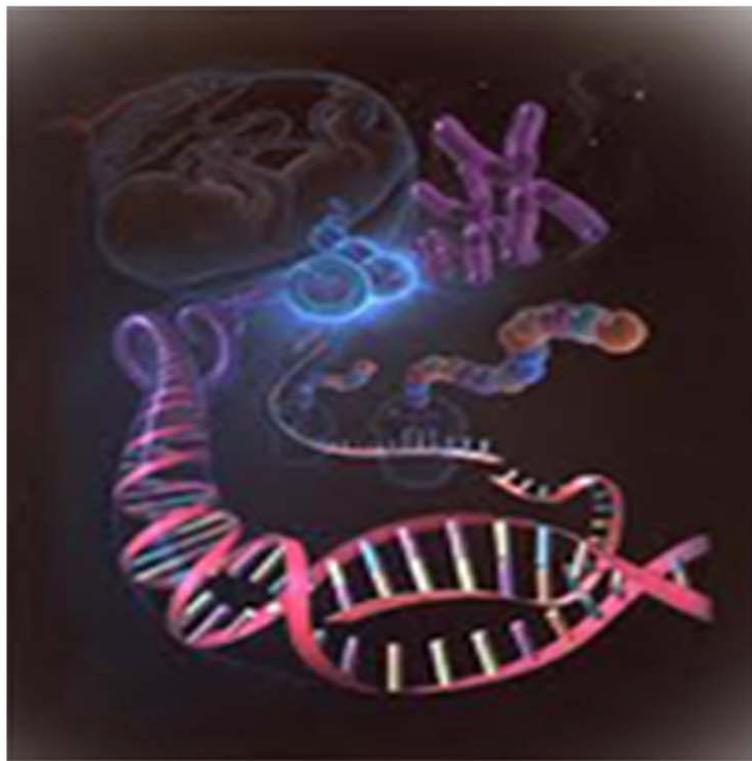




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Research Paper

# INVESTIGATION OF THE REPELLENCE ACTIVITY OF BIO-OUT, A NATURAL MOSQUITO REPELLENT

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Mosquito repellents are important tools for prevention of dreadful diseases as well as painful mosquito bites. Lemongrass essential oil (*Cymbopogon citratus*) has been investigated for its repellent activity against mosquitoes respectively. Tests were performed with out the contribution of human volunteers. Number of visible mosquitoes was noticed. Approximately 80% repellence activity has been observed. Lemongrass oil exhibited an average of 30 min protection time against mosquitoes to make them away from the observed area, All-out (50-80%) protection time in comparison to the activity of the best known chemical insect repellent, N, N-diethyl-m-toluamide (DEET). Determining the effectiveness of lemon grass (*Cymbopogon citratus*) as mosquito repellent is the main goal of the study. Specifically, it intends to apply the extraction procedure techniques to extract the essential oils of lemon grass. It can be concluded that lemon grass oil is a promising natural repellent due to its safety advantage over chemical repellents.

**Keywords:** Bio-out bottle, Cream formulation, Repellence, *Cymbopogon citratus*

## INTRODUCTION

Diseases transmitted by mosquitoes remain a major source of death worldwide. Mosquitoes alone transmit disease to more than 700 million persons annually (Osimitz and Grothaus, 1995). Mosquito borne diseases currently represent a greater health problem in tropical and subtropical climate. No part of the world is immune to their risks. The worldwide threat of mosquito transmitted diseases, with their associated morbidity and mortality, underscores the need for

effective mosquito repellents (World Health Organization, 1999; Moreira *et al.*, 2002)

Protection from mosquito bites is achieved by using insect repellents. Plant based mosquito repellents are an alternative to the use of insecticide based repellent. They may be applied to the skin to protect an individual from the bites of mosquitoes. A single bite from an infected mosquito can result in transmission of disease and the fact that people use synthetic chemical-based mosquito repellents to protect themselves

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from mosquito bite but in exchange get more serious problems. On an individual level, people adopt numerous personal protective measures to reduce the risk of mosquito bites (Monath *et al.*, 2001), it is important to know which repellent product can be relied on to provide predictable and prolonged protection from mosquito bites. Commercially available insect repellents are of synthetic and plant-derived essential oils. The best known chemical insect repellent is N, N-diethyl-3-methyl benzamide (DEET). People disagree to apply DEET to their skin, carefully seek out other repellent products. DEET is still the most widely used mosquito repellent. It has generally been regarded as safe, but toxic effects have been recorded, including encephalopathy in children, urticaria syndrome, anaphylaxis, hypotension and decreased heart rate (Peterson, 2001). DEET is a plasticizer which is capable of dissolving watch crystals, frames of glasses, and some synthetic fabrics. It has an unpleasant odor and leave skin feeling dry. Natural products derived plant-based essential oils have been gaining popularity as alternatives to DEET.

*Cymbopogon citratus* (lemon grass) belonging to the family Poaceae is a genus of about 55 species of grasses, native to warm temperate and tropical regions of the World. Lemongrass oil is the essential oil obtained from the aerial part of *Cymbopogon citratus*. The plant has been widely recognized for its ethno botanical and medicinal usefulness (Dalziel, 1937). It has several therapeutic properties (Akendengue, 1992) of its oil and extracts have been reported.

## METHODS

### Extraction of Lemongrass Essential Oil

Leaves of *Cymbopogon citratus* were screened

and unwanted leaves were removed before using them, a mixture of 900 g of the fresh leaves of *Cymbopogon citratus* and distilled water which was subjected to steam distillation. Volatile oil generated in the process was collected. The mixture of oil and water obtained from the steam distillation was separated with a separating funnel. The oil after the separation was dried over anhydrous sodium sulphate and kept in the refrigerator at 4 °C (Guenther, 1965).

### Preparation of Bio-Out

1 mL of the pure essential oil was diluted by mixing with 99 mL of white vinegar (5% acetic acid) and was perfectly mixed at room temperature which makes the final volume 100 mL. At this stage 45 mL of this oil was taken and fill this liquid in to an empty all-out and we named this as bio-out as it contains natural oil in an All-out bottle and tested for its mosquito repellent activity by comparing the results with All-out, a known mosquito repellent in the market and these two results were compared with the control by not keeping any of the repellents.

### Preparation of Repellent Cream

Pure aloe vera gel (20 g) was weighed accurately into wide-mouth glass bottle and melted in water bath at temperature of about 50 °C. At this stage, 1 g of the lemon grass oil was transferred into the bottle. The mixture was mixed properly that the oil is uniformly mixed with the aloe vera jelly. The product (5 % w/w cream) is then taken for repellency test.

### Methods of Conducting Repellent Test of Bio-out

Tests were undertaken to determine the effectiveness of the bio-out, all-out. No mosquito repellent was put as control. Our Bio-out repellent

was compared with all-out in eight separate test sessions. During each session, the two repellents were tested simultaneously. Each session lasted for each of the repellent for 180 min. After every session, the repellent was taken out from the test area and a minimum of 24 h elapsed for the next day tests. The test site used was the author's dark room which was the test area of similar surface area. Behavior of the mosquitoes and visible number were estimated approximately as events.

### Repellent Action of *Cymbopogon citratus* Cream

The formulation was screened for repellency against mosquitoes under laboratory conditions by using puppy dog during night period [9]. Before application of the repellent, the forearms and legs of puppy was washed and rinsed thoroughly with distilled water. The right arm was used for the treatment while the left arm was used for control. Considerable amount of the formulated cream was applied thinly to the puppy's bare skin on the right forearm as shown in the Figure 2. The mosquito repellence was determined in several experiments during same periods of time. After application, puppy was placed in a separate room in the author's home and tied with a belt, mouth cap and puppy was fed properly to allow her in sleep mood. The period of application of the

cream formulation to the time of first landing or bite of mosquitoes on the treated parts, behavior and landings for puppy was observed using Sony DCR 650E DVD handy cam very closely and compared the results using left arm control. To get the expected and genuine results, this type of experiments was performed for several days to a month

## RESULTS AND DISCUSSION

The result of the repellent activity of the pure lemon grass oil against mosquito behavior is shown in Table 1. The result obtained shows that during 6 to 7 pm numerous numbers of mosquitoes were gathered in the author's dark room, where this period was considered to be control period with no mosquito repellents and took the area as 100% attracted area for the mosquitoes (0% repellency). From 7 to 8 pm All-out, a positive control was used to check the repellence activity in that area as shown in the Figure 1, it was shown that up to 80% of the mosquito number was greatly reduced. Considering the same conditions from 8 to 9 pm again we have placed no repellent and numerous numbers of mosquitoes were gathered. From 9 to 10 pm Bio-out, our test sample was used to check the repellence activity in the area as shown in the Figure 1, it was shown that up to 70% of the mosquito number was greatly reduced. The

**Table 1: Repellent Activities of Lemon Grass Oil  
Number of Mosquitoes Attracted and Reduced to the Sample**

Time (min)	Type of Repellent Used	Observed Results
6.00-7.00 pm	NO ALLOUT	Mosquitoes were in numerous number
7.00-8.00 pm	ALLOUT	80% of the mosquito number was greatly reduced
8.00-9.00 pm	NO ALLOUT	Again mosquitoes number increased greatly
9.00-10.00 pm	BIO-OUT	70% of the mosquito number was reduced significantly

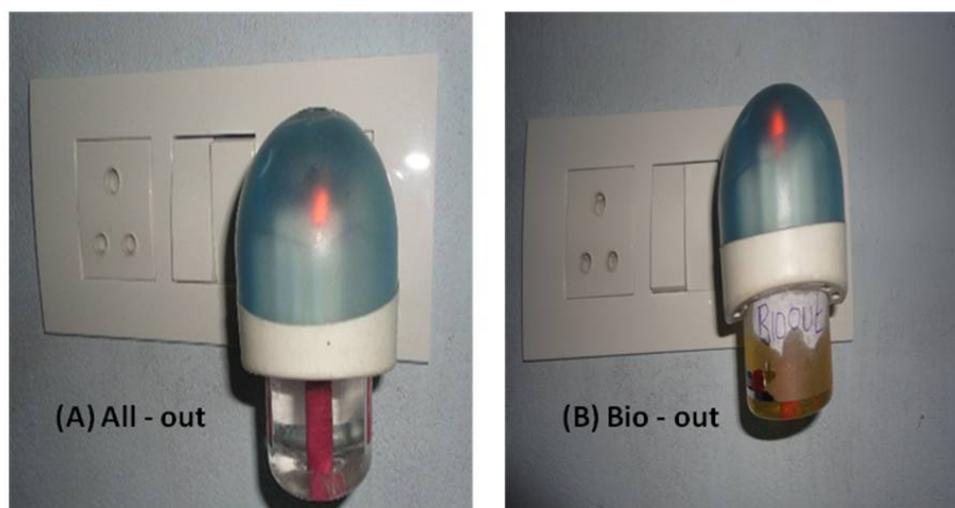
same procedure was treated and used immediately after treatment following the same procedure. After a period of 180 min, with our bio-out test sample repellent, we found that the highest number of mosquitoes was reduced significantly ( $H^*95\%$  repellency). The repellence activity is may be due to medicinal and insecticidal properties of the lemon grass oil.

### The Repellent Activity of Topical Cream Formulation of *Cymbopogon citratus* Essential Oil Against Mosquito Bite

The product produced is found to be 5% w/w and the repellent effect of the oil in the product reduces

with time. The essential oil fades with time due to diffusion of the vapor, resulting in its gradual reduction from the vicinity. Volatile oils diffuse faster from liquids (solutions) than from semisolid preparations (Oyedele *et al.*, 2000). The effect of the cream formulation of the oil is good for topical use, and lasts for longer period. Lactic acid is present in warm-blooded animal body odor and sweat, and is attractive to female mosquitoes. In behavioral studies lactic acid is essential to attraction of *Aedes aegypti* (Geier *et al.*, 1996), the mode of action of *Cymbopogon* oil is not yet established it might be that it masks lactic acid present in warm-blooded animal body odor and

**Figure 1: (A) All out, a Chemical Repellent to Compare our (B) Bio-out Results**



**Figure 2: Application of the Lemon Grass oil Repellent Cream to the Volunteer (Puppy)**



sweat thereby affecting the sensitivity of mosquitoes to the presence of host.

Several research studies show that the product containing DEET gives long lasting protection that is up to 90% protection for about 360 min after a single application. Although, the application of herbal essential oils do not usually last as long as chemicals which can protect up to 6 h, but as far as the health and safety concerned, essential oil repellents are safe for

human life, human and domestic animal skin with no side effects. Our research on puppy proved that our essential oil has great repellent activity which lasts for a minimum of up to 180 min as we observed. Although the length of time of a repellent remained effective depended on the rate at which it was lost by rubbing, evaporation or absorption, after that reapplication of the cream is required, when we compare our herbal cream with DEET based chemical repellent cream it was less cost effective. Continuous application of DEET causes in folding of the epidermis with fewer hairs and a thickened dermis with more vascularity (Al-Sagaff *et al.*, 2001). The lemon grass essential oil did not cause any kind of discomfort or skin irritation to the volunteer (puppy).

## CONCLUSION

From the tests we performed, the results obtained show that lemon grass essential oil provide great protection against mosquitoes like that of the DEET based ones. To make a good natural repellent that will last up to 6 h per application it is hoped that mixing lemon grass oil with other herbal oils like neem, eucalyptus, etc. Inorder to achieve good protection against mosquito bites it is necessary to make our surroundings clean and also wearing protective clothing can also provide protection. It is recommended that people should consider the use of lemon grass oil as a mosquito repellent apart from using chemical based repellents. We came to know the fact that lemon grass is a fast growing plant, and the extraction process is relatively cheaper. So we conclude our bio-out is a great promising natural mosquito repellent which provides you and your family safe.

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