study could be due to the presence of toxic components in the castor oil. The leaf ethanolic extracts of R. communis exhibited acaricidal activity against organophosphate and pyrethroid resistant ticks, R. microplus with dose-dependent manner. The efficacy of extract was killing 48.0, 56.7 and 60.0% diazinon, deltamethrin, and multi-acaricide resistant ticks, respectively. The synergistic acaricidal activity may be due to the active constituents in the extract like quercetin, gallic acid, flavones and kaempferol (Ghosh et al., 2013).

Ricinine is a toxic alkaloid isolated from the leaves and fruits of R. communis (Rana et al., 2012). The toxic effects of ricinine on the leaf cutting ant, Atta sexdens rubropilosa has been observed. The treated ants showed symptoms of intoxication, such as reduction or stoppage of locomotion, followed by disorientation, no coordination and death (Bigi et al., 2004). Ricinine exhibited an insecticidal activity against green peach aphids, Myzus persicae (Olaifa et al., 1991). The ethanol, hexane, and ethyl acetate extract of the seeds and leaves of R. communis, castor oil and ricinine were tested for insecticidal and insectistatic activities against S. frugiperda. The results demonstrated that castor oil and ricinine were shown to possess insecticidal and insectistatic activities, with the half maximum larvae viability values (LVC\textsubscript{50}) of 2.69×10\textsuperscript{3} and 0.38×10\textsuperscript{3} ppm, respectively (Ramos-López et al., 2010). Moreover, ricinine also reduced the survival times of female An. gambiae (Wachira et al., 2014).

CONCLUSION

In our study, castor oil showed larvicidal and adulticidal activities against Ae. aegypti. The results suggest that castor oil has potential in the practical control of both immature and adult stages of the mosquito vector. The results of this study should encourage further investigation on the possible mechanisms, and their active constituents, on mosquito development. Semi-field and field trials are also needed before recommendation of castor oil as an alternative natural insecticide for mosquito control.

Conflict of Interest
The authors declare that they have no conflict of interest.

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REFERENCES


