

THE EFFECTIVENESS OF REPELLENTS ON CLOTH AS DETERMINED BY OVIPOSITION OF *Aedes Aegypti* L.

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The main criterion in testing mosquito repellents has been the effective protection period they give on the skin of humans (Gilbert *et al.*, 1955); animals (Kasman *et al.*, 1953); or on cloth worn by humans (Smith and Cole, 1951). The effectiveness of repellents on skin is far lower than that on cloth, or other inert material, due mostly to absorption into the skin (Smith *et al.*, 1963). In some studies, mosquito repellents have been evaluated as regards their initial repellent effect without recourse to using living creatures as test animals (Bar-Zeev and Smith, 1959; Bar-Zeev, 1962; Frizel and Wright, 1962). The purpose of the present study was to develop a simple technique by

means of which both the initial and prolonged effects of repellents against *Aedes aegypti* L. can be compared on cloth, eliminating the need for humans or animals. This is desirable since living creatures vary in their attraction to the host and, therefore, cause large variations in results.

MATERIALS AND METHODS. Two 10 x 10 cm square pieces of poplin (100% cotton) cloth each having a hole 5 mm in diameter in its centre, were treated with different repellent solutions. The repellents were prepared as 10 percent solutions in ethanol. One ml of the solution was applied to each piece of cloth by dropping from a pipette so as to moisten the cloth

evenly with the repellent. The treated pieces of material were then hung to dry and kept at constant temperature of 28° C. and relative humidity of 65 percent. Twenty-four hours after treatment, each piece of material was fastened by means of a rubber band to the top of a plastic container 9 cm. high, 7.5 cm. top diameter, which was about half filled with water and lined inside with filter paper on which the mosquitoes laid their eggs (Fig. 1). Two such systems, the cloth of each

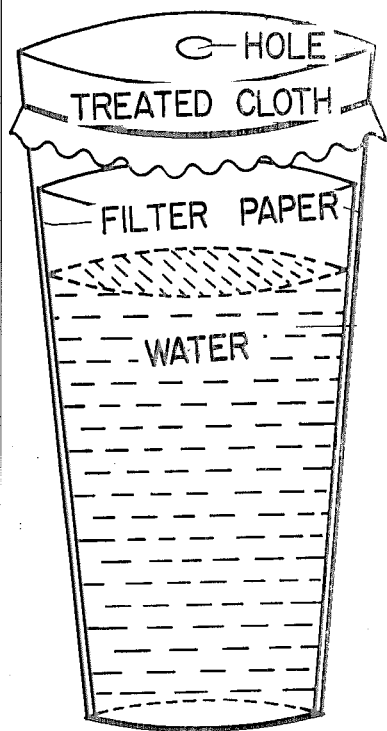


FIG. 1.—Diagram of container in which mosquitoes laid eggs.

treated with a different repellent, were placed 20 cm. apart, for 3 days, in a cage (30 x 30 x 30 cm.) containing about 1000 6-day old mosquitoes. The numbers were estimated by weighing at 4° C. in a cold room. About 50 percent were females. The mosquitoes were given their first

blood meal two days previously. A pad of cotton covered with honey was placed in each cage as a food source.

In order to lay eggs or drink water, the females had to enter the plastic container through the hole in the treated cloth and thus pass the repellent barrier. Furthermore in order to lay eggs, after entering the container, they had to stay inside the glass for some time and overcome the repellent effect. Under control conditions (non treated cloth) the mosquitoes laid a normal number of eggs. At the end of the experimental period, the plastic containers were removed, and the number of eggs on each filter paper was noted. The treated cloth was removed, and after being maintained at the above mentioned constant temperature and humidity, was re-used in tests repeated at 7-day intervals. It was assumed that the number of eggs laid could be taken as inversely proportional to the effectiveness of the repellent against the females. With each pair of repellents five replicated tests in most cases gave similar results.

The repellents were obtained through the courtesy of Dr. Carroll N. Smith of the Entomology Research Division, U.S.D.A., Gainesville, Florida. The names of the repellents are listed below, together with the ENT code numbers. In the tables, text and plates, the materials are

Ent. code No.	Compound
Ent-262	Dimethyl phthalate (DMP)
Ent-22542	Diethyltoluamide (DET) (= "DEET")
Ent-375 (Rutgers 612)	1,3 hexanediol, 2-ethyl (EHD)
Ent-6075	Anisic acid, propyl ester
Ent-9	Indalone (2 <i>H</i> -Pyran-6-carboxylic acid, 3,4-dihydro-2, 2-dimethyl- 4-oxo-, butyl ester)
Ent-6168	Succinamic acid- <i>N,N</i> -diethyl-, propyl ester
Ent-11558	1-Butanol, 3-phenyl-
Ent-1170	Anisyl alcohol
Ent-3916	Dimethyl carbate (Bicyclo [2,2,1]-5-heptene-2, 3-di- carboxylic acid, <i>cis</i> -, di- methyl ester)
Ent-949	Cinnamyl alcohol

identified by numbers or initials. All of these repellents were rated (King, 1954) as "4" or "4A" on cloth against *Ae. aegypti* on the classification scale of 1 (least effective) to 4, 4A (most effective).

RESULTS AND DISCUSSION. The repelling effect of Ent-262 (DMP), Ent-22542 (DET), and ENT-375 (EHD) as compared with the untreated (control) system are shown in Plate I. DMP and DET were effective until the 14th day after application, while EHD appears to be effective only until the 7th day. It can be seen that the effectiveness of the repellents gradually decreases with time. In subsequent tests comparisons were made between DMP, DET and EHD (Plate II). DET was more effective than DMP and EHD until the 14th day after application, and DMP to EHD until the 7th day after application. The superior performances of DET over DMP and EHD, and of DMP over EHD are in accordance with results obtained by Bar-Zeev (1959, 1962) using different methods. DET is considered as the outstanding mosquito repellent (Gilbert *et al.*, 1955).

The effectiveness of DMP and DET was compared with that of Ent-6168. Results are presented in Plate III. DMP and DET were superior on the 1st day after application, but from the 7th to the 28th day after application the effect was reversed, Ent-6168 being more repellent. Thus, Ent-6168 was much more persistent in its effect in spite of its initial poorer performance.

Results of a study in which DMP and DET were compared, in paired tests, with various other repellents, are given in Table 1. A summary of data on the persistence of the various repellents, as compared to data of DMP and DET is presented in Table 2. All compounds which were inferior (Ent-1170, Ent-3916) or equal to DMP (Ent-11558, Ent-949) as regards persistence of their effect were inferior to DET, again confirming the finding that DET is superior to DMP. One compound (Ent-6075) occupied an

intermediate position between DET and DMP. Ent-9 was similar to DMP and DET on the 1st or 7th day after application, but displayed a greater repellent effect in the subsequent period.

The advantages of the method described are as follows: The individual variations found when humans or animals are used as the test subject are eliminated. Two compounds are compared at the same time with the same insects under precisely the same conditions. The method is simple, and the amount of work involved in carrying out the experiments is relatively

TABLE 1.—Comparison of various repellents in paired tests with dimethyl phthalate (DMP) and diethyltoluamide (DET).

		0		+		++		+++		++++	
		indicates no eggs		indicates very few eggs		indicates few eggs		indicates medium number of eggs		indicates many eggs	
Days after application of repellent		DMP	vs.	Ent-11558							
1		+++		+++							
7		+++		+++							
14		++++		++++							
		DET	vs.	Ent-11558							
1		+		+++							
7		+		+++							
14		++++		++++							
		DMP	vs.	Ent-6075							
1		+++		+							
7		++++		+++							
14		++++		++++							
		DET	vs.	Ent-6075							
1		+		+							
7		++		+++							
14		+++		++++							
		DMP	vs.	Ent-1170							
1		0		++							
7		++++		++++							
		DET	vs.	Ent-1170							
1		0		+							
7		++		++++							
14		++++		++++							

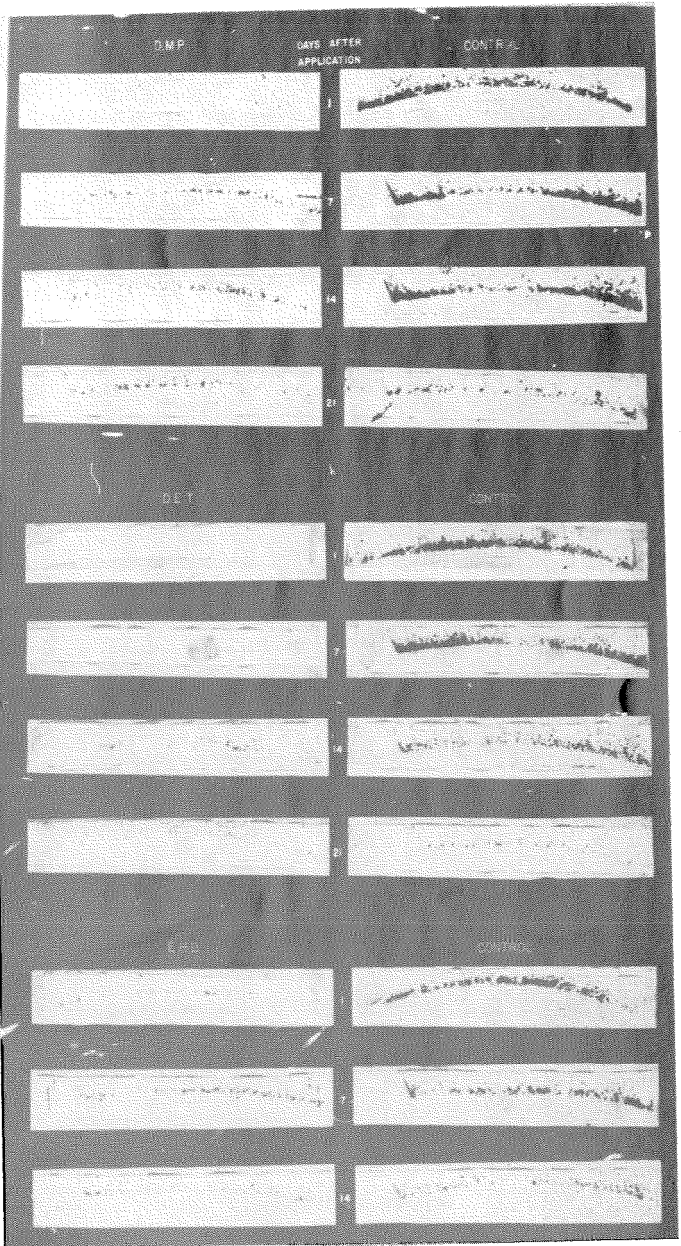


PLATE I.—Oviposition on filter paper by *Aedes aegypti* L. under the influence of the repellent effects of the compounds indicated.

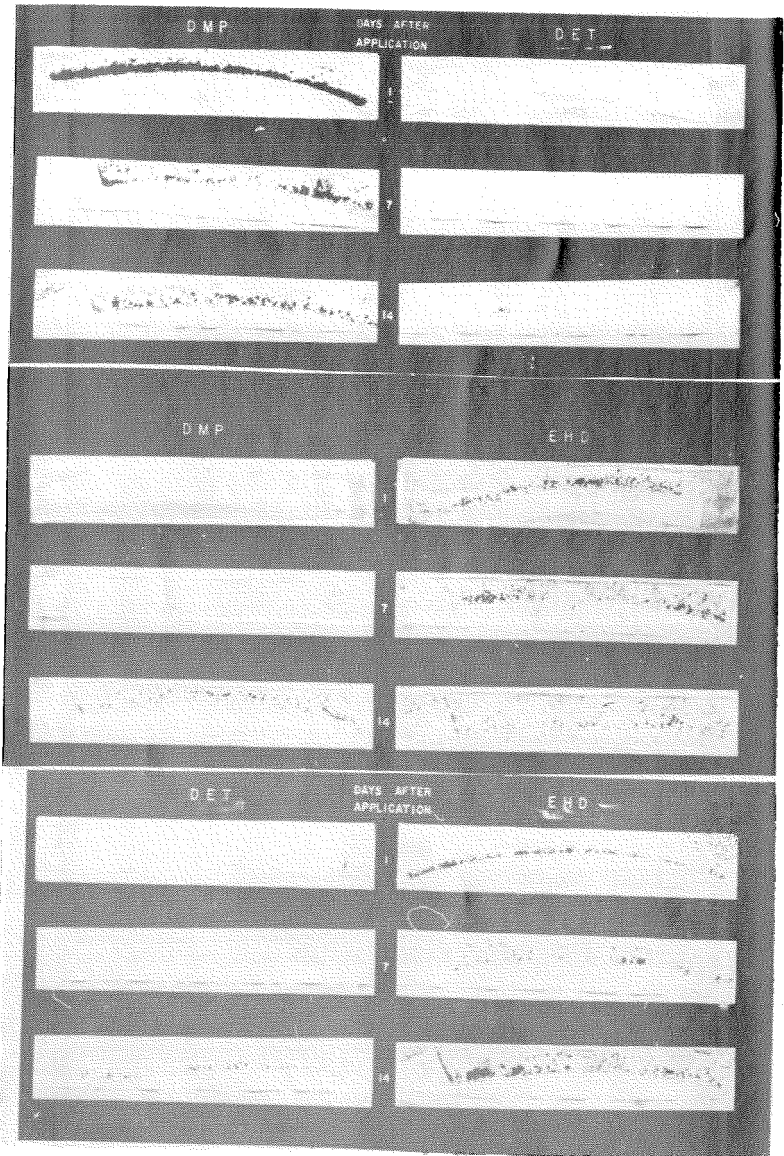


PLATE II.—Oviposition on filter paper by *Aedes aegypti* L. under the influence of the repellent effects of the compounds indicated.

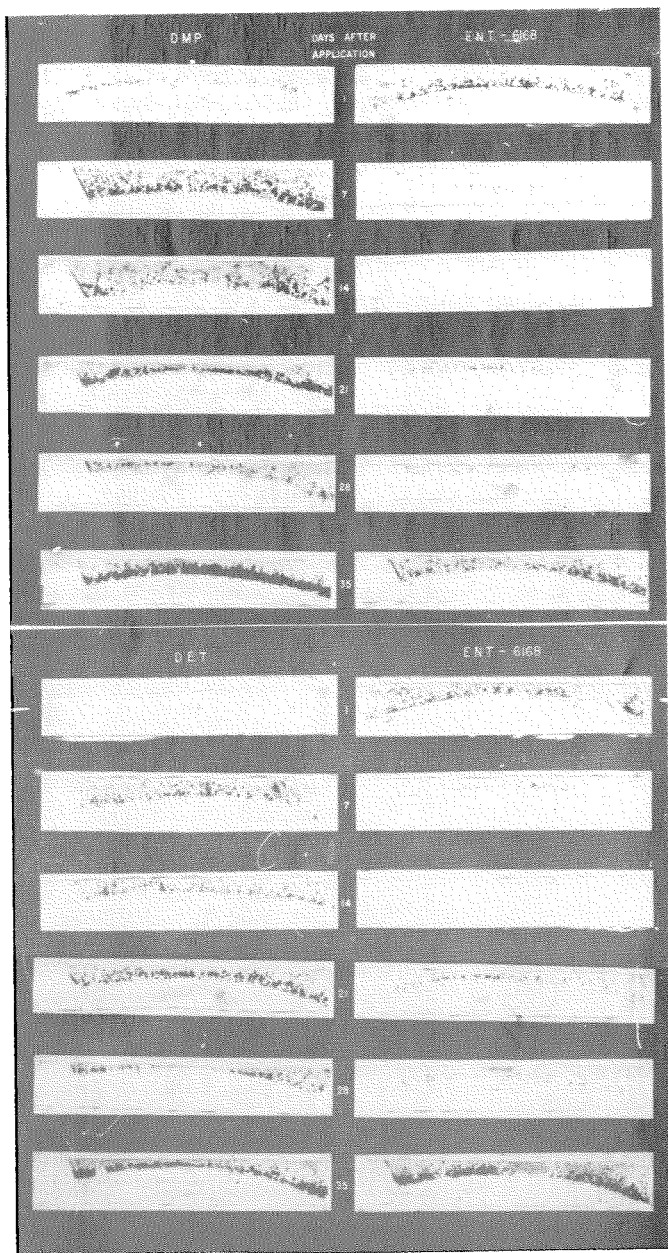


PLATE III.—Oviposition on filter paper by *Aedes aegypti* L. under the influence of the repellent effects of the compounds indicated.

Table 1.—Continued

Days after application of repellent	DMP	vs.	Ent-949
1	+++		+++
7	+++		+++
	DET	vs.	Ent-949
1	+		+
7	+		+++
14	+++		+++
	DMP	vs.	Ent-3916
1	+		+
7	+		++
14	++		+++
	DET	vs.	Ent-3916
1	0		++
7	0		++++
14	+		++++
	DMP	vs.	Ent-9
1	++		++
7	++++		0
14	++++		0
21	+++		+
28	++++		+++
35	++++		++++
	DET	vs.	Ent-9
1	+		+++
7	++		++
14	++++		+
21	++++		++
28	++++		+++
35	++++		++++

small. The method appears to be reliable since it confirms results obtained by other methods.

Ideally, it is preferable that screening of repellents for biting insects should be carried out by tests in which actual biting of the insects on a host are involved, reflecting the actual conditions in the field. The method described should, however, be useful as a complementary technique, i.e., when it is necessary to provide a further comparative rating of a group of repellents which have been found effective against mosquitoes by the usual biting methods. The described technique can detect, with relative ease, small differences in effectiveness.

The fact that some repellents (Ent-6168 and Ent-9) were similar or inferior to DMP and DET on the 1st and sometimes also on the 7th day after application, but superior on subsequent days, is probably due to their lower volatility or greater stability. Probably, therefore, a mixture of DET with either Ent-9 or Ent-6168 will be more effective on clothing than either one of them alone, giving a strong initial as well as a persistent effect.

SUMMARY. The effectiveness of various repellents on cloth against *A. aegypti* was compared. Gravid females had to withstand the effect of repellent-treated cloth, at various periods after treatment, in order to reach an oviposition site. Paired tests, each involving two different repellents,

TABLE 2.—Summary of results on the persistence of the various repellents as compared to dimethyl phthalate (DMP) and diethyltoluamide (DET).

Less persistent than DMP	Equally as persistent as DMP	More persistent than DMP
Ent-1170	Ent-11558	Ent-6075
Ent-3916	Ent-949	Ent-9
.....	Ent-6168
Less persistent than DET	Equally as persistent as DET	More persistent than DET
Ent-1170	Ent-9
Ent-6075	Ent-6168
Ent-3916
Ent-11558
Ent-949

were carried out, and the number of eggs laid in the vicinity of each repellent was compared.

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