Laboratory Evaluation of Traditionally made Coconut Oil as a Surface Larvacide for Malaria Vector Control

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Abstract

Environmental concerns have resulted in the search for environmentally friendly natural oils for use as mosquito larvacide. Methylated coconut oil (MCO) has been found to be toxic to mosquito larvae. However, the use of MCO is limited by resource constraints in rural communities in Papua New Guinea (PNG). In PNG and other Pacific Island countries, coconut oil is produced using traditional methods. This study evaluated the toxicity of traditionally made coconut oil to fourth instar *Anopheles stephensi* larvae. The results showed that traditionally made coconut oil is toxic to fourth instar *Anopheles stephensi* larvae. Traditionally made coconut oil has the potential for use as a larvacide for malaria vector control in community based programs utilizing community participation in the production and use of coconut oil.

Key words: mosquito larvacide, malaria vector control, coconut oil, toxicity, benchmark dose (BMD), methylated coconut oil (MCO)

Introduction

Mosquito-borne diseases can be controlled by reducing the larval stages of mosquito species. Larvae control or source reduction involves the use of biological control agents and the application of chemicals to breeding sites. In the 1930s and 1940s petroleum-based products were used in malaria controlled programs in Brazil and Egypt (Foley and Frances 2005). Oiling was also useful where the larval sites were limited in size and number (Foley and Frances 2005). The use of DDT to control the adult mosquito during the global malaria eradication program resulted in a decrease use of oils as a form of larvacide. Since then, costs, environmental concerns, and insecticide resistance have increased, making environmental management within integrated control operations more attractive (Gunasekaran et al. 2004, Thekkevilayil et al. 2004, Sivagnaname and Kalyanasundaram 2004, Foley and Frances 2005, Sengottayan 2006).

The toxicity of a larvacide depends on its volatility (Foley and Frances 2005). Although pure plant and vegetable oils are too viscous to be used as a mosquito larvacide, their physical and chemical properties can be modified to form methyl and ethyl esters of fatty acids (Foley and Frances 2005). The spreading

pressures of lipophylic products can also be increased by the addition of surfactants (Lampman et al 2000, Foley and Francis 2005). Methylated soy oil (MSO) mixed with the non-ionic surfactant Pyroter CPI-40 has been shown to be as effective as the petroleum-derived larvacide, Golden Bear Oil (GB-1111) in laboratory assays against *Culex pipiens* and *Anopheles stephensi* (Lampman et al. 2000). In a field trial, Dennet et al (2000) reported MSO with Pyroter to be comparable with *Bacillus thuringiensis* var. *Israelensis de Barjac* (Bti) in controlling *Anopheles quadrimaculatus* larvae. Foley and Francis (2005) evaluated the toxicity of methylated coconut oil (MCO) to *Anopheles farauti* and *Culex pipiens* and found MCO to be more toxic compared to GB-1111 after 24 hours. Furthermore, MCO without surfactant was also toxic to mosquito larvae (Foley and Francis 2005). However, for LD95 (lethal dose needed to kill 95% of test subjects), GB-1111 was more toxic than MCO for both *Anopheles farauti* and *Culex pipiens.* (Foley and Francis 2005).

The coconut palm *Cocos nucifera* L. is a native plant and abundant in many tropical countries where malaria is endemic. Judging from the effectiveness of MSO and MCO, the methylated form of coconut oil offers communities a local product for malaria vector control. However, in remote communities, even the simplest technology needed and the costs of producing MCO do not permit its use as a larvacide. Coconut oil is produced using traditional methods in the Pacific for cooking and cosmetic uses. From the studies on methylated coconut oil (Foley and Francis 2005) it can be hypothesized that traditionally made coconut oil will be toxic to mosquito larvae. To evaluate its toxicity, traditionally made coconut oil was bought from local markets in the Solomon Islands and transported to Japan for toxicity studies. The objectives of the study were to: (1) evaluate the toxicity of traditionally made coconut oil by calculating its LD50 (lethal dose needed to kill 50% of test subjects) and (2) formulate a regression equation to calculate the amount of coconut oil that may be needed for field evaluation studies.

Anopheles stephensi is a known malaria vector in Asia (Senthil 2006). Using the World Health Organization (WHO) protocol for testing new larvacide (WHO 2005), fourth instar *Anopheles stephensi* larvae were used to determine the toxicity of traditionally made coconut oil. The results of the study are presented and discussed.

Materials and methods

Mosquito

Fourth instar *Anopheles stephensi* larvae were used in the experiments. Larvae and adults were maintained using standard protocols in an insectary with temperature maintained at 26^{o}C and relative humidity 65% with a 15 hours 8 hours day-night cycle. Light was provided by four 40-watt fluorescent light bulbs. Eggs were hatched in 250ml of de-chlorinated tap water in plastic cups (surface area = 95 cm^{2}). At the late second to early third instar stage, larvae were transferred to 33- x 24- x 7-cm pans. Larvae were fed on ®Tetra Min

baby fish food (http//:www.tetra-jp.com). Water was changed every other day. Only fourth instar larvae were used in the toxicity experiments.

Evaluation of coconut oil against mosquito larvae

The trials were performed in a laboratory at room temperature. Humidity was controlled. The experiments were conducted during daylight hours. A total of twenty five fourth instar larvae were used per experiment with five larvae in a test cup. Each test had a corresponding control. Larvae were individually pipetted into 150ml of de-chlorinated tap water then varying amounts of coconut oil were added into plastic cups.

Traditionally made coconut oil was bought from local markets in Solomon Islands. Water was added in the controls. Food was not added in the cups to prevent bacterial overgrowth. The number of dead larvae were counted after 24 hours and compared to controls. The toxicity trials were conducted on four different occasions using four different generations of larvae. The data were collated and the averages of the data used to calculate the mortality rate and toxicity. The surface area covered by the film of coconut oil on water was also calculated. A regression equation was then formulated from this data to estimate the amount of coconut oil that may be required in field studies.

Statistical analysis

In assessing the toxicity of insecticides, the dose that kills 50% (LD50) or 90% (LD90) of the samples compared to the control has been the standard approach to determine how toxic a chemical is. The WHO recommends that the log-probit statistical model be used to calculate the LD50 or LD90 when assessing the toxicity of a new larvacide (WHO 2005). The use of the benchmark dose (BMD) is now increasingly being preferred over the traditional no-observed-adverse-effect-level (NOAEL) or lowest-observed-adverse-effect-level (LOAEL) approach in assessing the risk of chemicals to humans (Crump 1984, Filipsson and Victorin 2003, Foronda et al 2007). Several available softwares can be used to calculate the bench mark dose (Filipsson and Victorin 2003). The United States Environment Protection Agency (USEPA) has produced and released the benchmark dose software (BMDS) for use (USEPA 2007). The BMDS has been found to be user-friendly and has several statistical models to fit most study designs (Sand et al 2002, Filipsson and Victorin 2003).

The benchmark dose is defined as an exposure due to a dose of a substance associated with a specified low incidence or risk, generally in the range of 1% to 10%, of a health effect; or the dose associated with a specified measure or change of a biological effect (USEPA 2007). The extra risk of 1% or 10% is a function of the benchmark response (BMR). A BMR = 0.01 would account for 1% extra risk and BMR = 0.1 would give 10% extra risk. Therefore a BMR = 0.5, the BMD50 would be the dose at which the risk of incidence of the defined biological effect is 50% (personal communication, USEPA 2007), in other words the LD50, and the 95% confidence lower one sided limit on the BMD50 (BMDL50) calculated is the lower effective dose at which the defined

biological effect is observed. Using this method we assessed the toxicity of traditionally made coconut oil as a surface larvacide on fourth instar *Anopheles stephensi* larvae. Log-probit analysis was done using the model for dichotomous data (Sand et al 2002). The bench mark dose software (BMDS) version 1.4.1 was used. The BMDS can be freely downloaded from the United States Environment Protection Agency website (USEPA 2007). However, the software is continuously being updated.

The regression equation was formulated using computational tools set up on a website (Lowry 2006).

Results

The results of the experiments showed that traditionally made coconut oil was toxic to fourth instar *Anopheles stephensi* larvae. Over fifty percent mortality was observed with 10ul of coconut oil (Table 1.0). Ninety percent mortality was observed at 80ul.

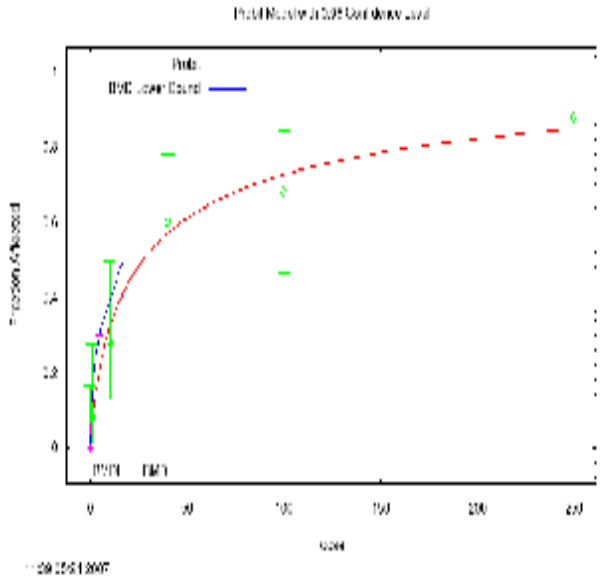
Table 1.0. *Anopheles stephensi* 4th instar larvae percentage mortality after 24 hours of exposure of coconut oil

Coconut oil (ul)	Dead*	Alive*	Mortality (%)
0	0	25	0
0.5	3.5	21.5	14
1	4.5	20.5	18
3	11.5	13.5	46
5	11	14	44
10	14	11	56
15	13	12	52
20	15	10	60
30	20	5	80
40	17	8	68
80	23	2	92
90	24	1	96

* Figures are the averages of four replicates of the experiment.

Using the benchmark dose approach, the BMD50 (or LD50) was 27.33ul (Figure 1.0) although fifty percent mortality was already seen at 10ul. This difference is due to the data input requirements of the BMDS software which has to generate the line of best fit to calculate the BMD50. The line of best fit is calculated by trial and error using several different values. We used the log-probit model. This is the recommended model for dichotomous data (USEPA 2006, Foronda et al 2007) as in this study.

Figure 1.0 Illustration of benchmark dose showing the BMD50 and the 95% confidence lower one sided limit (BMDS version 1.4.1). BMD50 = 27.33ul; BMDL50 = 16.92ul



The regression equation is shown in figure 2.0. It can be deduced from the graph that as the surface area increased, the quantity of coconut oil needed to cover the treatment surface area increased. This increase is exponential and begins at the 50cm^2 mark.

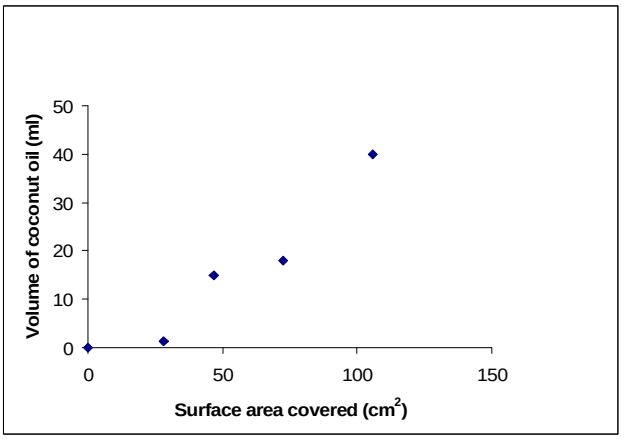


Figure 2.0 Amount of coconut oil needed in relation to surface treatment area. Regression equation: y = -4.39+0.38x. r = 0.96. Confidence interval for r (rho), 95% = 0.46 – 0.99. Confidence internal for slope of regression, 95% = 0.16 – 0.59.

Discussion

Traditionally made coconut oil has a higher LD50 (27.33ul) compared to MCO (8.6ul) and MSO (3.8ul, 6.2ul and 11.3ul) (Lampman et al 2000, Foley and Frances 2005). This difference may be due to the methylation of these two oils that makes them more volatile. Furthermore, the addition of a surfactant to MSO decreased the amount of oil needed to treat (Dennett et al 2000, Lampman et al 2000). Toxicity of natural oils depends on their volatility and the process of methylation increases the toxicicity of coconut oil (Foley and Frances 2005). Traditionally made coconut oil is not volatile. It kills by creating a film of oil on the water's surface preventing mosquito larvae from obtaining oxygen from the atmosphere.

The regression equation calculated showed that as the treatment surface area increased, the amount of coconut oil that is needed to cover the treatment area increased exponentially (Figure 2.0). This is because traditionally made coconut oil is viscous and heavy. As a result, as more and more coconut oil is added onto the water's surface, the coconut oil would begin to sink rather than spread. To overcome this chemical property of coconut oil, a suitable surfactant needs to be identified and added. The function of a surfactant is to reduce surface tension between the water and coconut oil molecules. Its addition would make coconut oil thinner and spread more easily over the treatment surface area. Thus a small amount of oil, pre-mixed with the suitable surfactant, would cover a large surface area.

Coconut oil can be produced cheaply using traditional methods in PNG. The technique and knowledge of producing coconut oil using traditional methods is well known, both in PNG and in other Pacific Island countries. Our study showed that traditionally produced coconut oil is toxic to *Anopheles stephensi* larvae. Methylated coconut oil (MCO) is also toxic to other *Anopheles* species but the cost and technology required to produce it makes it unsuitable for rural communities (Foley and Francis 2005). Furthermore, the production and use of MCO would be unsustainable. Other natural oils have also been shown to have larvacidal properties and are now commercially available (Lampman et al 2000, Foley and Frances 2005).

However, in PNG and in other Pacific Island countries, the use of commercially available oils to control malaria vectors in rural communities may not be sustainable due to finance and resource constraints. Coconut is abundant in the Pacific and traditionally made coconut oil can be produced with simple technology. It is commonly used for cooking and for cosmetic purposes but has the potential for use as a larvacide for controlling malaria in rural communities in PNG. Using community based programs; coconut oil can be produced with the participation of the community and used for malaria vector control. However, field studies are needed to evaluate its effectiveness in community based programs. A suitable surfactant also needs to be indentified.

Conclusion

Our study showed that traditionally made coconut oil is toxic to *Anopheles stephensi* fourth instar larvae. Although it has a higher LD50 compared to commercially available oils, its other advantages makes it a good candidate for use in community based programs for malaria vector control. However, its viscosity will need to be overcome with the addition of a suitable surfactant. Further studies are needed, including field studies, to continue to evaluate its effectiveness. A suitable surfactant will also need to be identified.

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Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research [KAKENHI (174060080)] from the Ministry of Education, Culture, Sports, Science and Technology. We would also like to thank the Government of Japan for financially supporting Dr Rodney L. Itaki and Dr Arif-Ul Hasan through its Monbusho scholarship program.

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