A SIMPLIFIED TEST FOR DETERMINING THE EFFECTIVENESS OF FIELD SPRAYING OPERATIONS AGAINST MOSQUITOES

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The WHO method for determining the susceptibility or resistance of adult mosquitoes to insecticides is well known. However, under certain conditions it has limitations, among which are the following.

(a) The mosquitoes may react to the impregnated blotting papers prepared and attached to the WHO kit, and not to the insecticides prepared and sprayed in practice in the field under different local conditions.

(b) In order to carry out any examinations, it is necessary to possess the above-mentioned kit, including the set of blotting papers impregnated for an appropriate period.

(c) There is a need for transportation facilities and a suitable place in which to carry out the examinations.

(d) It takes 24 hours to obtain and summarize the final results of each test.

While studying the bionomics of *A. sergenti* in the Dead Sea area over the past few years, we met extremely difficult conditions for applying this method of examining the susceptibility of the mosquitoes because, although the mosquitoes concentrate in large numbers inside caves, crevices and rock fissures, it is difficult to collect them; they die very quickly in the very high temperature and low humidity prevailing on the spot; there exist no suitable facilities for examinations lasting 24 hours, and the distance from any laboratory is considerable.

We therefore endeavoured to simplify the usually excellent, above-mentioned method, as described below.

1. *Susceptibility tests on the spot in small glass tubes.*

Instead of the plastic tubes, 125 mm long by 44 mm wide, we used small glass tubes, 62 x 17 mm (including a cap of 17 x 7 mm).

Squares of blotting paper, 55 x 50 mm, impregnated with insecticides—or left unimpregnated respectively—fitting into the glass tubes, were cut and inserted for examination purposes. Batches of 5 females each of *A. sergenti,* with blood, were collected from the caves, whenever required, and immediately transferred into the glass tubes fitted with impregnated blotting paper, whereas, for control purposes, 5 other females were placed into one glass tube with unimpregnated blotting paper.

After one hour's exposure on the spot in the cave, the living, moribund and dead mosquitoes were counted, the survivors transferred into other glass tubes fitted with similar squares of unimpregnated blotting paper and left therein for another hour. Our observations showed that—

(a) The longer the mosquitoes were exposed to insecticides, the less reliable were the results of the tests.

(b) The results of the contact of 5 females (with blood) with impregnated blotting paper in small glass tubes for 1 hour were similar to the results achieved in the bigger plastic tubes (see Table 1). In the small glass tubes we observed the same gradual dropping off of mortality and morbidity, proportionate to the reduction of the amount of insecticide in the blotting paper squares with which they were in contact, whereas in the control tubes containing unimpregnated paper *all mosquitoes invariably remained alive* after one or two hours' confinement therein, even when the tubes were hermetically closed (see Tables 2 and 3).

(c) After one hour's contact with insecticide-impregnated blotting paper, there was no drastic difference in morbidity and mortality between mosquitoes subsequently enclosed with unimpregnated paper for one hour and specimens thus confined for two hours.

TABLE 1.—Comparison of results of susceptibility tests with *A. sergenti* from a cave in the Dead Sea area, with regular impregnated blotting papers in plastic cylinders of the WHO kit (WK) and in small glass tubes (GT) in 1963—specimens counted immediately after one hour's exposure.

Date	T. C°	RH %	Container	DDT (%) 0.5 L, M+D*	DDT (%) 1.0 L, M+D	DDT (%) 2.0 L, M+D	DDT (%) 4.0 L, M+D	Dieldrin (%) 0.2 L, M+D	Dieldrin (%) 0.4 L, M+D	Dieldrin (%) 0.8 L, M+D	Dieldrin (%) 1.6 L, M+D	Dieldrin (%) 4.0 L, M+D
Feb. 4, 1963	26	32	WK	2/ 3	..	3/ 2	0/ 5	..	..	..	4/ 1	2/ 3
			GT	1/ 4	..	3/ 2	1/ 4	..	..	..	2/ 3	4/1 1
Mar. 4, 1963	24	50	WK	..	..	1/ 4	0/ 5	..	..	..	3/ 2	2/ 3
			GT	..	..	0/ 5	0/ 5	..	..	..	1/ 4	1/ 4
Mar. 19, 1963	25	50	WK	22/ 0	5/ 10	2/ 14	0/ 20	5/ 0	20/ 0	..	..	12/ 7
			GT	4/ 1	1/ 4	0/ 5	0/ 5	5/ 0	5/ 0	..	..	2/ 5
Apr. 28, 1963	29	58	WK	..	..	..	..	5/ 0	5/ 0	3/ 2	2/ 3	1/ 4
			GT	..	..	..	..	5/ 0	5/ 0	1/ 4	3/ 2	2/ 3

* L=Living; M+D=Moribund+Dead.

The results of contact of *A. sergenti* mosquitoes with impregnated blotting pa pers during one hour in the big plastic tubes of the WHO kit are similar to those in small glass tubes. In the control containers all specimens survived.

TABLE 2.—Susceptibility test of *A. sergenti* from an unsprayed cave in Wadi Bire (Jordan Valley) by one hour's exposure to impregnated blotting paper in small glass tubes—on August 1, 1962 (specimens counted immediately after exposure).

DDT (%) 0.5 L, M+D*	DDT (%) 1.0 L, M+D	DDT (%) 2.0 L, M+D	DDT (%) 4.0 L, M+D	Dieldrin (%) 0.1 L, M+D	Dieldrin (%) 0.2 L, M+D	Dieldrin (%) 0.4 L, M+D	Dieldrin (%) 0.8 L, M+D	Dieldrin (%) 1.6 L, M+D	Dieldrin (%) 4.0 L, M+D	Control
5/ 0	2/ 3	0/ 5	0/ 5	5/ 0	4/ 1	5/ 0	4/ 1	1/ 4	0/ 5	All five alive

* L=Living; M+D=Moribund+Dead. The mortality and morbidity is in direct proportion to the percentage of insecticides contained in the blotting papers.

TABLE 3.—Susceptibility tests of *A. sergenti* from an unsprayed cave at Neot Kikar (Dead Sea Area) counted immediately after one hour's contact with impregnated blotting papers in small glass tubes.

Date	T T.C.°	R.H. %	DDT (%) 0.5 L, M+D*	1.0 L, M+D	2.0 L, M+D	4.0 L, M+D	Dieldrin (%) 0.2 L, M+D	0.4 L, M+D	0.8 L, M+D	1.6 L, M+D	4.0 L, M+D	Control
Aug. 8, 1962	36	41	..	..	1/ 4	0/ 5	..	..	..	..	0/ 5	All five survived
Sept. 6, 1962	33	42	..	2/ 3	..	0/ 5	..	4/ 1	2/ 3	..	..	"
Oct. 4, 1962	33	52	4/ 1	3/ 2	2/ 3	0/ 5	3/ 2	2/ 3	2/ 3	1/ 4	1/ 4	"
Nov. 4, 1962	30	34	..	..	0/ 5	0/ 5	..	..	...	2/ 3	0/ 5	"
Dec. 4, 1962	26	42	5/ 0	..	0/ 5	1/ 4	5/ 0	5/ 0	5/ 0	1/ 4	1/ 4	"
Feb. 4, 1963	26	32	..	..	3/ 2	1/ 4	..	..	..	2/ 3	1/ 4	"
Mar. 19, 1963	25	50	4/ 1	1/ 4	0/ 5	0/ 5	5/ 0	5/ 0	..	..	2/ 3	"
Apr. 28, 1963	29	58	..	..	..	..	5/ 0	5/ 0	1/ 4	3/ 2	2/ 3	"

* L=Living; M+D=Moribund+Dead. The mortality and moribundity is directly proportionate to the percentage of insecticides contained in the blotting papers.

2. *Examination, by means of small test tubes, of the efficacy of a spraying operation under local conditions.*

Encouraged by Dr. Bruce-Chwatt, Chief of the Research and Technical Intelligence Division of Malaria, WHO, we carried out the following test.

On May 5, 1962, a piece of blotting paper, 25 x 21.5 cms in size, divided into 12 equal squares of 55 x 50 mm., was affixed to a wall in a room at Neot Kikar, Dead Sea area. The walls of the room, including the aforementioned blotting paper, were sprayed with a 5 percent solution of DDT in kerosene. Every month, from June, 1962 until April, 1963, we cut one square from the sprayed blotting paper and inserted it into one of the small glass tubes described above. A similar square of unsprayed blotting paper was placed into another such tube for control purposes (see Table 4).

(c) To observe the gradual changes, if any, in the physiological reactions of the local mosquitoes to the insecticides used.

Table 4 shows that the results of the spraying operation were satisfactory from beginning to end during the whole year.

Being afraid that the results of the spraying might be affected by the influence of the extreme climatic conditions prevailing in this area, the lowest on earth, in the past we used to spray DDT at two-month intervals; now, however, we started spraying only twice during the year.

Although we are well aware that the structures of the sprayed surfaces are different from the surface of blotting paper, we found it worth while to employ the method described above, for the following reasons:

(a) Blotting paper seems to be just as good a surface-spraying medium for standard field operations.

TABLE 4.—Monthly tests of susceptibility of *A. sergenti* to squares of blotting paper affixed to a wall and sprayed (on May 5, 1962) with a 5% solution of DDT in kerosene at Neot Kikar (Dead Sea) in small glass tubes (June 1962–April 1963).

Date	T T. C°	R.H. %	Contact with blotting paper: Sprayed: after one hour L, M+D	Contact with blotting paper: Unsprayed: after additional hour L, M+D	Control 2 hours (unsprayed)
June 5, 1962	..	..	0/ 5	0/ 5	All alive
July 5, 1962	..	..	2/ 3	0/ 5	"
Aug. 6, 1962	36	41	0/ 5	1/ 4	"
Sept. 6, 1962	33	42	2/ 3	0/ 5	"
Oct. 4, 1962	33	52	1/ 4	1/ 4	"
Nov. 4, 1962	30	34	2/ 3	0/ 5	"
Dec. 4, 1962	26	42	3/ 2	3/ 2	"
Feb. 4, 1963	26	32	5/ 0	1/ 4	"
Mar. 4, 1963	25	48	1/ 4	0/ 5	"
Apr. 17, 1963	29	50	2/ 3	0/ 5	"

* L=Living; M+D=Moribund+Dead.

The aim of this test was to measure roughly:

(a) The degree of effectiveness of the insecticides at the beginning of the spraying operation.

(b) To follow up the gradual falling-off of the efficacy of the crystal deposit of the insecticides on the blotting paper under local conditions.

(b) The results of the mosquitoes' contact with the surface of the impregnated blotting paper reflect, without doubt, the efficacy of the formula of the insecticide employed and of the method of its application in the field.

(c) The examinations, repeated once every month, can indicate the duration of the effectiveness of the insecticide under

local climatic conditions, reflecting simultaneously any changes in the susceptibility of the mosquitoes to the insecticide used.

The proposed method has several advantages:

1. The necessary equipment which is simple and cheap, is easily available and can be handled, used and transported anywhere without difficulty.

2. As the mosquitoes can be collected and immediately tested on the spot, all damage to them through transportation, climate or other negative influences is avoided, and the results of the tests are obtained within 1–2 hours only.

3. This method provides a means of measuring roughly but quickly the effectiveness of the spraying operations in the field under local conditions everywhere, even in the tropical regions of Africa.

METHOD FOR OBTAINING LARGE NUMBERS OF UNMATED *AEDES AEGYPTI* (L.)

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Males of *Aedes aegypti* (L.) are attracted to the females by the flight sound of the latter (Roth, 1948). Therefore both sexes must fly before mating occurs. Unmated individuals of both sexes often are needed for experimental purposes. The following is a method for obtaining such individuals readily and in large quantities. The principle is to rear them at a temperature sufficiently high to permit the adults to emerge from the pupae and to arrange that the adults are then immediately held at a temperature low enough to inhibit flying and thus prevent mating.

After several preliminary tests, the apparatus shown in Figure 1 was devised. The cage, A, is similar to that described by Nicholls (1963: Figs. 16 and 17) except that the dimensions are 10 inches by 10 inches by 16 inches high, one of the screen sides was replaced by masonite bearing a cotton sleeve and a hole 6 inches by 6 inches was cut in the bottom. The ledge left in the bottom rests on the top edge of the stainless steel rearing container, B, which is 6¾ inches by 6¾ inches by 10 inches high. Two holes in the side of B, 3 inches from the top, permit the insertion in a slanting manner of a thermostat, C (American Instrument Co., #4–235) and a dial type thermometer, D (Cole-Parmer

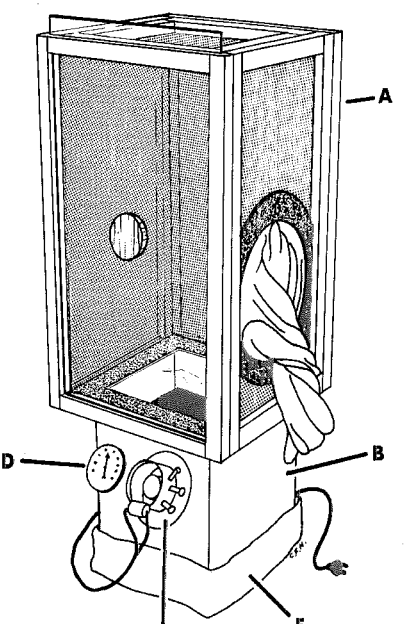

FIG. 1.—Temperature-controlled rearing container and cage.