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OBSERVATIONS ON DIELDRIN RESISTANCE IN ANOPHELES CULICIFACIES
IN THANA DISTRICT, BOMBAY STATE, INDIA

by

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Patel et al (1959) described a "probable" case of resistance exhibited by Anopheles culicifacies to dieldrin in six villages of Murbad Taluka, Thana District, Bombay State, India. The susceptibility tests which were carried out in October 1958 had shown an LC_{50} greater than 1.6%, that is, the highest concentration of impregnated paper used in the tests. Further detailed examination of the data indicated that the dosage mortality curve was not linear, inasmuch as the mortality rates did not show a corresponding increase with the increase of concentrations at higher levels. The wild population of Anopheles culicifacies at that time was obviously a mixture of susceptible and resistant individuals in the proportion of roughly 1:3.

History of insecticide application.

The use of insecticides in this area may be summarized as follows:

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|-----------|---|
| 1948-1952 | DDT emulsion, three rounds per year, at a dosage of 56 mg per square foot. |
| 1953-1956 | DDT 75% water wettable powder, two rounds each year, at a dosage of 100 to 112 mg per square foot. |
| 1957 | Dieldrin 50% water wettable powder, two rounds at 28 mg per square foot, once in May and once in September. |
| 1958 | One round of dieldrin, 50% water wettable powder in May-June, at 28 mg per square foot. |

In the second round of spraying in September-October 1958, dieldrin was replaced by DDT 75% wettable powder over the entire district, except in a compact group of 15 villages (total area about 33 square miles) which formed the venue of the studies reported in this paper. These villages were left unsprayed to enable us to watch the effect of the removal of selection pressure of the insecticide and to allow the natural mosquito population to take its own course. In May-June 1959, the entire district was sprayed with DDT 75% wettable powder, including these 15 villages.

The change-over from dieldrin to DDT was made necessary because of a serious recrudescence of malaria in two divisions (talukas) of this district between July and September 1958. Though the total population of the district was approximately 1.6 million in a total area of 3894 square miles, and dieldrin had been used in the entire district, the recrudescence of malaria occurred only in two divisions with a total approximate population of 89 198 and a total area of 432.3 square miles. This area was hyperendemic for malaria prior to 1948. The disease had been very effectively controlled till 1958 and the spleen rates at the end of 1957 were all extremely low, being below 1% in all cases. No positive blood smears had been found in the routine child parasite and infant parasite surveys over a period of two years. The increase in malaria prevalence in the two talukas, i.e., Murbad and Shahapur, was associated with a great increase in the densities of Anopheles culicifacies in sprayed structures. Such increase in densities was not noticed in other areas of Thana district where recrudescence of malaria had not occurred.

The increased malaria prevalence in this area in the year 1958 was rapidly and thoroughly brought once again under control by use of DDT and widespread use of antimalarial drugs. The details of this outbreak and the epidemiological features will be discussed in a separate report.

Susceptibility tests

Susceptibility tests with dieldrin have been carried out in this group of villages at intervals from October 1958 to December 1959. All specimens used in the tests were wild caught females which had taken full blood meals in the previous night. Owing to the general paucity of adult A. culicifacies, both because of a

decline in breeding immediately after the monsoon in 1958 and because of DDT spraying in May-June 1959, the numbers used in the tests have not been as large as one would have desired, though adequate for the purpose of following the trends in the susceptibility levels in the area as a whole throughout the period. The WHO standard test kits were used in all cases. Except for the papers with concentration of 3.2% and 6.4% which were prepared in our own laboratory, the other papers used were all standard WHO impregnated papers. Though we ourselves feel that the papers of 3.2% and 6.4% impregnated in our laboratory were satisfactory, those who may not wish to compare them with the data obtained with WHO impregnated papers may ignore them during the study.

Results of the tests have been summarized in Table I and illustrated in Chart I. The data presented show that with the efflux of time and with the diminishing dieldrin pressure, the field populations of A. culicifacies underwent a change indicating a reversal towards susceptibility. Mortalities at all concentrations have increased gradually and in December 1959 even the 0.1% paper has given a mortality as high as 70%, while in October 1958 it had given a kill of only 12.6%.¹

For purposes of comparison the data collected by this organization in the several other widely scattered places in Bombay State during the routine study of the susceptibility levels are presented in Table II and Charts II and III. The results of the tests at Chorbavli and Kada (both unsprayed areas) have already been

¹ In certain studies made subsequent to the preparation of this paper, it has been noticed that in one village (Nandni), where dieldrin pressure had been reapplied for experimental study, there was indication that the degree of susceptibility had again considerably decreased. For instance, the 1.6% paper yielded mortalities of only 19% in March 1960 and 15% in May 1960. These further studies are still in progress and will be reported in later communications. It may also be stated that in certain studies carried out subsequently in Poona district, to test whether the papers themselves had become old and ineffective, even 0.2% paper gave mortality of nearly 80% in A. culicifacies.

reported by Bhatia et al (1958). The remaining data were still unpublished and are from areas which have been sprayed. Though DDT was the main insecticide used in all these areas, dieldrin had also been used on a few occasions, for instance, in 1955 and 1956 in Manjri and Junnar, both in Poona district, and in 1958 and 1959 in Veraval of Saurashtra. In Aurangabad and Panchamahals districts no dieldrin has been used at all. Without going into details about the patterns of the mortality curves in several places, it may briefly be noted that the December 1959 figures in Thana district are approaching more and more close towards the pattern in the other sprayed areas, though still very different from the patterns in the totally unsprayed areas. In totally unsprayed area a 100% mortality of A. culicifacies was obtained with even a 0.1% paper. Obviously the reversal towards susceptibility in Thana district has not been complete even one year after the withdrawal of dieldrin selection pressure.

Discussion

The main object of this short note is to bring out the fact that in one small part of Bombay State, where a field population of Anopheles culicifacies had developed a measurable degree of resistance to dieldrin after three rounds of spraying with the insecticide, there was a gradual but significant reversal, over a period of about a year, towards susceptibility after discontinuance of dieldrin spraying. However, the susceptibility status even at the end of this period was not as high as in the case of original populations which had no contact with any insecticide.

A notable feature of the dosage mortality lines is that in most of them there is a marked tendency for levelling off at higher concentrations, strongly indicating heterogeneity. If the mortality percentage at which such a flattening occurs is taken theoretically to represent a point at which most of the susceptibles are killed off and most of the resistants survive, approximately 75% of the population was susceptible and 25% resistant to dieldrin in October 1959, as against 25% susceptible and 75% resistant in October 1958. These proportions should not be regarded as anything but rough estimates, but the change-over in the proportions is significant.

Brown (1958) has mentioned a few instances of such reversal to susceptibility to DDT in field populations of house-flies, particularly the observations of Keiding in Denmark. But such reversions in field populations are not common and few, if any, appear to have been reported for any species of mosquitos. In laboratory populations, however, it is known that with the removal of insecticidal pressure reversion to susceptibility may take place after some generations.

We are not yet in a position to give a reasonable explanation for the reversion, though incomplete, towards susceptibility and for the change-over in the proportions of resistant and susceptible individuals, in a natural field population. It is generally believed that in natural field populations where resistance has once become stabilized, reversion to susceptibility may not be expected. One should hesitate to express firm opinions in this regard in the absence of some knowledge of genetical aspects of dieldrin resistance in anophelines, particularly in A. culicifacies. Unfortunately, unlike A. gambiae, which has been the subject of so many interesting genetical studies by Davidson (1958), Elliot and Ramakrishna (1956), Armstrong, Ramakrishna and Ramsdale (1956) and others, A. culicifacies is not amenable to laboratory colonization and no mating experiments are possible.

Among the probable causes which one may postulate for such a reversion are:

(a) infiltration of susceptible individuals from neighbouring areas and the gradual reduction in the frequency of the resistant gene. This may not be a very probable reason in this area where it may be noted that dieldrin failure occurred in a total area of nearly 432 square miles, and the few villages which were the venue of these studies formed only a very small fraction of it. But the villages were not exactly in the centre of the area but towards the periphery and therefore there is a theoretical possibility of infiltration;

(b) changes due to mutations or other genetical mechanisms about which our knowledge is totally negative;

(c) a better survival value in nature for dieldrin susceptible individuals and a lower survival value in the resistants, leading to elimination of the resistant population in the absence of dieldrin pressure.

There are several questions which have been posed as a result of this study and which we have been unable to answer. One item of some interest is that the failure of dieldrin occurred only in a small part of the total area in which it was used. Unfortunately we have no data for the susceptibility tests in the other areas, and a few attempts to carry out the tests were unsuccessful because of paucity of wild caught A. culicifacies females. Apparently dieldrin had a good operational effect in the rest of the district. Therefore, it would be of interest to find out how the resistance arose within this area, whether it arose at a single point and spread to the entire affected area or whether it arose at several points simultaneously within the affected area. We hesitate to offer any views in the matter, in the absence of a deeper insight into the genetical mechanisms involved.

Summary

Tests for susceptibility to dieldrin using WHO standard kits were carried out with field collected A. culicifacies in a few villages of Murbad taluka, Thana district, Bombay State, India, between October 1958, when the species had developed a measurable degree of resistance to the insecticide, and December 1959, by which time there was a gradual but incomplete reversion towards susceptibility. While in October 1958 the proportions of the resistants and susceptibles in the populations were estimated to be approximately 75% and 25% respectively, the same in October 1959 were 25% and 75% respectively. The reversion towards susceptibility occurred after dieldrin was withdrawn from the area.

Acknowledgements

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TABLE I. PERCENTAGE MORTALITIES OF *A. CULICIFACIES* FEMALES (WILD CAUGHT AND FRESHLY ENGORGED) TO VARIOUS CONCENTRATIONS OF DIELDRIN IN VILLAGES OF MURBAD TALUKA, THANA DISTRICT, BOMBAY STATE, INDIA, AT DIFFERENT PERIODS OF THE YEAR (WITH WHO KIT)

Exposure for one hour and mortalities observed at the end of 24 hours

Date of test	October 1958 (12th to 15th)		December 1958 (21st to 25th)		March 1959 (12th to 16th)		May 1959 (5th to 11th)		October 1959* (17th to 25th)		December 1959 (22nd to 24th)	
	Number exposed	Percent-age kill	Number exposed	Percent-age kill	Number exposed	Percent-age kill	Number exposed	Percent-age kill	Number exposed	Percent-age kill	Number exposed	Percent-age kill
Control	88	1.4	126	0.0	-	-	45	0.0	65	4.6	45	0.0
0.05	65	3.5	72	38.9	-	-	95	5.3	40	32.5	-	-
0.1	66	12.6	74	31.1	-	-	44	38.6	45	44.4	40	70.0
0.2	82	22.3	77	45.3	-	-	45	48.9	44	62.2	22	68.2
0.4	82	30.9	72	69.4	10	40.0	48	62.5	45	73.3	40	78.0
0.8	103	26.4	73	45.2	12	58.3	45	75.5	45	68.8	40	82.5
1.6	94	39.7	71	69.0	25	40.0	210	73.3	62	75.8	40	95.0
3.2	-	-	35	74.3	43	90.1	36	91.7	-	-	40	100.0
6.4	-	-	36	88.9	-	-	35	100.0	-	-	-	-

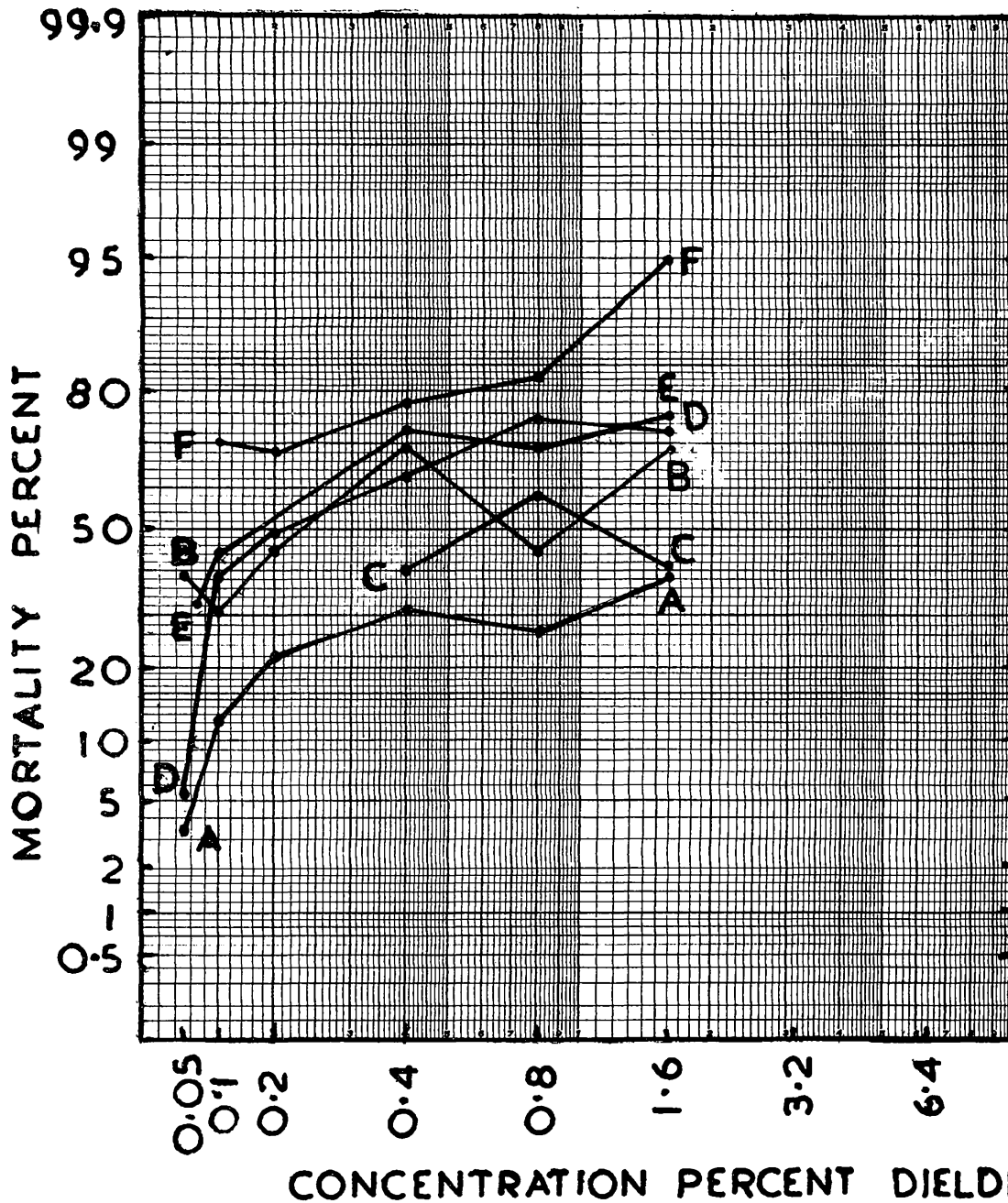
3.2 and 6.4 per cent. papers were those which were impregnated in this laboratory.

* Mosquitos collected from Talewadi and Amboli of Murbad Taluka, Thana District, about 20 miles away from Potgaon. But it is also one of the villages affected by malaria epidemic in 1958. Two rounds of DDT spraying at a dosage of 112 mg per sq.ft were given in 1959.

TABLE II. PERCENTAGE MORTALITIES OF A. CULICIFACIES FEMALE (WILD CAUGHT)
TO THE VARIOUS DOSAGES OF DIELDRIN IN DIFFERENT PARTS OF BOMBAY STATE

Year	1957	1957	1957	February 1959	August 1959	September 1959	September 1959	
Sprayed or Unsprayed	Unsprayed	Unsprayed	Sprayed with DDT	Sprayed with DDT and Dieldrin	Sprayed with DDT & Dieldrin	Sprayed with DDT & Dieldrin	Sprayed with DDT only	
Place & Dist.	Chorbavli (Nagpur)	Kada (Bhdr)	Godhra (Panchmahals)	Manjri (Poona)	Veraval (Junagadh)	Junnar (Poona)	Paradapur (Aurangabad)	
Dieldrin percent.	Total exposed	% kill	Dieldrin percent.	Total exposed	% kill	Total exposed	% kill	
Control	35	0.0	Control	27	0.0	Control	48	0.0
0.0125	39	46.0	0.0078	36	3.0	0.05	48	6.3
0.025	34	47.0	0.0156	33	9.0	0.1	49	24.4
0.05	29	76.0	0.0312	48	42.0	0.2	51	80.4
0.1	34	100.0	0.0625	41	88.0	0.4	51	80.4
0.2	18	100.0	0.125	36	100.0	0.8	50	84.0
			0.25	19	94.0			
			0.5	24	100.0			
				21	100.0			
Method	B U S V I N E - N A S H							W H O A D U L T K I T

CHART I

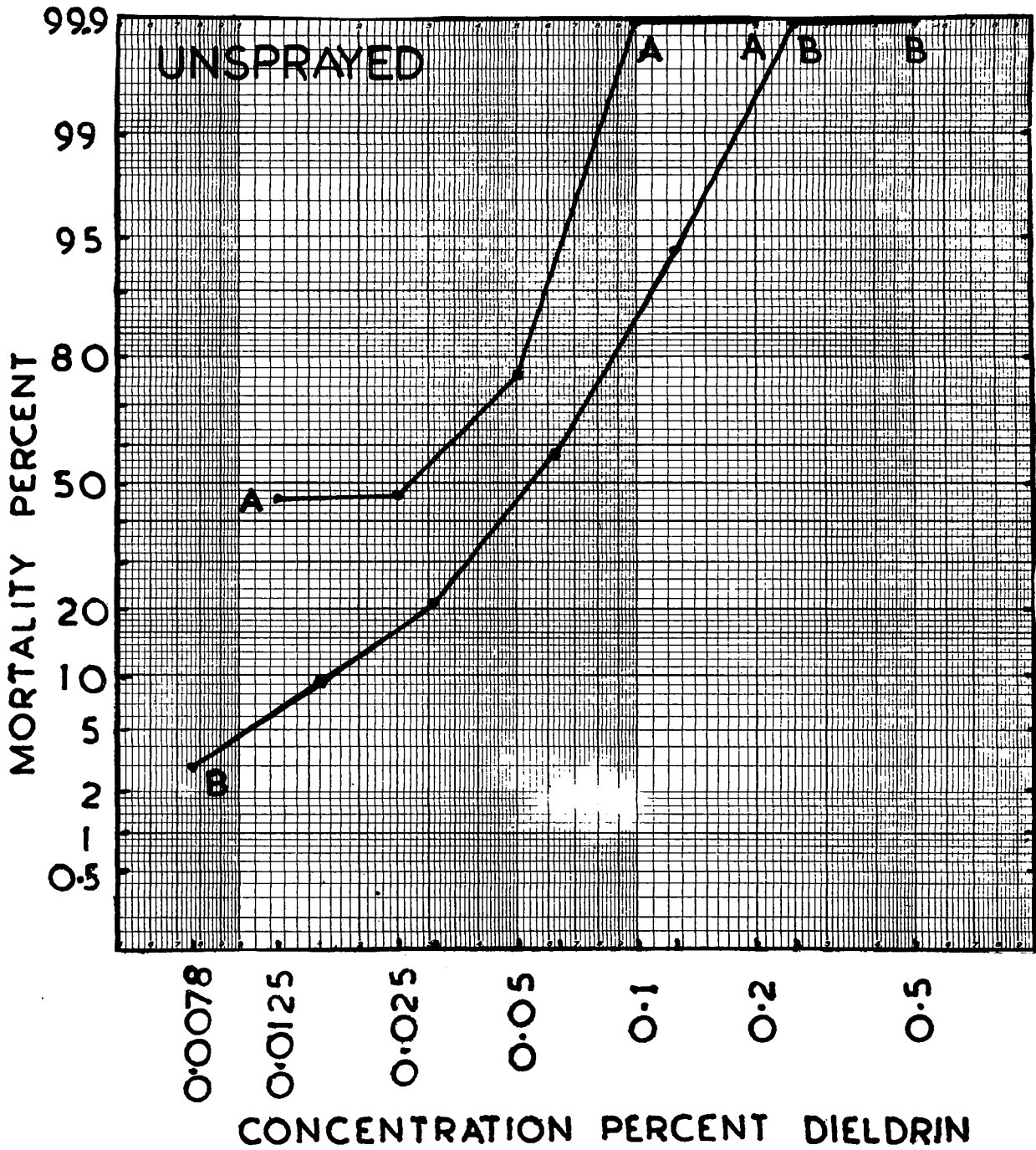


Dosage Mortality lines for *A. culicifacies*, Thana District (WHO Papers):-

A-A October 1958; B-B December 1958; C-C March 1959; D-D May 1959; E-E October 1959; F-F December 1959.

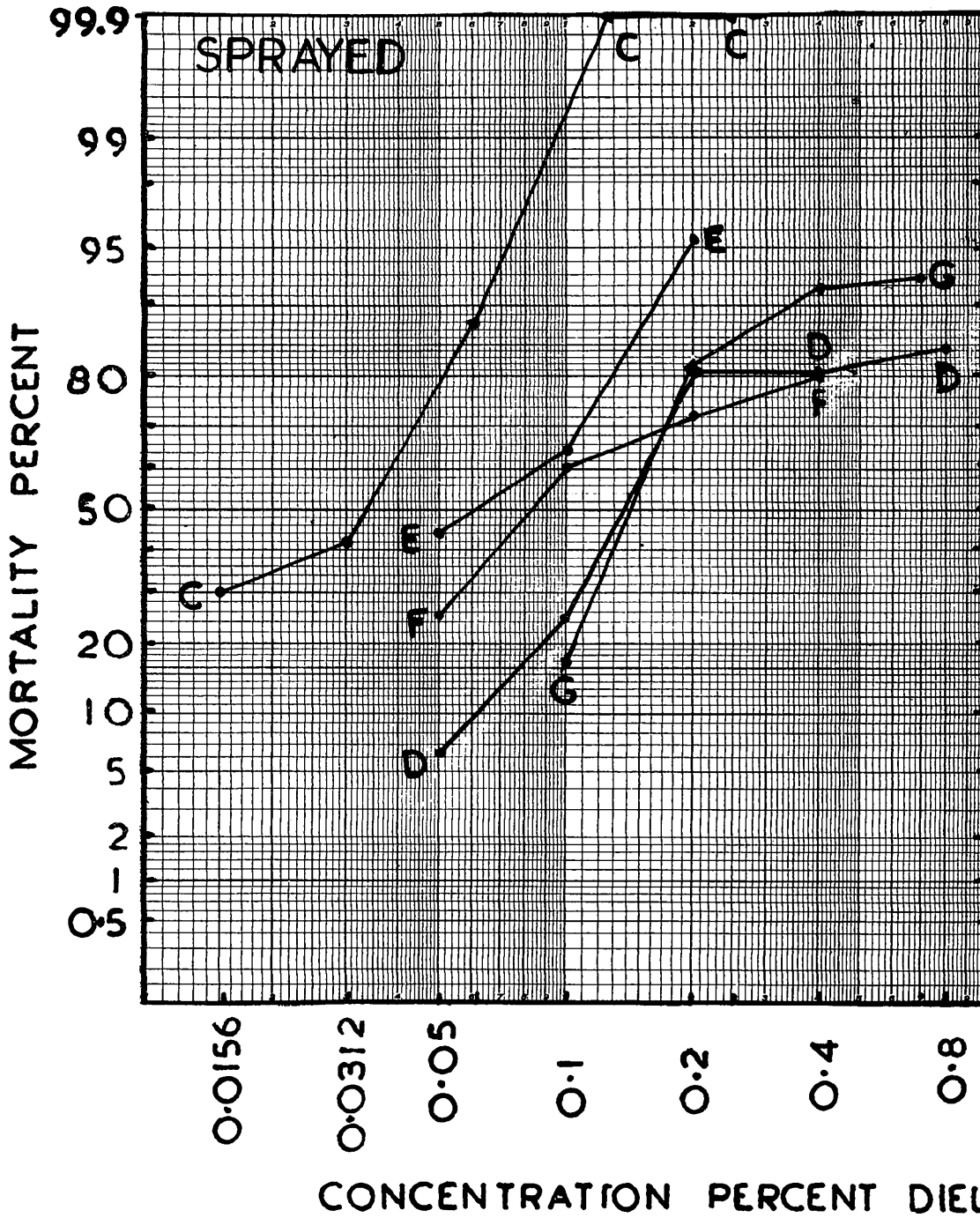
Note: Regression lines have not been drawn because of non-linearity, but the points have been directly connected with each other.

CHART II



Dosage Mortality lines for *A. culicifacies* from areas in Bombay State without any history of insecticide spraying (locally-impregnated papers). A-A Chorbavli (Nagpur); B-B Kada (Bhir).

CHART III



Dosage Mortality lines for *A. culicifacies* from areas in Bombay State with history of insecticide spraying (locally-impregnated papers).
 C-C Godhra (Panch Mahals); D-D Manjri (Poona); E-E Veraval (Junagadh); F-F Junnar (Poona); G-G Fardapur (Aurangabad).