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Laboratory Evaluation of Traditionally Produced Coconut Oil as A Surface Larvacide Against *Anopheles Stephensi* Fourth Instar Larvae.

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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 has been found to be toxic to mosquito larvae. However, the use of methylated coconut oil is limited by resource constraints in rural communities in Papua New Guinea and other Pacific Island countries where coconut oil is produced by traditional methods. This study evaluated the toxicity of traditionally produced coconut oil to fourth instar *Anopheles stephensi* larvae. The results showed that traditionally produced coconut oil is toxic to fourth instar *Anopheles stephensi* larvae.

The results showed that coconut oil produced by traditional method is toxic to fourth instar *Anopheles stephensi* larvae. The coconut oil can be used as a larvacide for malaria vector control in community based programs utilizing community participation in the production and use of coconut oil for large scale use of coconut oil. However, a suitable surfactant needs to be identified.

Key Words: *Anopheles stephensi*, methylated coconut oil, *cocos nucifera*, larvacide.
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INTRODUCTION:

Mosquito-borne diseases can be controlled by reducing the larval stages of mosquito species. Larvae control methods include source reduction, use of biological control agents and applying chemicals to breeding sites. In the 1930s and 1940s petroleum-based products were used in malaria controlled programs in Brazil and Egypt [1]. Oiling was also useful where the larval sites were limited in size and number [1]. The use of dichlorodiphenyltrichloroethane (DDT) to control the adult mosquito during the global malaria eradication program resulted in a decreased 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. The toxicity of a larvacide depends on its volatility [1]. 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 [1]. The spreading pressures of lipophilic products can also be increased by the addition of surfactants [1].

Methylated soy oil (MSO) mixed with polyoxyethylene (40) hydrogenated castor oil monopyroglutamate monoisostearate 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 [2]*. Field trials have shown that MSO with Pyroter to be comparable with *Bacillus thuringiensis* var. *Israelensis de Barjac* (Bti) in controlling *Anopheles quadrimaculatus* larvae [1]. Foley and Francis [1] 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 [1]. 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 [1]*.

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, 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. Based on previous studies [1] on MCO it can therefore be hypothesized that traditionally produced coconut oil will be

toxic to mosquito larvae. To evaluate its toxicity, traditionally produced coconut oil were bought from local markets in Solomon Islands and transported to Japan for toxicity studies. The objectives of the study were to evaluate the toxicity of traditionally made coconut oil by calculating its LD50 (lethal dose needed to kill 50% of test subjects) and to formulate a regression equation to calculate the amount of coconut oil that will be needed for field evaluation studies.

Anopheles stephensi is a known malaria vector in Asia [3]. Using the WHO protocol for testing new larvacides [4, 5], fourth instar *Anopheles stephensi* larvae were used to determine the toxicity of traditionally made coconut oil to mosquito larvae.

MATERIALS AND METHODS:

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 [1, 4, 5]. 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 33x 24 x7cm pans. Larvae were fed on ®Tetra Min baby fish food. Water was changed every other day. The traditionally produced coconut oil used in this study was purchased from local markets in the Solomon Islands. The trials were performed in an insectary with automated controls for room temperature and humidity. The experiments were conducted during daylight hours. The toxicity experiments were done following WHO protocol for laboratory evaluation of a new larvicide [4, 5]. A total of 25 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 and then varying volumes of the coconut oil were added into the plastic cups. Water instead of coconut oil was added in the controls. Food was not added in the cups to prevent bacterial overgrowth. After 24 hours the number of dead larvae was counted. The same procedures were repeated on four different occasions using four different generations of larvae.

The data were collated and used to determine the mortality rate and toxicity. The surface area covered by the film of coconut oil on water was also determined.

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 [4, 5]. 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 [6, 7]. The BMD is 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 [8].

The extra risk of 1% or 10% is a function of the benchmark response (BMR). A BMR of 0.01 would account for 1% extra risk and BMR of 0.1 would give 10% extra risk. Therefore at BMR of 0.5, the BMD50 would be the dose at which the risk of incidence of the defined biological effect is 50% [8], in other words the LD50 and the 95% confidence lower one sided limit on the BMD50 (BMD LD50) calculated is the lower effective dose at which the defined biological effect is observed.

This criteria was used in this study to determine the toxicity of the traditionally produced coconut oil as a surface larvacide on fourth instar *Anopheles stephensi* larvae. Log-probit analysis was done using the model for dichotomous data [8]. The bench mark dose software (BMDS) version 1.4.1 was used. The regression equation was formulated using the computational tools VassaStats [10].

RESULTS AND DISCUSSION:

Table 1 shows the percent mortality rates obtained after 24 hours exposures to the traditionally produced coconut oil. The 56% and the 92% mortality rates observed with 10ul and 80ul of the coconut oil respectively indicates effective toxicity to the fourth instar Anopheles stephensi larvae. Using the benchmark dose approach, the BMD50 (or LD50) was 7.54ul (Figure 1.0) although 50% 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 was calculated by using the dose of coconut oil and the mortality data from table 1. The lowest dose of the coconut oil at which toxic effect can be observed (BMD LD50) on *Anopheles stephensi* mosquito larvae was 4.44ul. The log-probit model was used because it is the recommended model for dichotomous data [7, 8]. Traditionally produced coconut oil has a higher LD50 compared to MCO and MSO [1, 2]. This difference may be due to the methylation of these two oils that makes them more volatile. Toxicity of natural oils depends on their volatility and the process of methylation increases the toxicity of coconut oil [1]. Traditionally produced coconut oil is not volatile thus it tends to kill only by suffocating mosquito larvae.

Table 1: *Anopheles stephensi* fourth instar larvae mortality rate after 24 hours of exposure to traditionally produced 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.

Figure 1: Illustration of BMD showing the BMD50 and the 95% confidence lower one sided limit (BMDS version 1.4.1) [8]. BMD50 = 7.54ul; BMDL = 4.44ul;

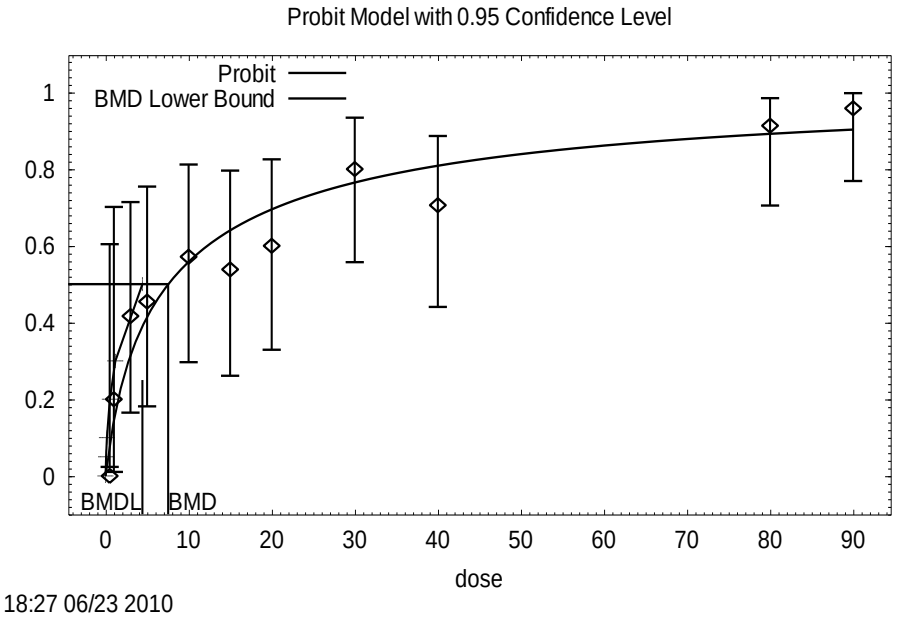

Figure 2: Amount of coconut oil needed in relation to surface treatment area. Regression equation: Y = -4.39+0.38x. r = 0.96. Confidence interval for r (rho), 95% = 0.46 – 0.99. Confidence internal for slope of regression, 95% = 0.16 – 0.59.

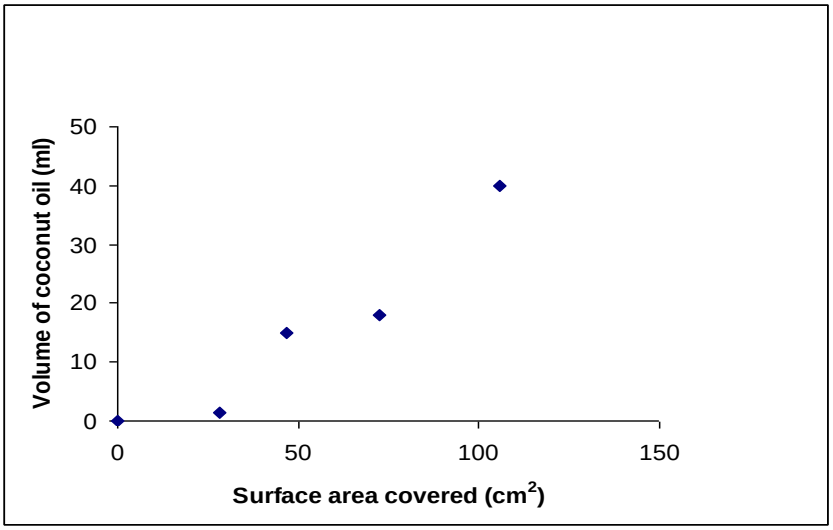

Figure 2 shows the regression equation (Y = -4.39+0.38x) obtained when our data were entered in the software VassaStats [10]. The regression equation indicated that as the treatment surface area increases, the amount of coconut oil needed to cover the treatment area increased exponentially. This behavior in the graph is due to the high viscosity and density of traditionally made coconut oil. 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 molecules and the 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 in PNG [1]. Furthermore, finance constraints and lack of technical expertise would make the

production and use of MCO unsustainable. Other natural oils have also been shown to have larvacidal properties and are now commercially available [1, 2]. Coconut is abundant in the Pacific and traditionally made coconut oil can be produced with simple technology and the methodology is common knowledge. It is commonly used for cooking and for cosmetic purposes but has a potential for use 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 to control malaria vectors. However, producing coconut oil using traditional methods is labour intensive (personal observation) and its production may not be cost-effective in terms of labour cost.

One of the main challenges of a community based program would be to keep people motivated to produce coconut oil. Since traditionally made coconut oil is a sought after product in PNG markets, people may feel that using coconut oil to kill mosquito larvae may be a waste of time and effort when they can easily sell the product and obtain an income to buy a mosquito net.

CONCLUSION:

Our study showed that traditionally made coconut oil is toxic to *Anopheles stephensi* fourth instar larvae. This finding agrees with current data that natural oils can be toxic to mosquito larvae. Although traditionally made coconut oil has a higher LD50 compared to commercially available oils, its advantage of using low cost technology to produce it makes it a good candidate for use in community based programs in rural communities to control malaria. However, its high viscosity will need to be overcome with the addition of a suitable surfactant.

Further studies are needed to continue to evaluate its effectives and find a suitable surfactant. The unavailability of a suitable surfactant makes this area of research more challenging.

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