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# Insecticidal effect of essential oils from *Boesenbergia rotunda* (L.) Mansf. and *Curcuma zedoaria* rosc against dengue vector mosquito, *Aedes aegypti* L.

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Essential oils from two Thai herbal plants, *Boesenbergia rotunda* (L.) Mansf. and *Curcuma zedoaria* Rosc were evaluated for their larvicidal, pupicidal, oviposition-deterrent potential against Dengue Vector Mosquito, *Aedes aegypti* L.. The larval mortality was recorded at 1, 5, 10, 15, 30, 60 minutes and 24 hours while pupal mortality was recorded at 15, 30 minutes, 1, 3, 6, 12, 24 and 48 hours. The results revealed that *C. zedoaria* essential oil proved to have greater toxicity against *Ae. aegypti* larvae and pupae with $LC_{50}$ value of 0.05% and 1.22%, respectively The essential oil of *C. zedoaria* exhibited higher larvicide activity than *B. rotunda* essential oil with $LT_{50}$ of 40.1, 3.2 and <1.0 minutes at 1, 5 and 10% conc., respectively. It was consistent with pupicidal activity which exhibited $LT_{50}$ values of 23.1, 1.3 and 0.5 hours at 1, 5 and 10% conc., respectively.
The results showed that gravid *Ae. aegypti* females preferred to lay eggs in control cups than in the cups treated with the essential oils. The oviposition activity index (OAI) of the essential oils indicated that there were more deterrant than the control. The mean number of eggs laid in three concentrations (1%, 5% and 10%) of *C. zedoaria* essential oils were lower than *B. rotunda* essential oils at the same concentration. Both essential oils in the three concentrations showed significant difference with controls.

**Keywords:** herbal essential oil, larvicide, pupicide, oviposition-deterrent, *Aedes aegypti*

## Introduction

Dengue fever is ranked by the World Health Organization (WHO) as the most important mosquito-borne viral disease in the world. In 2012, Outbreaks exerted a huge burden on populations, health systems and economics in most tropical countries of the world. (World Health Organization, 2012). An estimated 500,000 people with severe dengue require hospitalization each year, a large proportion of whom are children. About 2.5% of those affected die

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(WHO, 2013). *Ae. aegypti* is a major vector of dengue fever (DF) and dengue hemorrhagic fever (DHF). (Shultz *et al*., 2008). The common approach for the control of mosquito vectors and reducing the transmission of human pathogens is based on the chemical insecticide-based intervention measures (Paul *et al.*, 2006). Synthetic insecticides are used by the community because they are practical in use and rapid in action. However, the use of synthetic insecticides has not led to a reduction in DHF rates. On the contrary, there are reports from many countries about the occurrence of insecticide resistance, environmental pollution, and contamination of humans and animals. Most of the available synthetic insecticides kill only adult mosquitoes, and only a few kill mosquito larvae. (Muhlisin *et at*., 2006; Cavalca *et al.*, 2010 and Suwasono *et al.*, 2004).

Thus, the environmental friendly and biodegradable natural insecticides of plants origin have been receiving attention as an alternative green measure for controlling arthropods of public health importance (Koul *et al*., 2008). These natural products utilized as mosquito insecticides limit the environmental impact of pesticides due to shorter latency, which may be beneficial for preventing the evolution of resistance (Hardin and Jackson, 2009). There have been no reports of resistance by pests and vectors against botanicals (Madhumathy *et al*., 2007). The control of mosquito larvae using indigenous medicinal plants is beneficial in developing countries, such as Thailand and its Southeast Asian neighbors. Plant essential oils are natural volatile substances obtained from a variety of plants, in general have been recognized as an important natural resource of insecticides (Gbolade *et al*., 2000). Essential oils have received attention as potentially controlling vectors of mosquito-borne disease (Sutthanont *et al*., 2010).

Larviciding is one approach to vector control carried out at breeding centers of the vectors (Mohan and Ramaswamy, 2007). This is a successful way of reducing mosquito densities before they emerge as adults. They are killed before they disperse to human habitations. Larvae, unlike adult mosquitoes, cannot change their behavior to avoid mosquito control measures (Killeen *et al*., 2002).

One of the successful strategies for mosquito control is focused on targeting breeding sites of mosquitoes for regulation of their population density (Gubler, 1989). Oviposition is one of the most important events in the life cycle of mosquitoes. If oviposition is prevented, the mosquito life cycle is disrupted and population growth reduced (Xue *et al*., 2001).

In Thailand, many researchers have observed that biologically active materials derived from Thai indigenous plant sources can have larvicidal and pupacidal (Phasomkusolsil and Soonwera, 2010; Kaewnang-O *et al*., 2011; Phukerd and Soonwera, 2013), ovicidal and oviposition-deterrent (Tawatsin *et*

*al*., 2006; Phasomkusolsil and Soonwera, 2012) against mosquito vectors. A vast number of medicinal plants have not had their mosquitocidal potential assessed. Thus, the principal objectives of this study were to investigate the larvicidal, pupicidal, oviposition-deterrent of essential oils from two Thai herbal plants, *Boesenbergia rotunda* (L.) Mansf. and *Curcuma zedoaria* Rosc against dengue vector mosquito, *Aedes aegypti* L.

## Material and methods

### *Mosquitoes*

*Ae. aegypti* mosquitoes were raised in the Department of Entomology and Environment laboratory, Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok. Adult mosquitoes were maintained in cages (size 30x30x30 cm) and feed 5% glucose solution in water soaked on cotton pads. On day 5-7, the females were given a blood meal via artificial membrane method. Two to three days after the blood meal, the gravid mosquitoes laid their eggs. Larvae were reared in plastic trays (30x35x5 cm) containing 2 liters of tap water and fed on fine fish food. Fourth instar larvae and pupae were used for the larvicidal and pupicidal experiments.

Newly emerged pupae were transferred to screen cages (size 30x30x30 cm) and emerged as adults. Adults were provided with 5% glucose on saturated cotton pads. Females were blood-fed via membrane by artificial membrane methods (Nasirian and Ladonni, 2006) and used for the oviposition-deterrent experiment.

### *Plant materials*

Two Thai herbal plants used in this study were shown in Table 1. The essential oils (1,5 and 10%) in ethyl alcohol were used. These oils were provided by the medicinal plant laboratory, Faculty of Agricultural Technology, KMITL.

**Table 1.** List of Thai herbal plants tested in this study

| Scientific name | Common name | Used parts | Therapeutic property |
|---|---|---|---|
| *Boesenbergia rotunda* )L.)Mansf. | Finger root | Roots, Rhizomes | Anti-inflammatory, antioxidant, analgesic, antimutagenic, antitumour, antibacterial, antifungal, antipyretic, anti spasmodic, Insecticidal, mosquito larvicidal. |
| *Curcuma zedoaria* Rosc | Zedoary | Rhizomes | Anti-inflammatory, antioxidant, carminative, analgesic, antimicrobial, , antitumor, anticlastogenic, cytotoxicity, anti-tyrosinase, perfumery, insecticidal. |

### *Bioassay procedures*

### *Larvicidal activity*

The test procedures were done according to World Health Organization (2005). One milliliter of test oil was added to 99 ml distilled water in a 250 ml plastic cup, which was shaken lightly to ensure a homogeneous test solution. Twenty-five specimens each of fourth instar larvae, and pupae of *Ae. aegypti* were divided into respective groups and placed in cups. No food was provided during the treatment. The larval mortality was recorded at 1, 5, 10, 15, 30, 60 minutes, and 24 hours while pupal mortality was recorded at 15, 30 minutes, 1, 3, 6, 12, 24 and 48 hours. Larvae were considered dead if they were incapable of rising to the surface or did not show the characteristic dicing reaction when the water was disturbed (Tiwary *et al*., 2007). The mean mortality was recorded. Each experiment was performed in five replicates with a simultaneous control (1 ml ethyl alcohol in 99 ml water). The $LC_{50}$ values were calculated at 1 hour for larvicidal activity and 24 hours for pupicidal activity. $LT_{50}$ and $LC_{50}$ values were calculated using probit analysis. The mortality data was analyzed by Duncan's multiple range test using SPSS for window (version 16.0).

### *Oviposition deterrent activity assay*

The bioassay was evaluated by using the following method (Phasomkusolsil and Soonwera, 2012). Two black plastic cups (250 ml in capacity) were filled with 99 ml of well water and were placed at diagonally

opposite corners of the cage. An aliquot (1 ml) of essential oil dissolved in ethyl alcohol (1%, 5% and 10%) was added to one bowl whilst an equivalent quantity of ethyl alcohol was added to the second (control). Their positions were switched between replicates to avoid positional effects. A piece of filter paper was placed on the internal surface of each bowl to provide a support for oviposition. The paper was located in each cup so as the lower half of the paper was submerged in water. Meanwhile fifteen gravid female mosquitoes (5-7 days old) were mated. They were simultaneously exposed, in a bioassay cage (30 x 30 x 30 cm). After 96 hours, the eggs laid in each cup were counted after removal of the oviposition paper. Five replicates were performed. The oviposition experiments were expressed as mean number of eggs and oviposition activity index (OAI), which was calculated using the following formula (Kramer and Mulla, 1979).

$$OAI=\frac{NT-NC}{NT+NC}$$

Where NT is the total number of eggs in the test solution, and NC is the total number of eggs in the control solution. The OAI ranges from -1 to +1, with 0 indicating neutral response. The positive index values indicate that more eggs were deposited in the test cups than in the control cups, and that the test solutions were attractive. Conversely, more eggs in the control cups than in the test cups result in negative index values and the test solutions were a deterrent. The percent effective repellency (ER%) for each essential oil was calculated in case of the test solution as a deterrent using the following formula (Rajkumar and Jebanesan, 2009).

$$ER\%=\frac{NT-NC}{NT}\ x\ 100$$

In addition, the percent effective attractancy (EA%) was calculated in case of the test solution as a attractant using the following formula (Govindarajan *et al*., 2008).

$$EA\%=\frac{NT-NC}{NT}\ x\ 100$$

Where ER is effective repellency / EA is effective attractancy, NC is the total number of eggs in the control solution and NT is the total number of eggs in the test solution.

### *Statistical analysis*

The mean number of eggs deposited in test and control cups were analyzed using a paired *t*-test. One-way analysis of variance (ANOVA) and Duncan's multiple comparisons were used for the hatching percentage to determine significant treatment differences by SPSS for Windows (version 16.0).

## Results

The $LT_{50}$ and $LC_{50}$ values of *B. rotunda and C. zedoaria* essential oils against fourth instar of *Ae. aegypti* were shown in Tables 2. The result of the experiment revealed that *C. zedoaria* oils proved to have greater toxicity against *Ae. aegypti* larvae and pupae with $LC_{50}$ value of 0.05% and 1.22%, respectively while *B. rotunda* induced toxicity with $LC_{50}$ value of 4.28% and 8.06%, respectively. The larvicidal and pupicidal activities of the two essential oils correlated with concentration. The essential oil of *C. zedoaria* at 1,5, 10% conc. exhibited larvicidal activity with $LT_{50}$ values of 40.1, 3.2 and <1 minutes , respectively while essential oil of *B. rotunda* showed lower effective with $LT_{50}$ values of 6959.5, 37.0 and 3.1 minutes, It was consistent with pupicidal activity, *C. zedoaria* essential oil induced higher pupicidal activity with $LT_{50}$ values of 23.1,1.3 and 0.5 hours, respectively while *B. rotunda* essential oil showed lower effective with $LT_{50}$ values of 252.2%, 184.6 and 2.7 hours, respectively. According to larvicidal and pupicidal activities, the 5% of *B. rotunda* essential oil induced 100% mortality against *Ae. aegypti* larvae and pupae at 1 hour and 24 hours, significant differences over *B. rotunda* essential oil (Table 3).

Additionally, the oviposition deterrant of the two essential oils at three concentrations (10%,5% and 1%) has been shown in table 4. The results showed that the different concentration of essential oils reduced number of eggs deposited by gravid *Ae.aegypti* of treatment concentrations at 1%,5% and 10% as compared with ethyl alcohol control. The mean number of eggs laid in three concentrations (1%, 5% and 10%) of *C. zedoaria* essential oils showed 76.0±52.3, 18.7±16.9 and 9.2±17.3 eggs per cup, respectively and showed significant difference with control while *B. rotunda showed* lower property with 162.0±34.1, 52.0±13.5 and 59.2±13.7 eggs per cup, respectively and also showed significant difference with control. Furthermore, the highest percentage of effective repellency (ER%) was shown in 10% conc. of *C. zedoaria* and followed by5% conc. with 98% and 96%, respectively. The oviposition activity index (OAI) of the two essential oils at the three concentrations when being paired with control ranged from 0.7 to -1.0 (*C. zedoaria*) and -0.5 to -0.8 (*B.*

*rotunda*). It showed that gravid *Ae. aegypti* females preferred to lay eggs in control cups than in the cups treated with the two essential oils.

**Table 2.** $LT_{50}$ and $LC_{50}$ values of essential oils from *B. rotunda* and *C. zedoaria* against fourth instars larvae and pupae of *Ae. Aegypti*

| Essential oils | Conc. | Larvicidal activities | | Pupicidal activities | |
|---|---|---|---|---|---|
| | | $LT_{50}$ (min) | $LC_{50}$ (%) at 1 hr | $LT_{50}$ (hr.) | $LC_{50}$ (%) at 24 hr |
| *B. rotunda* oil | 1% | 6959.5(-) | | 252.2 (-) | |
| | 5% | 37.0(34.4-39.8) | 4.28 | 184.55 (-) | 8.06 |
| | 10% | 3.14(0.7-5.3) | | 2.7 (-) | |
| *C. zedoaria* oil | 1% | 40.1(34.5-47.4) | | 23.1 (20.9-29.4) | |
| | 5% | 3.2(2.6-3.6) | 0.05 | 1.3(1.1-1.6) | 1.22 |
| | 10% | <1 (-) | | 0.5 (0.3-0.7) | |

**Table 3.** Larvicidal and Pupicidal activities of essential oils from *B. rotunda* and *C. zedoaria* against fourth instars larvae and pupae of *Ae. Aegypti*

| Essential oils | % Mortality±SD | | | | | |
|---|---|---|---|---|---|---|
| | Larvicidal activities | | | Pupicidal activities | | |
| | 1% | 5% | 10% | 1% | 5% | 10% |
| *B. rotunda* oil | $0(\pm0)^{b\,1}$ | $82.4(\pm6.1)^{b}$ | $100(\pm0)^{ns}$ | $11.2(\pm3.4)^{b}$ | $17.6(\pm6.1)^{b}$ | $69.6(\pm6.1)^{b}$ |
| *C. zedoaria* oil | $80(\pm13.0)^{a}$ | $100(\pm0)^{a}$ | 100(±0) | $39.2(\pm6.6)^{a}$ | $100(\pm0)^{a}$ | $100(\pm0)^{a}$ |
| Control (Ethyl alcohol) | $0(\pm0)^{b}$ | $0(\pm0)^{c}$ | 0(±0) | $0(\pm0)^{c}$ | $0(\pm0)^{c}$ | $0(\pm0)^{c}$ |
| CV% | 28.06 | 5.8 | NA | 25.3 | 8.9 | 6.2 |

[1]Means in each column followed by the same letter are not significantly different ($P<0.05$, by one-way ANOVA and Duncan's multiple range test).
ns = not significant

**Table 4.** The oviposition deterrent/ attractant/ neutral of the essential oils in three concentrations (1%, 5% and 10%) against *Ae. Aegypti*

| Essential oils | Conc. | Number of eggs ±SD | | OAI** | ER% | EA% | No.of tested eggs laid per female |
|---|---|---|---|---|---|---|---|
| | | Tested | Control | | | | |
| *B. rotunda* | 1% | 162±34.1* | 534±51.1 | -0.5 | 69.7 | - | 10.8 |
| | 5% | 59.2±13.7* | 617.2±41.4 | -0.8 | 90.4 | - | 3.9 |
| | 10% | 52±13.5* | 628.2±42.2 | -0.8 | 91.7 | - | 3.5 |
| *C. zedoaria* | 1% | 76±52.3* | 400±54.5 | -0.7 | 81.0 | - | 5.1 |
| | 5% | 18.67±16.9* | 468.4±58.4 | -0.9 | 96.0 | - | 1.2 |
| | 10% | 9.2±17.3* | 459.7±68.0 | -1.0 | 98.0 | - | 0.6 |

*Significant differences between tested and control by paired *t*-test ($P< 0.05$)
**The OAI ranges from -1 to +1; the positive index values (+) indicated that the test solutions were attractants; the negative index values (–) indicated that the test solutions were deterrents and 0 indicating neutral response
OAI = Oviposition Active Index; ER = Effective Repellency; EA = Effective Attractancy

## Discussions

Essential oils from plants may be an alternative source of mosquito control, since they have a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in integrated management programs of mosquito control. This study aimed to reveal two Thai essential oils from *B. rotunda* and *C. zedoaria* against dengue vector mosquito, *Aedes aegypti* L. The two plants are in Zingiberaceae family. The Zingiberaceae plants well known for its immense medicinal values and therapeutic properties are distributed widely throughout the tropics, particularly in Southeast Asia (Jantan *et al*., 2003). Chemical analysis on *Curcuma zedoaria* rhizome volatile oil, demonstrated the presence of β-tumerone (19.88%), 1,8-cineole (8.93%), and 7-zingiberene (7.84%) as major constituents. (Pitasawat *et al.*, 2007). Furthermore, 4-hydroxypanduratin A and panduratin A, isolated from the rhizomes of B. rotunda were found to show high inhibitory activity towards dengue-2 virus protease at 120 ppm (Kiat *et al*., 2006). The result of this study indicated that *C. zedoaria* essential oil showed greater toxicity against *Ae. aegypti* larvae and pupae than *B. rotunda* essential oil with $LC_{50}$ value of 0.05% and 1.22%, respectively while *B. rotunda* essential oil exhibited $LC_{50}$ value of 4.28% and 8.06%, respectively. In fact, many researchers have reported on the effectiveness of plant essential oils against mosquito larvae. Pitasawata *et al.* (2007) reported *C. zedoaria* exerted significant larvicidal activity against *A. aegypti* after 24 hours exposure. Phukerd and Soonwera (2013) reported that 10% Conc. of *B. rotunda* and *C. zedoaria* oils showed high toxicity against *Cx. quinquefasciatus* larvae with $LT_{50}$ value of 1.7 minutes and 100% mortality at 10 minutes and 5 minutes, respectively. The two essential oils induced 100% mortality against *Ae. aegypti* larvae at 60 minutes. Furthermore, Sutthanont *et al*. (2010) suggested the use of the oil as a potential alternative source for developing novel larvicides to be used in controlling vectors of mosquito-borne disease. Medicinal plants with larvicidal properties have paramount importance for the local control of mosquito (Debella *et al.*, 2007). Champakaew *et al*. (2007) reported Zedoary oil exhibited pronounced potential against the fourth instar larvae of *A. aegypti* with an $LC_{50}$ and $LC_{99}$ of 33.45 and 83.39 ppm, respectively. Zedoary oil-impregnated sand granules provided remarkably longer activity, with a larval mortality of 100% for a period of 9 days; and mortality below 50% was obtained in week 3 of application. The efficacy in killing *A. aegypti* larvae and good biological stability of zedoary oil-impregnated sand granules make this product promising as an alternative to essential oil in the development of new botanical natural larvicide for use in mosquito control programs.

In the present study, it was found that both *B. rotunda* and *C. zedoaria* showed the potential for oviposition-deterrent activity against *Ae. Aegypti*. The 10% in ethyl alcohol of *C. zedoaria* showed the best activity with effective repellency (ER) of 98%. Thus, effective oviposition-deterrent could be useful and developed further in the integrated approach to mosquito control programmes against container-inhabiting mosquitoes (Xue *et al*., 2005).

Although our investigation demonstrated the larvicidal, pupicidal and oviposition-deterrent potential of the two herbal plants essential oils against *Ae. Aegypti*. Further investigations are needed to elucidate these oils against a wide range of mosquito species and study their efficacy to be utilized in preparing a commercial products to be used as a mosquiticidal agents. These results could encourage the search for new active natural compounds offering an alternative from other Thai indigenous plants.

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## References

Cavalca, P., Mançano, A., Gomes, Ml., de Assumpcao L., Reis B. and Bonato, CM. (2010). Homeopathic and larvicide effect of *Eucalyptus cinerea*essential oil against *Aedes egypti. Brazilian* Archives of Biology and Technology. 53:835-843.

Champakaew, D., Choochote, W., Pongpaibul, Y., Chaithong U., Jitpakdi, A., Tuetun, B. and Pitasawat, B. (2007). Larvicidal efficacy and biological stability of a botanical natural product, zedoary oil-impregnated sand granules, against *Aedes aegypti* (Diptera, Culicidae). Journal of Parasitology Research. 100(4):729-737.

Debella, A., Taye, A., Abebe, D., Mudi, K., Melaku, D.and Taye, G. (2007). Screening of some Ethiopian medicinal plants for mosquito larvicidal effects and phytochemical constituents. Pharmacologyonline 3:231-243

Gbolade, A.A., Dyedele, A.D., Sosan, M.B., Adewayin, F.B. and Soyela, O.I. (2000). Mosquito repellent activities of essential oils from two Nigerian *Ocimum* species. Journal of Tropical Medicinal Plants 1:146-148.

Govindarajan, M., Jebanesan A., Pushpanathan, T. and Samidurai, K. (2008). Studies on effect of *Acalypha indica* L. (Euphorbiaceae) leaf extracts on the malarial vector, *Anopheles stephensi* Liston (Diptera:Culicidae). Parasitology Research. 103(3):691-695.

Gubler, D.J. (1989). *Aedes aegypti* and *Aedes aegypti*-borne disease control in the 1990s: top down or bottom up. Charles Franklin Craig Lecture. The American Society of Tropical Medicine and Hygiene. 40(6):571-578.

Hardin, J.A. and Jackson, F.L.C. (2009). Applications of natural products in the control of mosquito transmitted diseases. African Journal of Biotechnology 8:7373-7378.

Jantan, I., Ping, W.O., Visuvalingam, S.D. and Ahmad, N.W. (2003). Larvicidal Activity of the Essential Oils and Methanol Extracts of Malaysian Plants on A*edes aegypti*. Pharmaceutical Biology 41:234-236.

Kaewnang, O, E., Ngampongsai, A., Subhadhirasakul, S. and Teerapol, S. (2011). Toxicity of fixed oil and crude extract from sa-dao-thiam, *Azadirachta excels* (Jack) seed kernel to *Aedes aegypti* (L.).Songklanakarin Journal of Science and Technology 33(1):43-49.

Kiat, T.S., Pippen, R., Yusof, R., Ibrahim, H., Khalid, N. and Rahman, N.A. (2006). Inhibitory activity of cyclohexenyl chalcone derivatives and flavonoids of fingerroot, *Boesenbergia rotunda* (L.), towards dengue-2 virus NS3 protease. Bioorganic and Medicinal Chemistry Letters 16:3337-3340.

Killeen, G.F., Fillinger, U. and Knols, BG. (2002). Advantages of larval control for African malaria vectors : low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. Malaria Journal 1: pp. 8.

Koul, O., Walia, S. and Dhaliwal, G.S. (2008). Essential oils as green pesticides: Potential and constraints. Biopesticides International 4(1):63-84.

Kramer, L.W. and Mulla, M.S. (1979).Oviposition attractants and repellents: Oviposition responses of *Culex* mosquito to organic infusions. Environmental Entomology 8:1111-1117.

Madhumathy, A.P., Aivazi, A.A. and Vijayan, V.A. (2007). Larvicidal efficacy of *apsicum annum* against *Anopheles stephensi* and *Culex quinquefasciatus.* The Journal of Vector Borne Diseases 44:223-226.

Mohan, D.R. and Ramaswamy, M. (2007). Evaluation of larvicidal activity of the leaf extract of a weed plant, *Ageratina adenophora*, against two important species of mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*. African Journal of Biotechnology 6:631-638.

Muhlisin, A. and Arum, P. (2006). Penanggulangan Demam Berdarah Dengue (DBD) di Kelurahan Singopuran Kartasura Sukoharjo Warta 9:123-129.

Nasirian, H. and Ladonni, H. (2006). Artificial bloodfeeding of *Anopheles stephensi* on a membrane apparatus with human whole blood. Journal of the American Mosquito Control Association 22(1):54-56.

Paul, A., Harrington, L.C. and Scott, J.G. (2006). Evaluation of novel insecticides for control of dengue vector *Aedes aegypti* (Diptera: Culicidae). Journal of Medical Entomology 43:55-60.

Phasomkusolsil, S. and Soonwer, M. (2012). The effects of herbal essential oils on the ovipositiondeterrent and ovicidal activities of *Aedes aegypti* (Linn.), *Anopheles dirus* (Peyton and Harrison) and *Culex quinquefasciatus* (Say). Tropical Biomedicine. 29(1):138–150.

Phasomkusolsil, S. and Soonwera, M. (2010). Potential larvicidal and pupacidal avtivities of herbal essential oils against *Culex quinquefasciatus* Say and *Anopheles minimus* (Theobald). Southeast Asian Journal of Tropical Medicine and Public Health 4:1342-51.

Phukerd, U. and Soonwera, M. (2013). Larvicidal and pupicidal activities of essential oils from Zingiberaceae plants against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus.* Southeast Asian Southeast Asian Journal of Tropical Medicine and Public Health 44:761-771**.**

Pitasawat, B., Champakaew, D., Choochote, W., Jitpakdi, A., Chaithong, U., Kanjanapothi, D., Rattanachanpichai, E., Tippawangkosol, P., Riyong, D., Tuetun, B. and Chaiyasit, D. (2007). Aromatic plant-derived essential oil: an alternative larvicide for mosquito control. Fitoterapia 78(3):205-210.

Rajkumar, S. and Jebanesan, A. (2009). Larvicidal and oviposition activity of *Cassia obtusifolia* Linn (Family: Leguminosae) leaf extract against malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitology Research 104(2):337-340.

Shultz, R.G., Blackmore, C. and Stanek, D. (2008). Mosquito-borne disease in Florida. Northeast Florida Medicine. 59:31-35.

Sutthanont, N., Choochote, W. and Tuetun, B. (2010). Chemical composition and larvicidal activity of edible plant-derived essential oils against the pyrethroid-susceptible and -resistant strains of *Aedes aegypti* (Diptera: Culicidae). Journal of Vector Ecology 35:106-115.

Suwasono, H., Mardjan and S. Uji (2004). Coba beberapa insektisida golongan Pyrethroid sintetik terhadap vektor demam berdarah dengue *Aedes Aegypti* Di Wilayah Jakarta Utara. J Ekol Kes 3:43-47

Tawatsin, A., Asavadachanukorn, P. and Thavara, U. (2006). Repellency of essential oils extracted from plants in Thailand against four mosquito vectors (Diptera: Culicidae) and oviposition deterrent effects against *Aedes aegypti* (Diptera: Culicidae). Southeast Asian Journal of Tropical Medicine and Public Health. 37:915-931.

Tiwary, M., Naik, S.N., Tewary, D.K., Mittal, P.K. and Yadav, S. (2007). Chemical composition and arvicidal activities of the essential oil of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito vectors. Journal of Vector Borne Diseases. 44:198-204.

WHO (World Health Organization). (2013) "Dengue and severe dengue." Fact sheet N°117 Updated September 2013.

World Health Organization (2012) Global strategy for dengue prevention and control 2012-2020: pp. 1-35.

World Health Organization (WHO). Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/2005.13

Xue, R.D., Ali, A. and Barnard, D.R. (2005). Effects of forced egg-retention in *Aedes albopictus* on adult survival and reproduction following application of DEET as an oviposition deterrent. Journal of Vector Ecology 30(1):45-48.

Xue, R.D., Barnard, D.R. and Ali, A. (2001). Laboratory and field evaluation of insect repellents as oviposition deterrents against the mosquito *Aedes albopictus*.Medical and Veterinary Entomology 15(2):126-131.

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