

## ADDITION OF VANILLIN TO MOSQUITO REPELLENTS TO INCREASE PROTECTION TIME

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**ABSTRACT.** Vanillin was studied as a prolongator of insect repellents. Seven insect repellents were tested combined with vanillin in 4 different ratios. Vanillin was effective with all. The increases in protection time with different ratios of vanillin to repellents were not significant

except with diethyl toluamide (deet). In most cases increase in protection time was more than 100%. The study suggests that with some repellents mixed with vanillin, it is feasible to obtain protection against mosquito bites for almost 24 h.

**INTRODUCTION.** Previously we reported the potentiating effect of synthetic musks on the protection time of diethyl toluamide (Khan et al. 1975). The protection increase ranged from 12 to 88% depending on the musk and the ratio it was mixed with the repellent. This study describes the effect of vanillin on the protection time of several insect repellents.

**METHODS AND MATERIALS.** Vanillin (4-hydroxy-3-methoxy-benzaldehyde) is a white powdery substance slightly soluble in water but very soluble in organic solvents (alcohol, acetone etc.). It has low volatility (vapor pressure 0.0031-0.0056 mm Hg at 20° C). We tested vanillin combined with the following repellents: diethyl toluamide (deet), ethyl hexanediol, dimethyl phthalate, butyl 3,4-dihydro-2,2-dimethyl 4-oxo-2H-pyran-6-carboxylate (Indalone®), triethylene glycol monohexyl ether, triethylene glycol monoheptyl ether and triethylene glycol-2-ethylhexyl ether. The last 3 repellents have been described by Johnson et al. (1974). In formulating repellent with vanillin, the latter was added at one half, equal, 2, or 3 times the quantity of repellent. Vanillin at ½ the quantity of repellent was used only where the repellent alone lasted a good part of the day (6-8 h). In such cases, the repellent and vanillin in 1:3 ratio was dropped.

The test method was modified from Granett (1940). A known quantity of repellent was applied on a 5 x 25 cm area on the ventral surface of one forearm and the same quantity formulated with vanillin on an equal area on the other forearm

of a male human subject. Each subject was his own control. A long plastic sleeve sealed at one end and with a cut-out matching the repellent treated area was pulled over the forearm. It was held in place with a wire clamp, exposing only the treated surface. The forearm was introduced through a sleeve into a 1 cu. ft. cage containing 500 female yellow fever mosquitoes, *Aedes aegypti* (L.). The mosquitoes had no previous blood meal and had been fed on 5% sugar solution. After 3 min exposure the forearm was withdrawn. This was repeated every 30 min until a total of 2 bites were obtained. The time from application to 2 bites was designated protection time. Tests were performed in a room at 27 ± 1° C and 60% RH.

**RESULTS AND DISCUSSION.** Table 1 shows significant ( $P < .01$ ) increases in protection time with repellent-vanillin mixture compared to repellent alone. With deet mixed with vanillin at 1:1 ratio the protection time increased by 95% and at 1:2 and 1:3 ratio by 142 and 176% respectively. The last two increases differ significantly from that at 1:1 ratio. With the other 6 repellents, addition of vanillin in different ratios increased the protection time significantly compared to control ( $P < .01$ ) but differences among formulations for the same repellent were not significant.

The use of musks as prolongators of mosquito repellents was reported previously (Khan et al. 1975). Of the 7 musks tested, musks Tibetine®, ambrette, give-ambrol, and musk xylol increased the

Table 1. Mean protection time (h) of several repellents on formulation with vanillin in different ratios.

Repellent	Formulations				
	Repellent 0.16 mg/cm <sup>2</sup> (control)	Repellent 0.16+van. 0.08 mg/cm <sup>2</sup>	Repellent 0.16+van. 0.16/cm <sup>2</sup>	Repellent 0.16+van. 0.32 mg/cm <sup>2</sup>	Repellent 0.16+van. 0.48 mg/cm <sup>2</sup>
Deet	4.0±1.2 <sup>a</sup> (21, ..)* 5.2±0.9 <sup>a</sup> (4, ..)	.....	7.8±3.7 <sup>b</sup> (21, 95)*	.....	.....
Ethyl hexanediol	2.0±0.2 <sup>a</sup> (4, ..)	.....	3.5±0.6 <sup>b</sup> (4, 75)	12.6±0.7 <sup>b</sup> (4, 142)	14.5±1.3 <sup>b</sup> (4, 178)
Dimethyl phthalate	1.5±0.2 <sup>a</sup> (4, ..)	.....	4.2±0.5 <sup>b</sup> (4, 180)	4.2±0.8 <sup>b</sup> (4, 180)	3.6±0.2 (4, 140)
Indalone	1.5±0.2 <sup>a</sup> (4, ..)	.....	2.8±0.5 <sup>b</sup> (4, 87)	3.3±0.5 <sup>b</sup> (4, 120)	3.5±0.8 <sup>b</sup> (4, 133)
Triethylene glycol monohexyl ether	7.3±0.9 <sup>a</sup> (6, ..)	.....	14.8±1.6 <sup>b</sup> (6, 103)	.....	.....
	7.0±0.9 <sup>a</sup> (5, ..)	13.6±1.2 <sup>b</sup> (5, 94)	.....	.....	.....
	6.9±0.9 <sup>a</sup> (4, ..)	.....	.....	14.6±1.0 <sup>b</sup> (4, 112)	.....
Triethylene glycol monohexyl ether	8.6±2.3 <sup>a</sup> (5, ..)	15.5±3.0 <sup>b</sup> (5, 80)	14.0±2.4 <sup>b</sup> (5, 63)	19.5±3.3 <sup>b</sup> (5, 127)	.....
Triethylene glycol ethylhexyl ether	7.3±2.6 <sup>a</sup> (6, ..)	.....	17.1±5.3 <sup>b</sup> (6, 134)	.....	.....
	8.3±2.8 <sup>a</sup> (3, ..)	21.0±4.9 <sup>b</sup> (3, 153)	.....	22.0±4.9 <sup>b</sup> (3, 165)	.....

Means with standard deviations along rows not followed by same letter significantly different ( $P < .01$ ).

Figures in parentheses represent number of replicates and percent increase in protection time respectively.

\* Used many times as additional controls with other repellents and their formulations with vanillin, hence more replicates.

protection time of deet significantly. The increases ranged from 12 to 88%. None of the 7 musks was effective on combination with dimethyl phthalate, ethyl hexanediol, or Indalone®. Vanillin is more potent than musks in increasing the repellents' protection time. Musks were more specific and were effective in combination with deet only, whereas the effect of vanillin is more general. It increased the protection time of the 7 repellents tested. The percent increase varied with repellent and the ratio it was mixed with vanillin (Table 1). By itself, vanillin was minimally repellent on some subjects and completely non-repellent on most. In an *in vitro* study, vanillin reduced the evaporation rate of deet by one-half (Spencer, 1974). Whether it also affects absorption or penetration of deet into the skin is not known.

Vanillin at 0.32 and 0.48 mg/cm<sup>2</sup> mixed with 0.16 mg/cm<sup>2</sup> of deet was more effective than 0.16 mg/cm<sup>2</sup> of vanillin with an equal quantity of deet per unit area. With the other repellents vanillin combined in different ratios did not cause a significant difference in the extension of protection times.

Considering duration, vanillin combined with triethylene glycol monoheptyl ether protected up to 19.5 h and with triethylene glycol-2-ethylhexyl ether up to 22 h. It appears that at least with these 2 repellents it is feasible to obtain protection for almost 24 h against mosquito bites with one application.

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