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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 LC_{50} 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 LD_{50} 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% (LC_{50}, LC_{90}, and LC_{95}, respectively) were calculated by using probit analysis program (Finney, 1971).

Adulticidal 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 anaesthetized 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 anesthetized mosquitoes with no treatment served as untreated. After the application, all mosquito groups were maintained at 27 ± 2 ºC, 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).

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

Concentration (ppm)	% Mortality (Mean±SE)	Larvicidal activity (95% C.I., ppm)		
		LC_{50}	LC_{90}	LC_{95}
10	1.00±0.58			
25	11.67±4.63			
50	53.00±2.89	51.38	116.26	146.87
75	73.33±10.20	(47.94-53.10)	(103.70-123.41)	(127.89-158.18)
100	85.33±4.26			
Control	0.33±0.33			
Untreated	1.00±1.00			

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 aromatic*a 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 was 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*,

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

Concentration (µg/mg female)	% Mortality (Mean±SE)	Adulticidal activity (95% C.I., µg/mg female)		
		LD_{50}	LD_{90}	LD_{95}
1	13.33±1.33			
5	21.33±1.33			
10	62.67±7.05	6.03	25.07	37.55
15	86.67±2.67	(2.43-14.90)	(6.09-106.11)	(6.50-225.63)
20	93.33±2.67			
Control	0±0.00			
Untreated	0±0.00			

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_{50}) of 2.69×10^3 and 0.38×10^3 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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