Journal of Bioscience and Bioengineering
VOL. 121 No. 3, 299–302, 2016

www.elsevier.com/locate/jbiosc

Utilization of biodiesel by-products for mosquito control

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Received 28 January 2015; accepted 24 July 2015
Available online 18 August 2015

The current paper has elaborated the efficient utilization of non-edible oil seed cakes (NEOC), by-products of the biodiesel extraction process to develop a herbal and novel mosquitocidal composition against the *Aedes aegypti* larvae. The composition consisted of botanical active ingredients, inerts, burning agents and preservatives; where the botanical active ingredients were karanja (*Pongamia glabra*) cake powder and jatropha (*Jatropha curcas*) cake powder, products left after the extraction of oil from karanja and jatropha seed. The percentage mortality value recorded for the combination with concentration, karanja cake powder (20%) and jatropha cake powder (20%), 1:1 was 96%. The coil formulations developed from these biodiesel by-products are of low cost, environmentally friendly and are less toxic than the synthetic active ingredients.

[**Key words:** Biodiesel; By-products; Biopesticides; Phorbol esters; Mosquito-coil; Karanja; Jatropha; *Aedes aegypti*; Knockdown]

Mosquitoes are known to spread diseases like Malaria and Dengue fever in tropical countries. A number of mosquito repellents like coils, vaporizers, aerosol sprays are used to control indoor biting of mosquitoes. When compared with mats and liquid vaporizers, coils have been considered as an effective mosquito repellent, because when they are burnt, they continuously emit smoke along with the active material used, which prevents mosquito biting for a considerable period of time (1).

Numerous plant extracts and phytochemicals including essential oils are known to be potential sources of commercial mosquito control agents/products (2,3). Among the natural pyrethrins, those incorporating the alcohol pyrethrolone, pyrethrin I and II, are the most abundant and account for most of the insecticidal activity (4).

Green pesticides refer to a number of naturally derived and environment friendly pest control materials that can contribute to reduce the pest population and increase food production as they have more compatibility with the environmental components than the synthetic ones (5). In this paper, attempts have been made to utilize biodiesel by-products (left-outs of the biodiesel extraction process) as green pesticides to develop coil formulations for mosquito control.

Considering the extraction of oil from the non edible seeds, karanja (*Pongamia pinnata*) and jatropha (*Jatropha curcas*) have been selected as major source of non-edible oil for production of biodiesel (6). The technology of biodiesel production consumes only extracted vegetable oil from non-edible seeds and renders a large amount of unutilized biomass as seed cake. Disposal of cake after expelling oil from seed has become a major concern in the past few years. Due to its toxic nature, the cake neither can be used for animal feeding nor can be used in agricultural farming. The presence of non-edible oil seed cakes in the open atmosphere would generate gases (CH_4, N_2O, H_2S, NH_3, and CO_2) and volatile organic compounds (VOCs) due to self decomposition of biomass over the action of various microorganisms. Familiar strategies adopted for the management and efficient utilization of the biomass is the generation of biogas by their anaerobic digestion, fertilizer for soil enrichment as it has fine N, P, K ratio (7).

The non-edible seeds of jatropha (*Jatropha curcas*) and karanja (*Pongamia glabra*) contain some toxic components, i.e., phorbol esters (Fig. S1) in *Jatropha curcas* and karanjin (Fig. 1) in *P. glabra*, which can be exploited to function as bio-pesticides (8). Literature survey does give some information about the insecticidal activity of neem and karanja oil cakes in combination against mosquito vectors (9–11).

In the present study, the knocking down and insecticidal activity of the coil formulation containing biodiesel by-products based active ingredients individually and in combination has been compared with the blank coil as well as with a commercial mosquito repellent.

MATERIALS AND METHODS

Karanja (*Pongamia glabra*) and jatropha (*Jatropha curcas*) cakes were procured from Centre for Rural Development and Technology, Indian Institute of Technology, Delhi. Saw dust (Wood dust), jigat (*Machilus macrantha*), sodium benzoate, cow dung and guar gum (*Litsea glutinosa*) were purchased from Burari, New Delhi (Fig. 2).

Preparation of karanja and jatropha cake based mosquito coil Mosquito coils were prepared using the ingredients in different compositions, i.e., saw dust as organic filler, jigat and guar gum as binding materials, sodium benzoate as preservative and cow dung as burning material along with karanja and jatropha cake as the

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http://dx.doi.org/10.1016/j.jbiosc.2015.07.010

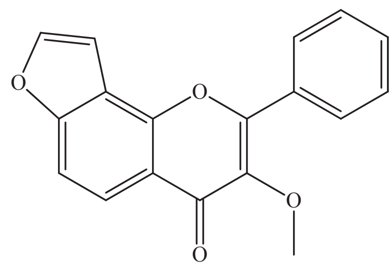

FIG. 1. Structure of karinjin (3-Methoxyflavone).

active ingredients. In case of blank coil, burning material used was cow dung only (Table 1). The individual ingredients were washed properly, dried at room temperature for two days and minced in a mixer into a fine powder (50 μm) and finally extruded through a coil machine to get the final product, i.e., mosquito coil with desired shape and size. The mixture of the ingredients thus ground was used to prepare the coils immediately or preferably within 12 h of obtaining the same.

Mosquito culture and bioassay Eggs of *Aedes aegypti* were collected from National Institute of Malaria Research (NIMR), Delhi, India. They were colonized and eighteen generations were maintained at 27 ± 2°C, 75–85% RH under a photoperiod of 14:10 h (light/dark) continuously in the laboratory, free of exposure to pathogens, insecticides or repellents. Under these conditions, full development from egg to adult lasted for about 3–4 weeks. Larvae were fed on finely ground dog biscuit and yeast extract in the ratio of 3:1. Water was changed every day to avoid scum formation, which might kill the larvae. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages (30 × 30 × 30 cm dimension) for adult emergence. The adults were reared in respective glass/plastic cages (30 × 30 × 30 cm dimension) (12). The adult colony was provided with ten percent sucrose solution and it was periodically blood-fed on restrained rabbits. After 72 h, ovitrap was kept in the cages for egg laying and the eggs were collected and transferred to enamel trays. The developmental stages, larvae and adults (females), were incessantly available for conducting the experimental trials. They were maintained at the same condition.

The bioassay was conducted in a peet-grady chamber measuring 120 × 120 × 120 cm following the method of Chadwick (13). Mosquitoes in the polyethylene cup and coils, both were introduced into the chamber through a 30 × 30 cm sliding window at the mid-bottom on one side of the chamber. The mosquito coil was kept on a stand in the middle of the chamber and allowed to burn for 2 min before 200 sucrose-fed mosquitoes were released into the chamber. Knocked-down mosquitoes (i.e., those that no longer maintained normal posture and were unable to fly or were on their backs) were recorded at l-rain intervals up to 6 h or until total knock-down was achieved. Knocked-down mosquitoes were placed in a clean container containing cotton wool soaked with 5% sucrose solution and the mortality of the mosquitoes was observed after 24 h. The above procedures were carried out in triplicate for each coil formulation. Control was performed by exposing the mosquitoes to the smoke of a blank coil. Knock-down times (KD_{50} and KD_{90}, as the minutes needed to knock-down 50% and 90% of mosquitoes, respectively) were determined by the probit analysis (14).

RESULTS

The mortality value, in case of coil containing 20% karanja and 20% jatropha cake powder, was found to be 96% (Table 2). It was found that KD_{50} and KD_{90} values gradually increased with the coil composition containing karanja (KD_{50} = 73.91 min) and jatropha (KD_{50} = 88.03 min) cake powder individually in the recipe, while the values showed a decrease for compositions containing active ingredients in combination, i.e., KD_{50} = 43.18 min. The KD_{50} values of the coils prepared of the cakes individually and in combination were also compared with the KD_{50} value of commercial mosquito repellent which was reported to be 16.17 min (15). The value was obviously less due to toxic nature of the active ingredient, i.e., trans allethrin.

Table 2 results reveal that with the increase in concentration of karanja and jatropha cake, i.e., 16–40%, mortality value also increases with respect to concentration of active ingredient in the coil prepared. After 28% concentration of both cakes, i.e., karanja and

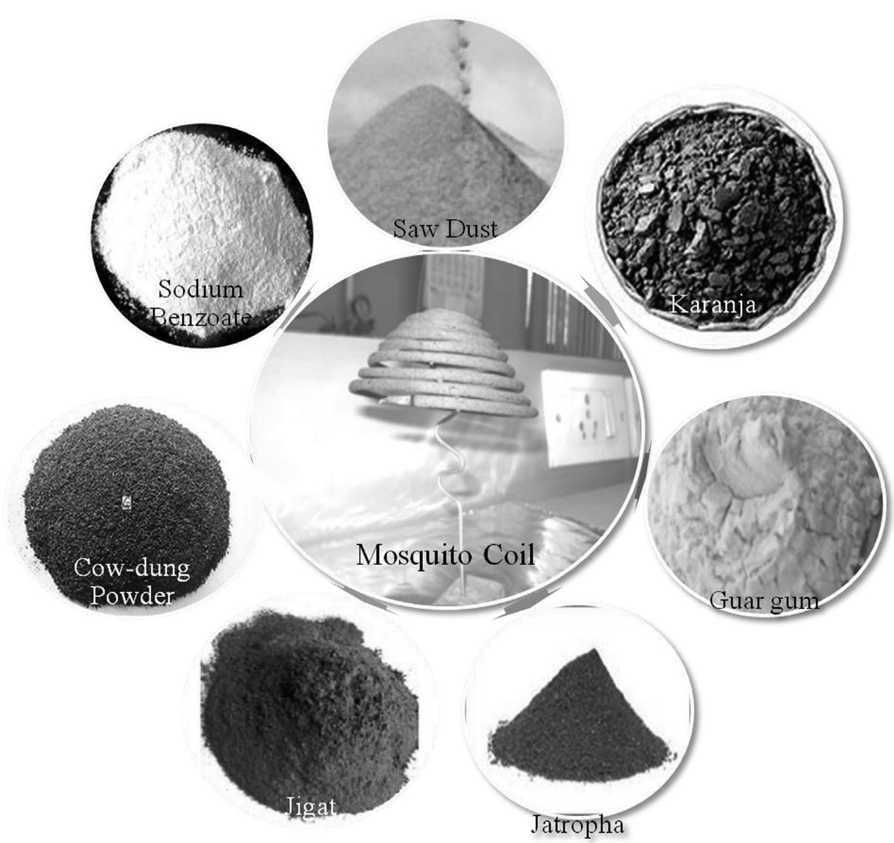

FIG. 2. Various ingredients used in the preparation of mosquito coil.