SUSCEPTIBILITY TO INSECTICIDES OF CERTAIN ANOPHELINES ACCORDING TO THE TIME OF CATCH AND TESTING

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INTRODUCTION. It is general practice to perform insecticide susceptibility tests of adult mosquitoes during the morning hours of the day. For this, the mosquitoes utilized for the tests are collected normally between the hours 0700 and 0900, and the tests are carried out immediately after.

In early 1960, *Anopheles annularis* Wulp, a common anopheline in Panchmahals district of Gujarat State in India, was found resistant to DDT, when mortalities of less than 3.5 percent only were obtained in the 4.0 percent DDT concentration, thus giving an LC_{50} much greater than 4.0 percent (Shalaby, in press).

In the course of conducting district-wide DDT susceptibility tests on *A. annularis*, it had been possible to carry out two replications at Bandibar village of Limkheda Taluka in the late part of the evening of 29 February 1960 on mosquitoes collected between the hours 2000 and 2100. In the morning of the same day two replications were conducted on mosquitoes collected between the hours 0700 and 0900, as it is the common practice. My attention was drawn to a distinct difference between the susceptibility to DDT of the *A. annularis* collected and tested in the late part of the evening and of those collected and tested in the morning. Significantly high mortalities were recorded in the susceptibility tests which were conducted in the late part of the evening. In October and November of the same year, further investigations designed to explain this phenomenon were carried out.

This article pertains mainly to *A. annularis*. Similar susceptibility test results with *Anopheles vagus* Donitz against dieldrin had been reported in January 1961 by Badawi (personal communication) in Indonesia and which have been incorporated in the present article.

MATERIAL AND METHODS. The *A. annularis* used for the susceptibility tests had been captured from one mixed dwelling in Dangaria village of Baria Taluka in Panchmahals district. For the purpose of these studies, three series of susceptibility tests to DDT were undertaken.

First, seven test replicates were carried out at intervals between the 3rd and 13th of November 1960. The *A. annularis* used for these tests were collected between the hours 0700 and 0900, and the tests were conducted at 1000 hours. The mosquitoes utilized for the tests were uninjured fully fed females caught while resting on the ceiling and on the walls.

Second, seven test replicates were carried out at intervals from the 31st of October to the 15th of November 1960. For these tests, the *A. annularis* were collected between the hours 2000 and 2100, and the tests were conducted in the laboratory at 2200 hours. It was especially noticeable that the *A. annularis* invaded the dwellings during the hours 1900 and 2300, hence no difficulty was encountered in collecting sufficient numbers of mosquitoes in a relatively short time. In most instances, the mosquitoes were carefully aspirated off the cattle soon after they finished their feed. A few were also captured while resting on the wall immediately after they had the blood meal.

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In this way, the females utilized for these tests were all fully engorged with bright red fresh blood.

Third, eight replicated tests were carried out at intervals from 1 to 16 November 1960. For the purpose of these tests, the *A. annularis* were collected between the hours 2000 and 2100 but the tests were conducted at 1000 hours of the next day. In the meantime the mosquitoes were placed in spacious cages 15 x 15 inches covered with damp cloth and were kept in the laboratory throughout the night. Only the uninjured fully fed females were utilized for testing.

For all these tests, the mosquitoes were exposed to the DDT concentrations one, two and four percent and the knockdown counts after the one hour exposure period were recorded. Otherwise, the standard test method of the World Health Organization was employed in each instance.

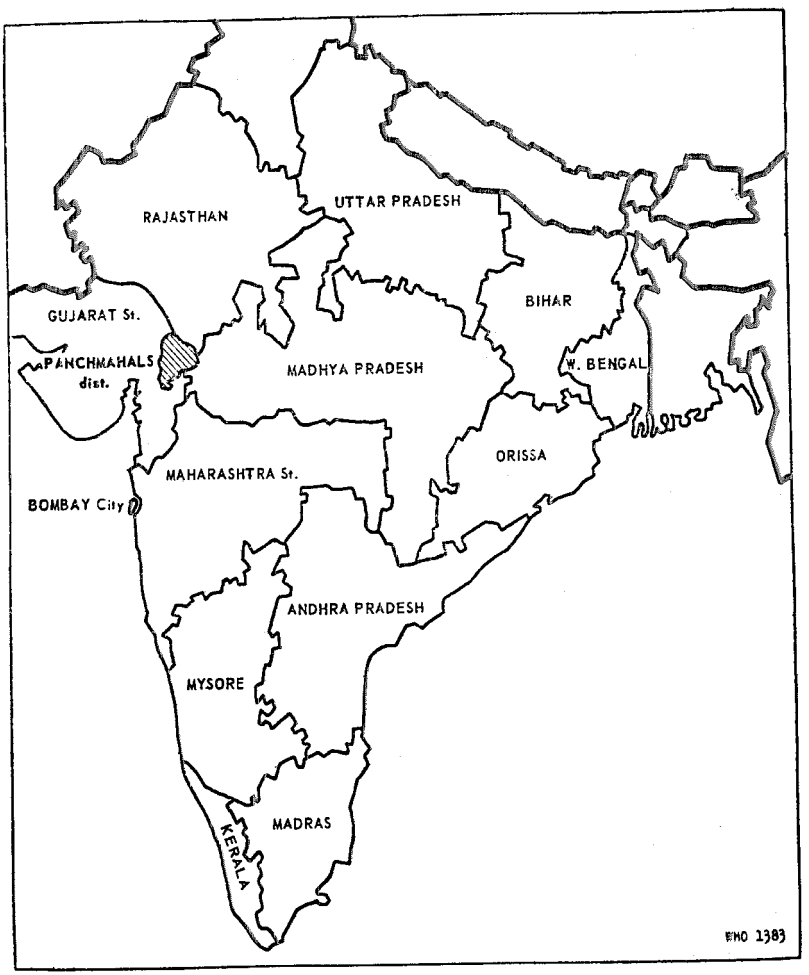

FIG. 1.—Map wih part of Gujarat State of India shaded to indicate the District of Panchmahals.

Susceptibility tests with *Anopheles vagus* against dieldrin were performed at Tambak Wedi in the Surabaya zone of East Java in Indonesia. Five replicated tests were carried out at 1000 hours with mosquitoes that had been collected between the hours 0700 and 0900 of the same morning. Five more replication tests were performed at 2200 hours with mosquitoes that had been collected between the hours 2000 and 2100 of the same evening. The *A. vagus* utilized for the tests were uninjured fully fed females and the procedures observed in conducting those tests are those of the World Health Organization.

SPRAY HISTORY OF LOCALITIES. Panchmahals district which is situated 350 miles north of the city of Bombay (Fig. 1) has been under continuous residual DDT spraying since 1950. It has received three rounds of spraying with a dosage of 56 mgm./sq.ft. every year for the first three years which was followed by two rounds of spraying each year with a dosage of 112 mgm./sq.ft. except for the years 1956 and 1957 when only one round of spraying was given with a dosage of 112 mgm./sq.ft. In 1958 and 1959, an additional third round of the dosage 112 mgm./sq.ft. was applied in Baria and Limkheda Talukas which comprise one fifth of the area of Panchmahals district.

Surabaya zone of East Java in Indonesia has received three years of residual DDT spray from 1954 to 1956 which was followed with dieldrin spraying from 1957 onwards.

RESULTS. The susceptibility to DDT of *A. annularis* that were collected and tested against the insecticide in the morning

TABLE 1.—Adult susceptibility tests on *A. annularis* collected between 0700 and 0900 hours and tested against DDT at 1000 hours.

Concentration (% DDT)	Replicate-I (% mortality)	Replicate-II (% mortality)	Total (% mortality)
0.25	0 (20)	0 (20)	0 (40)
0.5	0 (20)	0 (20)	0 (40)
1.0	0 (20)	0 (20)	0 (40)
2.0	0 (20)	10.0 (20)	5.0 (40)
4.0	5.0 (20)	15.0 (20)	10.0 (40)
Control	0 (20)	0 (20)	0 (40)

N.B.: 1—The figures in parentheses represent the number of mosquitoes tested at each concentration.
2—The temperature during exposure=27° C.
3—Range of temperature during following 24 hours=24° C.–27° C.
4—Relative humidity during exposure=51%.

TABLE 2.—Adult susceptibility tests on *A. annularis* collected at 2100 hours and tested against DDT at 2200 hours.

Concentration (% DDT)	Replicate-I (% mortality)	Replicate-II (% mortality)	Total (% mortality)
0.25	0 (20)	5.0 (20)	2.5 (40)
0.5	0 (20)	10.0 (20)	5.0 (40)
1.0	5.0 (20)	20.0 (20)	12.5 (40)
2.0	20.0 (20)	15.0 (20)	17.5 (40)
4.0	40.0 (20)	75.0 (20)	57.5 (40)
Control	0 (20)	0 (20)	0 (40)

N.B.: 1—The figures in parentheses represent the number of mosquitoes tested at each concentration.
2—The temperature during exposure=25° C.
3—Range of temperature during following 24 hours=24° C.–27° C.
4—Relative humidity during exposure=57%.

hours of 29 February 1960 at Bandibar village of Limkheda Taluka was compared with their susceptibility when they were collected and tested in the late hours of the evening (Tables 1 and 2).

It can be readily seen from table 1, that this species is highly resistant to DDT. Average mortalities of 5 and 10 percent only were obtained in the 2.0 and 4.0 percent DDT concentrations, respectively. The mortalities obtained in the lower concentrations were nil. On the other hand, the figures presented in table 2 show a lesser degree of resistance to DDT in *A. annularis*. Average mortalities of 17.5 and 57.5 percent were obtained in the 2.0 and 4.0 percent DDT concentrations, respectively. Mortalities of 2.5, 5.0 and 12.5 percent were also encountered in the 0.25, 0.5 and 1.0 percent DDT concentrations respectively.

It will also be seen from Table 3 that the mortality rates of *A. annularis* that were collected and tested in the morning from Dangaria village of Baria Taluka did not exceed 15 percent in the 4 percent DDT concentration, thus giving an average of 8.5 percent. Furthermore, the mortality rates have ranged between zero and 5.0 percent in the 1 percent DDT concentration, giving an average of 0.7 percent; and between zero and 10.0 percent in the 2 percent DDT with an average of 6.4 percent. It should be noted also that the knocked down mosquitoes during the exposure period were considerably fewer (Table 3), in addition to the fact that a number of them recovered.

It is also evident that higher mortality rates were obtained when the *A. annularis* were collected in the later hours of the evening and tested in the same evening which confirm the observation of 29 February 1960 and which was noted at Bandibar village. As indicated, the mortality rates in the 4 percent DDT had ranged between 20 percent and 75 percent, giving an average of 44.6 percent. In the 1 and 2 percent DDT concentrations, the mortality rates had ranged between zero and 30 percent and between 15 and 45 per-

TABLE 3.—Results of susceptibility tests with DDT on adult *A. annularis* from Dangaria village, Baria Taluka, Panchmahals district (WHO method).

Concentration DDT	Number mosquitos	Percent knocked down (after 1 hour)	Percent mortality (after 24 hours)
Mosquitos collected between 0700 and 0900 hrs. and tested at 1000 hrs.*			
1.0%	140	0.0	0.7
2.0%	140	2.1	6.4
4.0%	141	7.1	8.5
Control	140	0.0	0.0
Mosquitos collected at 21 hrs. and tested at 2200 hrs.**			
1.0%	138	1.5	8.7
2.0%	141	12.1	28.4
4.0%	139	35.3	44.6
Control	140	0.0	0.0
Mosquitos collected at 2100 hrs. and tested at 1000 hrs. the next day ***			
1.0%	136	0.0	3.7
2.0%	141	2.1	9.9
4.0%	160	13.1	20.0
Control	158	0.0	0.0

* Tests conducted during 3–12 November 1960. Temp. during exposure 25–26° C. RH during exposure 36%–40%. Temp. during following 24 hrs. 21–27° C.

** Tests conducted during 31 October–15 November 1960. Temp. during exposure 23–25° C. RH during exposure 45%–55%. Temp. during following 24 hrs. 21–29° C.

*** Tests conducted during 1–16 November 1960. Temp. during exposure 23–27° C. RH during exposure 38%–60%. Temp. during following 24 hrs. 21–29° C.

cent with average mortalities of 8.7 and 28.4 percent, respectively. Most striking of all was the high number of knocked down mosquitoes during the period of exposure with none recovering. Out of a total of 140 *A. annularis* which were exposed to the 4 percent DDT concentration, 49 (35.0 percent) were knocked down.

It is of interest to note, however, that when the *A. annularis* were collected in late hours of the evening and the tests were conducted the morning of the next day, the figures obtained demonstrated a level of susceptibility which seemed to be intermediate if compared with the results shown in Tables 3 and 4. The mortality rates in the 4.0 percent DDT concentration ranged between 5 and 35 percent giving an average of 20 percent. In the 1 and 2 percent DDT, the average mortality rates were 3.7 and 9.9 percent, respectively. It was of interest also to note that the number of the knocked down mosquitoes during the period of exposure was more or less parallel to those encountered during the morning tests, except in the 4 percent DDT when 21 (13.3 percent) were knocked down out of a total of 158 mosquitoes that had been exposed. Only 7.2 percent were knocked down during the morning tests.

Table 4 summarizes the tests carried out with *A. vagus* against dieldrin. Although the differences between the results of tests conducted in the morning and those conducted during the late evening were not as pronounced as encountered in the case of *A. annularis* the mortalities were sufficiently higher when the *A. vagus* were collected in the late hours of the evening and tested against the insecticide in the same evening. In the 1.6 percent dieldrin, a mortality rate of 17.6 percent was recorded during the tests that were conducted at night, while a mortality rate of 3.6 percent was encountered during the tests that were conducted in the morning (Table 4).

DISCUSSION. Hadaway and Barlow (1958) reported that the nutritional state, age and sex are factors which influence the susceptibility of the mosquitoes to the different insecticides. In 1946, David and Bracey showed a progressive decrease in resistance with age in adult female *Aedes aegypti* (Linnaeus) exposed to spray mists containing pyrethrins or DDT. They also reported that the *A. aegypti* males were more susceptible than females both to pyrethrins and DDT.

The data presented in this paper give evidence which is quite conclusive that a relationship does exist between the susceptibility of the mosquitoes and the time of their catch and their exposure to the insecticide. The differences between the susceptibility to DDT of the *A. annularis* which were collected at 2100 hours and those which were collected between the hours 0700 and 0900 can be correlated with the possibility that their composition is different. Also, the differences indicated in the case of *A. vagus* against dieldrin (Table 4), although not as pronounced as those indicated in the case of *A. annularis* against DDT due probably to innate dissimilarities in the physiologies of these two species, render substantial support to this observation.

TABLE 4.—Results of susceptibility tests with dieldrin carried out during the morning of January 10 and during the night of January 11, 1961 on *A. vagus* from Tambak Wedi, Surabaya, East Java, Indonesia.

Concentration (dieldrin)	Percent mortality	
	Morning tests	Night tests
0.2%	6.8(96)	15.0(104)
0.4%	4.7(114)	11.4(98)
0.8%	4.6(92)	14.2(84)
1.6%	6.2(124)	17.6(94)
Control	3.9(104)	3.5(106)

N.B. 1) Numbers in parentheses represent the total numbers tested at each concentration.
2) Temp. during exposure period: In the morning 28° C.; At night 27° C.–28° C.
3) RH during exposure: In the morning 78%–80%; At night 82%–86%.
4) History of spray in the area: 3 years DDT from 1954 to 1956 and 4 years dieldrin from 1957 to 1960.

It is highly probable that the *A. annularis* population that enter the dwellings during the late part of the evening is of a heterogeneous nature, constituting resistant as well as susceptible individuals; and those that are collected from the dwelling in the early hours of the morning are the selected resistant strain of the population. With a history of 11 years of intensive DDT residual spray in the area, there is every reason to believe that the pressure exerted by DDT during the hours of the night has resulted in the mortality of the susceptible elements after they acquired a lethal dose of the insecticide. This would naturally lead to the selection of the resistant elements which remain resting on the sprayed surfaces throughout the night. This seems to offer a reasonable explanation for the high mortalities recorded in the mosquitoes which were collected and tested during the late hours of the evening as compared to much less mortalities in those which were collected and tested in the hours of the morning.

On the other hand, it is of particular interest to note that the mortality rates recorded in the *A. annularis* which were collected in the late hours of the evening and tested in the morning of the next day, thus eliminating the factor of the DDT selection pressure occurring during the hours of the night (Table 3), were noticeably lower than those recorded in the mosquitoes which were collected and tested during the late part of the evening. At the same time they were considerably higher than those recorded in the mosquitoes that were collected and tested in the early hours of the morning. For example, an average mortality of 20 percent was recorded in the 4 percent DDT concentration, while average mortalities of 44.6 percent and 8.5 percent were recorded in the susceptibility tests conducted in the late hours of the evening and those conducted in the early hours of the morning, respectively.

A comparison of the values given therefore, shows that, (1) while the pressure exerted by the DDT during the hours of the night plays a definite role in the selection of the resistant and the susceptible elements in the population, hence these marked differences between the susceptibility level of the mosquitoes that were collected and tested in the hours of the morning and those that were collected and tested in the late hours of the evening; it is also obvious that (2) other factors also are, perhaps, responsible for the unusually high mortalities recorded in the mosquitoes which were collected and exposed to the insecticide in the late part of the evening.

As described above the susceptibility tests which were conducted in the evenings were performed on mosquitoes the majority of which were aspirated off the cattle immediately after taking the blood meals. In such condition, the females manifest considerably less activity due to the substantial increase in the body weight owing to the stomach being full with undigested blood. As a result, their strength may be inadequate for overcoming the toxicity of the insecticide which may so hamper the process of digestion in its initial stage. This would lead to the knockdown of the less vigorous individuals, as the metabolic activities of this stage demand more energy expenditure of which those individuals, already weakened, have become incapable. This is supported by the appreciable number of the mosquitoes that were knocked down when they were tested in the evening soon after they took the blood meal. Thirty-five percent, out of 140 *A. annularis* tested, were knocked down during their exposure to the 4 percent DDT concentration. Hadaway and Barlow (1956) reported that female *Aedes aegypti* and *Anopheles stephensi* Liston which were tested against DDT after 3 hours of taking the blood meal were more susceptible than those which were tested after 24 hours of taking the blood meal.

The nutritional state of the mosquitoes and the DDT selection pressure exerted throughout the night acting jointly, among

other factors, are probably responsible for the variations in their susceptibility when they were collected during the late hours of the evening and when they were collected during the early hours of the morning. These observations are of interest and further investigations along the same lines are indicated since the information available on this subject is limited.

SUMMARY. In experiments on the effect of the time of the mosquito collection on their susceptibility to the insecticides, it was found that the *A. annularis* which were collected between the hours 2000 and 2100 and tested at 2200 hours were more susceptible to DDT than those which were collected between the hours 0700 and 0900 and tested at 1000 hours. The same was found with *A. vagus* against dieldrin, but not as pronounced.

The susceptibility of *A. annularis* which were collected between the hours 2000 and 2100 and tested against DDT at 1000 hours of the next day was found to be intermediate between their susceptibility when they were collected and tested in the late part of the evening and their susceptibility when they were collected and tested in the hours of the morning.

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