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PRELIMINARY NOTE ON THE DEVELOPMENT OF DDT RESISTANCE IN A. CULICIFACIES GILES IN PANCHMAHALS DISTRICT OF GUJERAT STATE (FORMERLY PART OF BOMBAY STATE) INDIA

by

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Intensive susceptibility testing of A. culicifacies against DDT has been carried out in Panchmahals District of Gujerat State since September 1959 (Fig. 1).

Panchmahals District, which is situated about 350 miles north of Bombay city has been exposed to residual DDT spraying since 1950 as follows:

1950)
1951) Three cycles/year, each of 56 mg DDT/sq. ft.
1952)

1953)
1954) Two cycles/year, each of 112 mg DDT/sq. ft.
1955)

1956)
1957) One cycle/year, of 112 mg DDT/sq. ft.

1958)
1959) Two cycles/year, each of 115 mg DDT/sq. ft.
1960)

In 1958 and 1959 an additional (third) cycle of DDT at 112 mg/ft^2 was applied in Baria and Limkheda talukas which comprise about one fifth of the area of the district.

The malaria vectors encountered in the district are A. culicifacies, A. fluviatilis and A. stephensi. The most important is considered to be A. culicifacies which is prevalent throughout the year and has been found by us in the highest densities during the months of November, December and January.

The malaria transmission season lasts from July to November generally throughout the district and probably until February in Baria and Limkheda talukas.

The first tests carried out in September 1959 showed an appreciable reduction in the normal susceptibility of A. culicifacies to DDT. These tests performed in the villages of Motipura in Baria taluka and Sindbhall Mehta in Halol taluka which are 30 miles apart, gave LCs_{50} of between 2.3 and 2.5% DDT.

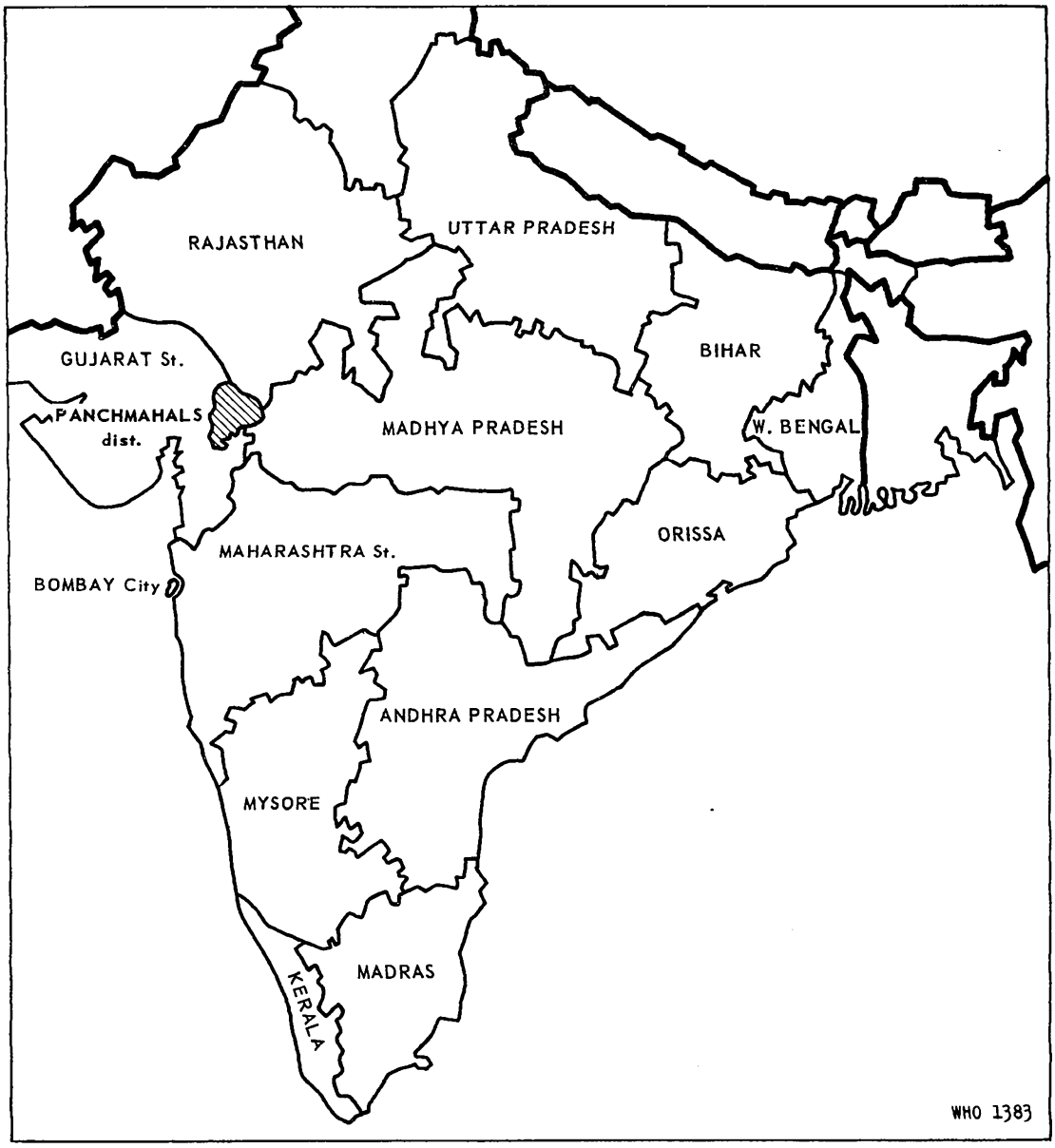
Unfortunately no DDT susceptibility tests on A. culicifacies had been carried out previously in these areas. However, at two other locations in Panchmahals district previous tests on A. culicifacies had demonstrated an LC_{50} of 0.5% DDT at Khojwa village (Rao and Bhatia, 1957) and of 0.5% at Popatpura (Bhatia et al. 1958). Prior to our tests the highest LC_{50} DDT recorded for A. culicifacies was 0.84% at Potgoan village in Thana district of Bombay State (Patel et al., 1959) about 300 miles south of Panchmahals. Previously published information on the DDT susceptibility status of A. culicifacies at various places in India is gathered in Table I. From this it will be seen that until August 1959 the recorded LC_{50} DDT varied from 0.4 to 0.84% in DDT sprayed areas and from 0.16% to 0.43% in unsprayed areas. The tests at Motipura and Sindbhall Mehta, showing an appreciable increase in the LC_{50} DDT of A. culicifacies coupled with the fact that 100% mortality was not produced at the highest impregnated paper concentration (4.0% DDT), gave the impression that we were dealing with a heterogeneous A. culicifacies population developing DDT resistance.

Susceptibility tests were continued at intervals until February 1960 when the work was interrupted by the shortage of DDT impregnated papers. Until that time there had been no further increase in the LC_{50}. In fact the LC_{50} of A. culicifacies at Motipura village dropped to 1.4% during the month of January 1960 but it showed an increase to 1.8%-2.1% DDT during the month of February as indicated in Table II.

With the arrival of fresh DDT impregnated papers in May 1960, susceptibility tests were continued at monthly intervals. Tests carried out in June 1960 indicated more definitely the development of DDT resistance in this species. In Motipura village (Baria taluka), for example, the highest LC_{50} recorded up to February 1960 was 2.1% DDT. In the series of tests performed in June, July and August 1960 the LC_{50} had risen to a figure greater than 4% (Table II). In 1959 the lowest mortality

FIG. 1

MAP OF INDIA, WITH THE SHADED PART OF GUJARAT STATE INDICATING THE DISTRICT OF PANCHMAHALS



recorded to 4.0% DDT was 65.0%. In the June, July and August 1960 tests it had fallen to 34% - 38%. In Rampura village (Godhra taluka) a similar situation is developing. The LC_{50} DDT was 2.8% in June 1960, 3.7% in July 1960 and 4.0% in August 1960 compared to the previous highest figure of 1.6% in November 1959 (Table III).

Susceptibility testing with lengthened exposure periods of 2, 3, 4 and 8 hours was carried out. Eight hours' exposure to 4.0% DDT gave mortalities of 90%, 79.0% and 75.0% in July, August and September 1960 compared with 99.0% in November 1959 (Table IV).

Tests on the progeny of the Anopheles survivors of 4.0% DDT exposed for one hour and 4% DDT exposed for eight hours have been attempted. Owing to difficulties in breeding A. culicifacies in the laboratory, no conclusive results were obtained.

Susceptibility tests of A. culicifacies on dieldrin have been performed. At Motipura village the LC_{50} was 0.12% in December 1959 and 0.065% in March 1960 (Table VIII).

Larval tests with DDT were carried out in February and October 1959 and in April and October 1960 (Table VI). At Godhra in October 1959 the LC_{50} DDT was 0.03 p.p.m. At Motipura during the period from February 1959 to October 1960, the LC_{50} DDT has risen from less then 0.02 p.p.m. to a figure between 0.13 and 0.16 p.p.m. indicating a significant decrease in susceptibility.

At Motipura village in May 1960, the LC_{50} for dieldrin was less than 0.0008 p.p.m. (Table VII).

FIELD OBSERVATIONS

Parallel with the series of adult susceptibility tests, careful observations have been made on variations in density and on the resting habits of A. culicifacies, in order to detect any sign indicating the failure of DDT residual spraying to control the vector effectively.

Until the end of 1959 field observations showed no indication of a possible breakdown in control. Densities of A. culicifacies in well-sprayed structures were very low and no A. culicifacies could be found resting even on old DDT deposits.

However, in early 1960 it was noticed that significant increases in densities of A. culicifacies were occurring in houses three to four weeks after they had been sprayed. At the same time it was noticed that a few A. culicifacies were found resting on old visible DDT deposits (at Motipura on a 7-month-old deposit; at Rameshra, Halol taluka, on 4-month-old deposits).

During July and August 1960 it was noticed at Motipura village that a round of DDT spraying had little effect on the day-time resting densities of A. culicifacies. Houses which were sprayed on 3 July were found to yield large numbers of mosquitos, equal to pre-spraying densities, on 12 July. These houses have continued to yield high catches of A. culicifacies, estimated at about 100 per man hour. At the same time it was noticed that an occasional A. culicifacies could be observed resting on fresh deposits of DDT. It should be pointed out that the DDT spraying in these houses was of very poor quality.[1]

DISCUSSION

The results of the first tests carried out at Motipura and Sindbhall Mehta in September 1959 raised the question of whether we were dealing with the first instance of physiological DDT resistance in A. culicifacies. Although the dosage/mortality curve given by these tests showed no plateau effect at the higher dosages, it was felt likely that, since no 100% mortality could be obtained with 4% DDT and as the LC_{50} had risen to at least five times that recorded in unsprayed areas, we were dealing with heterogeneous A. culicifacies population developing DDT resistance.

The series of tests carried out at approximately monthly intervals until February 1960 did not demonstrate any further increase in resistance levels in the population, nor was a plateau effect evident in the dosage mortality curve in any of the tests.

During November and December 1959 two series of tests on A. culicifacies were carried out using an 8 hour exposure to 4% DDT. The survival rate was just over 1% (6 out of 495 tested). Zulueta (1959), in his work on A. sacharovi in Greece and Turkey, considered that the presence of survivors after 8 hours exposure to 4% DDT indicated the presence of physiological resistance.

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[1] It would have been of much interest to see if the decreased insecticidal effect of DDT was accompanied or followed by an increased transmission of malaria assessed by epidemiological methods. Ed.

The tests in June, July and August 1960 indicated with much more certainty the appearance of DDT resistance. At Motipura the mortalities on 4% DDT had fallen to 34-37%, the LC_{50} had risen to over ten times that of a normally susceptible population and the dosage mortality curve was much flattened although still not demonstrating the plateau effect expected in a mosquito population developing resistance. In addition, it was shown that the proportion of A. culicifacies surviving 8 hours exposure to 4% DDT had increased from 1% in December 1959 to 9% in July, 21% in August and 25% in September 1960.

It is of interest to notice that DDT resistance in A. culicifacies has taken the long period of ten years to develop in the area. The tests reported by Bhatia et al. (1958), in which at two places in Panchmahals the LC_{50} of DDT to A. culicifacies was found to be 0.5%, indicate that there had been little decrease in the susceptibility during the eight years of DDT residual spraying from 1950 to 1957. However, since 1957, the LC_{50} had risen from 0.5% to between 1.5% and 2.5% in about two years and to a figure greater than 4.0% nine months later.

This long delay in the development of resistance in A. culicifacies could be attributed to the reaction of excito-repellency exhibited by the mosquito on contact with DDT sprayed surfaces. Observations in two experimental huts at Motipura village demonstrate the highly irritant effect of DDT on A. culicifacies. Viswanathan et al. (1955), as a result of their work on the nocturnal behaviour of A. culicifacies in Poona district of Bombay State, had suspected the excito-repellency of DDT on this species. This irritability, by causing the mosquito to leave a sprayed surface very quickly, may reduce the pressure exerted by the DDT deposits to a level below that which is necessary to select out the resistant individuals in the population. This has probably slowed down the appearance of physiological resistance.

The delayed appearance of resistance in A. culicifacies (only during the tenth year of continuous presence of residual DDT) might also be due to the fact that the period from 1958 to 1960, during which the LC_{50} DDT had been rising, coincides with the intensified DDT spraying during the attack phase of the National Malaria Eradication Programme. From 1950 to 1955 inclusive, two cycles a year of DDT spraying were given to the area. In 1956 and 1957 only one cycle was given in each year.

When the eradication programme commenced in 1958, the number of cycles per year was increased to two in some parts of the district and to three in others. This has increased the number of months per year in which the A. culicifacies population is exposed to DDT selection pressure and it has reduced the periods without selection pressure, during which a dilution of the resistant genes could occur.

SUMMARY

The results of susceptibility tests in Panchmahals district since September 1959 have demonstrated physiological resistance to DDT on the part of A. culicifacies after 10 years of DDT spraying.

It seems likely that the development of resistance within the last 2 years has been caused by the increased DDT selection pressure consequent on the intensification of DDT spraying operations during the attack phase of the National Malaria Eradication Programme (1958-60). The high degree of irritability exhibited by A. culicifacies to DDT may also help to account for the delay in appearance of DDT resistance and for the fact that the populations are still heterogeneous in their response to DDT.

(Since the original note was submitted in October 1960, tests carried out at three localities in Panchmahals district have demonstrated a higher degree of resistance to DDT than that previously recorded. These localities are Omidpura and Bania villages of Limkheda taluka and Mandhra village of Baria taluka. At Omidpura the mortality of A. culicifacies in 4% DDT exposed for 1 hour was 6.0%, at Bania - 3.7% and Mandhra - 17.8%.

Despite the increase of the levels of resistance and its persistence in the district, there has been no noticeable evidence of A. culicifacies invading the houses after thorough DDT spraying. Also there has been no increase in the tendency of A. culicifacies to rest on fresh DDT deposits).

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TABLE I. PREVIOUSLY PUBLISHED RESULTS OF DDT SUSCEPTIBILITY TESTS OF A. CULICIFACIES

Location	LC_{50}	Spray history	Investigators
BOMBAY STATE			
PANCHMAHALS			
Khojwa	0.501	7 yrs DDT	T.R.Rao & S.C.Bhatia (1957)
Popatpura	0.5	7 yrs DDT	Bhatia et al. (1958)
Sindbhai Mehta	2.6	10 yrs DDT	Rahman et al. (1959)
POONA			
Manjari	0.453 0.536	4 yrs DDT	T.R.Rao & S.C.Bhatia (1957)
Manjari	0.2	2 yrs DDT & 2 yrs Dieldrin	S.C.Bhatia et al. (1958)
Dhond	0.598	1 yr DDT	T.R.Rao & S.C.Bhatia (1957)
Dhond	0.24	Unsprayed	T.R.Rao & S.C.Bhatia (1957)
Kolwadi	0.6	2 yrs DDT & 2 yrs Dieldrin	S.C.Bhatia et al. (1958)
Baramati	0.535	4 yrs DDT	T.R.Rao & Bhatia (1957)
KOLABA			
Dasagaon	0.365	Unsprayed	T.R.Rao & Bhatia (1957)
Ladapur	0.41	4 yrs DDT	" "
THANA			
Revti	0.412	7 yrs DDT	" "
	0.571		" "
Potgaon	0.84	9 yrs DDT	Patel et al. (1959)
NAGPUR			
Chorbarvli	0.2	Unsprayed	Bhatia et al. (1958)
BHIR			
Kada	0.3	Unsprayed	" " "
DHARWAR			
Bellary	0.379	Unsprayed	T.R.Rao & Bhatia (1957)
BIJAPUR			
Haldur	0.472	7 yrs DDT	" "
MYSORE STATE			
MANDYA			
Nelligeri	0.158	Unsprayed	* M.I.I. Team (M.L.Mamman)
Mandya District	0.25	Unsprayed	Sunder Rao et al. (1958)

* The findings of the Malaria Institute of India (M.I.I.) teams were quoted by Pal (1958).

TABLE I. PREVIOUSLY PUBLISHED RESULTS OF DDT SUSCEPTIBILITY TESTS OF A. CULICIFACIES (Continued)

Location	LC_{50}	Spray History	Investigators
RAJASTHAN STATE			
UDAIPUR			
Kailashpuri	0.78	DDT twice	* M.I.I. Team (J.S.Khamre)
"	0.19) 0.22)	Unsprayed	Sharma et al. (1957)
MADHYA PRADESH			
MORENA			
Palad Pur	0.35) 0.34)	Unsprayed	* M.I.I. Team (Babu Ram)
UTTAR PRADESH			
MEERUT			
Arthala	0.43	Unsprayed	Sharma et al. (1957)
PUNJAB			
MARNAL			
Uchana	0.31	Unsprayed	* M.I.I. Team (M.I.D.Sharma)

* The findings of the Malaria Institute of India (M.I.I.) teams were quoted by Pal (1958).

TABLE II. ADULT SUSCEPTIBILITY TESTS WITH DDT ON A. CULICIFACIES FROM MOTIPURA VILLAGE (WHO METHOD)

Date	Temp. during exposure (C°)	Relative humidity during exposure (%)	Temp. during following 24 hrs (C°)		Percentage mortality at DDT concentrations					Control	LC_{50}
			Max.	Min.	0.25%	0.5%	1%	2%	4%		
3 to 16 Sep. 59	27.5	94	32	26	9(55)	20(45)	24(55)	30(40)	65(55)	2(55)	2.0-2.5%
22 Sep. to 6 Oct. 59	27	80-90	27½	24	-	-	21.5(78)	44.5(74)	81(77)	5.3(78)	2.1%
1 to 5 Dec. 59	23	50-70	25	21	5(100)	9(80)	14(80)	55(80)	88(76)	0(80)	1.9%
19 to 21 Jan. 60	18	63-67	19	17	1.7(60)	5(80)	37.5(80)	64(78)	96(72)	0(80)	1.4%
18 to 28 Feb. 60	22-26	40-52	26	21	1.2(78)	6.5(78)	20(80)	48(71)	91(67)	0(76)	1.8-2.1%
15 to 22 Jun. 60	31	80	35	30	5(80)	11(81)	15(80)	24(79)	34(79)	0(79)	> 4%
12 to 19 Jul. 60	29-31	84-88	31	29	4(125)	3(101)	2(102)	5(104)	35(105)	3(105)	> 4%
8 to 10 Aug. 60	27-28	82-85	28½	27	-	-	4(185)	13(184)	37(183)	0.5(188)	> 4%
2 to 6 Sep. 60	28	75-83	29	27	-	-	3(77)	22(81)	45(79)	0(79)	> 4%

Note 1. The figures in brackets are the numbers of mosquitos tested at each concentration.
2. The mortalities in the 22/9-6/10/59 tests are corrected for control mortality.

Spray History

1950)
1951) 2 cycles DDT at 56 mgs/sq.ft
1952)

1953)
1954) 2 cycles DDT at 112 mgms/sq.ft
1955)

1956)
1957) 1 cycle at 112 mgms/sq.ft

1958)
1959) 3 cycles at 112 mgms/sq.ft

1960 -1 cycle July 1960

TABLE III. ADULT SUSCEPTIBILITY TESTS WITH DDT ON A. CULICIFACIES FROM GODHRA (WHO METHOD)

Date	Temp. during exposure (C°)	Relative humidity during exposure (%)	Temp. during following 24 hrs (C°)		Percentage mortality at DDT concentrations					Control	LC_{50} %
			Max.	Min.	0.25%	0.5%	1%	2%	4%		
28/29 Sep. to 10 Oct. 59	28	85-95	29	26	-	-	7.5(69)	85.5(68)	96.5(65)	8.8(68)	1.5
19 to 28 Nov. 59	25	60	25	23	-	1.8(56)	30.5(76)	55(69)	93.7(80)	0(76)	1.6
29-30 Jan. 60	18	41	21	19	1.2(80)	5(80)	37(78)	78(79)	97.5(80)	1.2(80)	1.2
21-27 Feb. 60	21-28	30-55	28	20	5(80)	11(82)	48(77)	73(74)	97.4(76)	0(78)	1.2
29-30 Jun. 60	30	92-94	31	30	5.4(56)	10(71)	16.5(73)	33(72)	65(74)	1.4(72)	2.8
26-28 July 60	30-32	78-89	32	29½	1.7(60)	5.3(75)	2.2(92)	30(91)	53(90)	2.2(92)	3.7
24-25 Aug. 60	27-28	84-87	28½	27	-	3.5(56)	7.5(80)	40(80)	49(80)	2.5(80)	4.0

Note 1. The figures given in brackets are the numbers of mosquitos tested at each concentration.
2. The mortalities for the 28/9-10/10/59 tests are corrected for control mortality.

Spray History

1950)
1951) 2 cycles DDT at 56 mgms/sq.ft
1952)

1953)
1954) 2 cycles DDT at 112 mgms/sq.ft
1955)

1956)
1957) 1 cycle DDT at 112 mgms/sq.ft

1958)
1959) 2 cycles at 112 mgms/sq.ft

1960 - 1 cycle at 112 mgms/sq ft

TABLE IV. ADULT SUSCEPTIBILITY TESTS WITH 4% DDT ON A. CULICIFACIES FROM MOTIPURA USING LENGTHENED EXPOSURE TIMES

Date	PERCENTAGE MORTALITIES							
	2 hours		3 hours		4 hours		8 hours	
	4% DDT	Control	4% DDT	Control	4% DDT	Control	4% DDT	Control
11 to 26 Nov. 59	92.5(40)	0(20)	-	-	-	-	98.6(220)	0(120)
1 to 8 Dec. 59	-	-	-	-	-	-	98.9(275)	0(120)
18 to 27 Feb. 60	-	-	96.2(53)	4(56)	-	-	-	-
2 to 5 July 60	-	-	-	-	-	-	**91%(259)	6.5(77)
11 to 18 Aug. 60	50(79)	0(20)	-	-	59(101)	2.5(40)	79(118)	2.5(39)
1 to 5 Sept.60	50(80)	0(40)	-	-	72.5(80)	2.5(40)	75(161)	2.5(41)

* Corrected mortality

TABLE V. OTHER TESTS ON A. CULICIFACIES AGAINST DDT IN GUJERAT STATE

Location	LC_{50}	Spray History	Date
PANCHMAHALS			
Sindbhai Meta	2.4	10 yrs DDT	September 1959
Omidpura	> 4.0	11th yr DDT	September 1960
KAIRA			
Vasad	0.8	3 yrs DDT	October 1959
SABARKANTHA			
Himatnager	0.9-1.0	7 yrs DDT	February 1960
BARODA			
Kevdi	1.8-2.0	7 yrs DDT	October 1959
"	1.0	7 yrs DDT	December 1959

TABLE VI. LARVAL SUSCEPTIBILITY TESTS WITH DDT ON A. CALICIFACIES IN PANCHMAHALS DISTRICT (WHO METHOD)

Locality	Date	Temperature at which test was performed (C°)	Percentage mortalities at DDT concentrations (parts per million)						LC_{50} (ppm)
			0.004	0.02	0.10	0.50	2.50	Control	
Motipura (Baria Tal)	2-3 Feb. 1959	18-21	10(20)	100(20)	100(20)	100(20)	100(20)	0(20)	Between 0.004 and 0.02
"	14-15 Oct. 1959	27-28	*5.25(40)	*10.5(40)	*58(40)	100(40)	100(26)	5.25(38)	0.08
"	26 Apr. 1960	22-23.5	20(30)	32(28)	45(29)	89(35)	100(38)	0(29)	0.06-0.07
"	5 Oct. 1960	24-25	2.0(49)	8.3(48)	28.6(49)	90.0(50)	100(50)	0(49)	0.13-0.16
Godhra (Godhra Tal)	7-8 Oct. 1960	26-28	14(78)	35(89)	85(92)	100(91)	-	13.75(80)	0.03

Notes on tables

1. *Corrected mortalities
2. The figures in brackets are the number of larvae tested.
3. All larvae tested were in late III or early IV instar.

TABLE VII. LARVAL SUSCEPTIBILITY TEST WITH DIELDRIN ON A. CULICIFACIES FROM MOTIPURA VILLAGE (WHO METHOD)

Date	Temperature at which test was performed (C°)	Percentage mortalities at Dieldrin concentrations (parts per million)						LC_{50} (ppm)
		0.0008	0.004	0.02	0.1	0.5	Control	
11 May 1960	22-23	82.5(40)	95(40)	100(40)	100(40)	100(40)	2.5(40)	<0.0008

Notes on tables

1. *Corrected mortalities
2. The figures in brackets are the number of larvae tested.
3. All larvae tested were in late III or early IV instar.

TABLE VIII. ADULT SUSCEPTIBILITY TESTS WITH DIELDRIN ON A. CULICIFACIES FROM MOTIPURA (WHO METHOD)

Date	Temp.during exposure (C°)	Relative humidity during exposure (%)	Temp.during following 24 hrs (C°)		Percentage mortalities at DLD concentrations						Control	LC_{50} %
			Max.	Min.	0.05%	0.1%	0.2%	0.4%	0.8%	1.6%		
14-16 Dec. 59	23-24	50-60	24	20½	6.5(77)	32(76)	83.5(58)	98.6(73)	98.6(73)	100(75)	1.3(78)	0.12
9-18 May 60	27	50	28	22	31(99)	79(100)	98(103)	100(101)	100(101)	-	2(100)	0.065

Note The figures shown in brackets are the numbers of mosquitos tested at each concentration.