

# 천연 아로마 모기 기피제 (시트로넬라와 시트로넬롤)의 기피력 효과 측정

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## Evaluation of Repellency Effect of Two Natural Aroma Mosquito Repellent Compounds, Citronella and Citronellal

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### Abstract

Repellent efficacies of two natural aroma compounds, citronella and citronellal, against mosquitoes were evaluated both in field and *in vitro*. *In vitro*, the experiment was conducted with three controlled bands impregnated with 30% citronella extract, 15% citronella extract and 30% citronellal extract, and with band impregnated 30% citronella in field. Raw data was obtained by the means of counting numbers bitten by mosquitoes per unit time, namely human bait method. Comparative repellent efficacies of above three controlled bands were calculated at 86%, 73%, and 78%, respectively *in vitro*, and 80% in field, showing high repellent effectiveness against mosquitoes. This estimation was also confirmed by t-test compared between control group and each experimental group.

### 1. Introduction

Mosquitoes are currently one of the most principal measures to mediate vector borne diseases: *Anopheles sinensis*, *Aedes togoi* and *Culex tritaeniorhynchus* mediate malaria, filariasis and Japanese encephalitis, respectively. And recently, *Culex pipiens* was reported as a nuisance pest (with their frequent biting human body) even in urban areas.

The use of mosquito repellent might be practical and economic means that decrease or prevent these problems. The widely used synthetic (or chemical) insect repellent is dimethyl phthalate (DMP) and dimethylbenzamide (also known as diethyltoluamide, DEET). The chemical is effective against other biting insects as well as many species of mosquitoes [1,2]. However, its adverse effects such as contact urticaria syndrome [3], toxic encephalopathy in children [4,5], skin eruption [6] have been reported. To prevent its

malfunctions, there was an attempt to use chloropyrifos as a alternative chemical. However it was also learned to have adverse effect when used in children, hypotensive patients and person with high skin sensitiveness. And these two chemicals cause not only drug-resistance and general toxicity to human and animal but environmental pollution. Such as, studies on substitution repellent which is safe for human and environment have been required. Recently, extracts from neem (*Azadirachta indica* A. Juss) and citronella grass (*Cymbopogon nardus* Rendle) have been known as such possible natural compounds. These repellents (compounds) have low level toxicity to human and animals and high repellent effect against insects [7-10].

The main aim of the present study is to evaluate the actual repellent efficacy of these two natural compounds, citronella and citronellal, *in vitro* and field.

## 2. Material and Methods

### 2.1. Preparation of Insects

The mosquito vectors used in laboratory experiment were laboratory-reared *Culex pipiens pallens* Coquillett. These mosquitoes were reared according to the standard protocol of the Medical Zoology Section, National Institute of Health, Korea, and maintained in the insectary of the institute. The larvae were fed a diet consisting of chow and brewer's yeast (1:1 ratio). The adults were kept in mosquito cages at 27±1°C, 75±5% relative humidity, and 14 hrs light and 10 hrs darkness. They were fed 10% glucose solution.

### 2.2. Preparation of Test Repellents

In the sake of experimental convenience, we used band type of instruments for releasing aroma repellent produced by Hannaeum Co. (Asan, Korea). The band act as good as a reservoir for the active ingredient for expected time, i.e. it can release effect repellent compound continuously during expected time.

*In vitro*, three bands impregnated with 30% citronella extract, 15% citronellal extract and 30% citronellal extract were used. And in field, the band impregnated with 30% citronella extract was used. The band for control group is same in size and shape as the experimental band except for non-treating of the compounds.

### 2.3. In Vitro Repellent Determination

The repellency of the repellent was measured using the human-bait technique [10-13]. Before testing, the mosquitoes were starved for 24 hrs. And mosquitoes were tested for their will to bite by placing an untreated bare arm of each volunteer. If at least 3 mosquitoes landed or bite the arm during 30 sec., the test was carried out. Experiments were carried out in a chamber maintained at temperature 27±1°C and 75±5% relative humidity. After treatment, every 1 hr, each volunteer put the arm into a mosquito cage (25 x 25 x 25 cm) containing 600 mosquitoes for 1min. The tests for each aroma repellent against mosquitoes were conducted on separate days. All tests were replicated 5 times: the raw data measured by number of being bitten by mosquitoes are tabulated in Table 1.

### 2.4. Field Repellent Determination

Two groups (control and experimental group), each composed of 20 persons were organized. Test was carried out in their normal outdoor active during 1 hr exposure time, and then the bitten number was

counted. The band was fasten in forearm as in Fig. 1 band impregnated with 30% citronella just be used. Expected 40 variables were not fulfill because several volunteers failed to keep appropriate experimental condition.

### 2.5. Statistical Analysis

The repellency effect was compared using the Duncan's multiple range test. Percentage repellency was calculated as follows [10,14,15]: % Repellency = 100 x (C-T)/C, where C is the bitten number by mosquitoes counted from control group and T is the number counted from the experimental group of volunteers.

Averaged t-test, adopted in less than 30 testing trials, was done to determine the statistical significance. Raw data from control group and experimental group in field was compared. As to experiment *in vitro*, control group and the three experimental groups (with treated by different natural chemicals as mentioned above) were compared pair-wisely.

## 3. Results and Discussion

Whole the raw data obtained in this study is given in Table 1. Comparative repellent efficacies on three controlled bands (impregnated with 30% citronella extract, 15% citronella extract and 30% citronellal

In field		In vitro			
C	30% citronella	C	30% citronella	15% citronellal	30% citronella
2	0	20	3	5	4
1	0	15	4	7	4
3	2	14	3	3	4
3	0	15	1	4	4
1	0	11	2	2	2
1	0	17	2	5	4
2	0	15	0	3	4
4	2	17	3	1	6
5	0	16	3	4	3
2	0	12	1	4	3
10	0	14	2	3	4
1	0	17	3	7	5
	0	14	3	1	3
	0	10	1	5	4
	2	13	1	0	1
	0	14	1	1	7
	1	16	3	2	6
		15	2	2	6
		13	1	3	3
		13	1	1	3
n=12	n=17	n=20	n=20	n=20	n=20

(Table 1.) Raw data on experimental test *in vitro* and field.

extract) were calculated at 86%, 73%, and 78%, respectively *in vitro*, and 80% on the band impregnated with 30% citronella extract in field.

	variables treat	DF	t Value	Pr >  t
In field	Control vs. 30%citronella	27	3.79	0.0008
In vitro	Control vs. 30% citronella	38	22.06	<.0001
	Control vs. 15%citronella	38	16.77	<.0001
	Control vs. 30%citronellal	38	17.32	<.0001

(Table 2.) The result of t-test.

Both comparative repellency value and the resulted t-test value tell us that natural aroma compounds used in this study have high effectiveness at least in mosquito repellency (Table 1).

As a result, 1) both of two aroma chemicals, i.e. citronella and citronellal, have repellent effect against mosquito vectors not only in vitro but in field. It means that they might be somewhat useful protector to decrease the negative effect brought about by mosquito vectors. 2) High dose of aroma chemical with strong odor sometimes bring about the discomfort depending on users: as shown in the case of citronellal of the present study, the dose of impregnating on band measured as 15% as enough to show repellent efficiency.

### References

- [1] Thavara, U., Y. Malainual, C. Chansang, and Phan-Urai, "Evaluation on the use of soap" *Bull. Dept. Med. Sci.* 4, 203-207, 1990.
- [2] Coleman, R.E., L. L. Robert, L. W. Roberts, A. Glass, D. C. Seeley, A. Laughinghouse, V. Perkins, and R. A. Wirtz, "Laboratory of repellents against fouranopheline mosquitoes (Diptera : Culicidae) and phlebotomine sand flies (Diptera : Psychodidae)" *J. Med. Entomol.* 30, 499-502, 1993.
- [3] Maibach, H. I., and H. L. Johnson, "Contact syndrome" *Arch. Dermatol.* 111, 726-730, 1975.
- [4] Zadikoff, C.M., "Toxic encephalopathy with use of insect repellent" *J. Pediatr.* 95, 140-142, 1979.
- [5] Edwards, D.L., and C. E. Johnson, "Insect repellent -induced toxic encephalopathy" *Clin. Pharm.* 6, 496-498, 1987.
- [6] Reuveni, H., and P. Yagupsky, "Diethyltoluamide -containing insect repellent : adverse effects in use" *Arch. Dermatol.* 118, 582-583, 1982.
- [7] Schmutterer, H., "Properties and potential of pesticides from the neem tree, *Azadirachta indica*" *Ann. Rev. Entomol.* 35, 271-279, 1990.
- [8] Sharma, V.P., M. A. Ansari, and R.K. Razdan, "Mosquito repellent action of neem (*Azadirachta indica*) oil" *J. Am. Mosq. Control Assoc.* 9, 359-360, 1993.
- [9] Lindsay, L.R., G. A. Surgeoner, J. D. Heal, G. J. Gallivan, "Evaluation of the efficacy 3% citronella candles and 5% citronella for protection against field populations *Aedes* mosquitoes" *J. Am. Mosq. Control* 12, 293-294, 1996.
- [10] Lee, W.J., H.S.Lee, Y.J. Ahn, and D.K. Lee, "Laboratory Evaluation of Controlled-release Repellent Treated Pulp Fabric on Human Volunteers Against Mosquito Vectors. *Entomol. Res.* 34, 37-42, 2004.
- [11] Schreck, C.E., and T.P. McGovern, "Repellents and other personal protection strategies against *Aedes albopictus*" *J. Am. Mosq. Control Assoc.* 5, 247-252, 1989.
- [12] World Health Organization, "Report of the WHO informal consultation on the evaluation and testing of insecticides CTD/WHOPES/IC/96.1" World Health Organization, Geneva, Switzerland 1996.
- [13] Thavara, U., A. Tawatsin, J. Chomposri, W. Suwonkerd, U-R. Chansang, and P. Asavadachankern, "Laboratory and field evaluations of the insect repellent 3535 (ethylbutylacetylaminopropionate) and deet against mosquito vectors in Thailand" *J. Am. Mosq. Control Assoc.* 17, 190-195, 2001.
- [14] Yap, H.H., K. Jahangir, A.S.C. Chong, C.R. Adaman, N.L. Chong, Y.A. Malik, and B. Rohaizat, "Field efficacy of a new repellent, KBR 3023, against *Aedes albopictus* (Skuse) and *Culex quinquefasciatus* (Say) in a tropical environment" *J. Vector Ecol.* 23, 62-68, 1998.