

## SUSCEPTIBILITY OF SIX ANOPHELINE SPECIES FROM THAILAND TO INSECTICIDES

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**INTRODUCTION.** The development of resistance by mosquitoes to insecticides has posed a considerable threat to the success of public health efforts in combating mosquito-borne diseases in many parts of the world. In Thailand, insecticides have been widely used against agricultural pests and in the national malaria eradication program. Levels of susceptibility have been reported for some species of culicines in Thailand, but the only data published on the susceptibility of anophelines in this country are those reported by Scanlon and Sandhinand (1965) for adults of *Anopheles balabacensis* Baisas.

This paper presents tests conducted between 1965 and 1967 on the susceptibility of *Anopheles balabacensis* Baisas, *An. maculatus* Theobald, *An. minimus* Theobald, *An. splendidus* Koizumi, *An. tessellatus* Theobald, and *An. vagus* Donitz to DDT and/or dieldrin. All of these species are known to feed freely on man and have, except for *An. splendidus* and *An. tessellatus*, been incriminated in the transmission of human malaria either in this country or elsewhere.

**MATERIALS AND METHODS.** Material representative of all the six species tested was obtained from one or more of the following localities—the experimental farm of Kasetsart University in the Bang Khen district of Bangkok and from a variety of localities in provinces of Saraburi, Chon Buri and Nakornrajisima (Fig. 1). For several years dwellings in these provinces have been treated once or twice yearly with DDT residual sprays under

the national malaria eradication program. At the Kasetsart experimental farm insecticides, including parathion, several chlorinated hydrocarbons and paris green have been in more or less continuous use. Specific locations for any single species tested will be mentioned under its appropriate section in the discussion. *Anopheles balabacensis* was the only species tested with material provided from a laboratory colony maintained by the SEATO Medical Research Laboratory (SMRL).

The standard WHO adult and larva tests were used throughout this study (WHO, 1963). Observed mortalities of mosquitoes obtained in the various tests were corrected for control mortality using Abbott's Formula. Log-dose probit mortality regression lines were constructed as the method of Litchfield and Wilcoxon (1949) was followed for the calculation of the limits of confidence.

In order to reduce the high mortality often encountered with field-collected anophelines the following handling procedures were used: (1) engorged mosquitoes were collected in vials, from human bait; (2) identifications of mosquitoes were made in the field soon after capture, using ether to immobilize specimens, when necessary; (3) identified mosquitoes were anaesthetized with ether transferred to WHO holding tubes, held in test groups and provided with sugar-water pads or slices of any available fruit; (4) mosquitoes were safeguarded against desiccation by covering holding tubes with wet towels; (5) the condition of the males was checked closely on the following morning so that moribund and dead specimens could be removed from test groups prior to exposure to the toxicant.

**RESULTS AND DISCUSSION.** *Anopheles*

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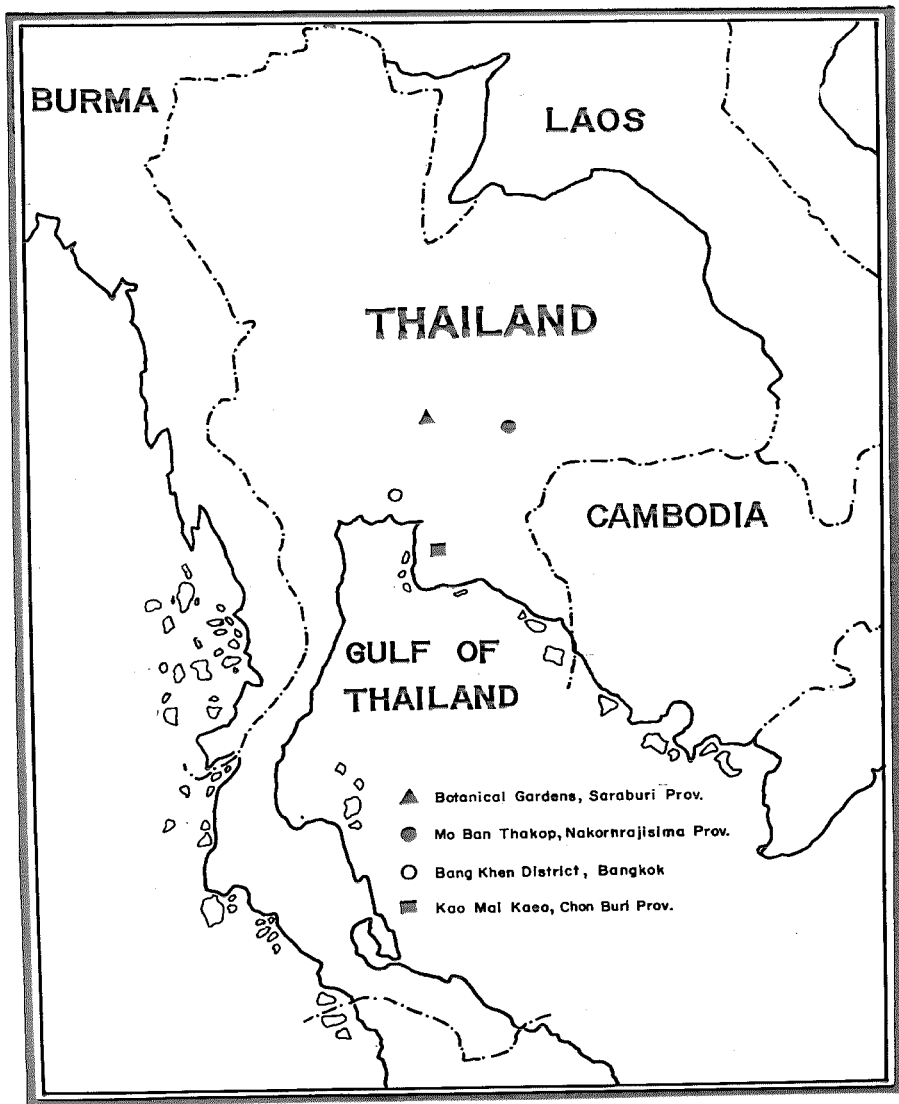


FIG. 1.—Map of Thailand indicating localities where material was collected for testing.

*balabacensis*.—Scanlon and Sandhinand (1965) tested adults of this species with material collected at Kao Mai Kao, Chon Buri Province, and they reported susceptibility to DDT to be within the normal range ( $LC_{50}=0.3$  percent). The susceptibility of larvae of this species from the SMRL colony was tested during the tests reported here. The  $LC_{50}$  obtained was 0.016 ppm indicating that larvae were normally susceptible to this insecticide. The small slope value (Table 1) of the mortality regression line (Fig. 2) obtained reflected the heterogeneity of the larvae of this colonized strain with respect to their response to DDT.

*Anopheles maculatus*.—Adults of this species were tested for their susceptibility to DDT and dieldrin. Blood-fed females were collected while biting man at Kao Mai Kao, Chon Buri Province during February 1967. Some adults were held over for egg-laying and as a result testing of larvae against DDT was possible. The  $LC_{50}$  with dieldrin for adults was 0.056 percent and the  $LC_{90}$  was 0.1 percent.

These values indicated that adults of this species were highly susceptible to dieldrin.

In the DDT tests,  $LC_{50}$  values of 0.65 percent and 0.010 ppm obtained for the adults and larvae, respectively, indicated that *An. maculatus* were also susceptible to this insecticide. The  $LC_{50}$  for adult *An. maculatus* was two times greater than that (0.3 percent) determined for adult *An. balabacensis* tested from the same area by other workers. An MLC value of 0.40 percent of DDT was obtained for adult *An. maculatus* when tested by Wharton (1955) in Malaya.

*Anopheles minimus*.—Adults and larvae of this species were tested for their susceptibility to DDT. Blood-fed females were collected while biting man in the vicinity of houses near the Botanical Gardens near Saraburi, Saraburi Province. Eggs were also recovered from some of the adults to support larval testing. Both adults and larvae of *An. minimus* were susceptible to DDT (Table 1). The  $LC_{50}$  value of 0.31 percent approximated that (0.282 percent) reported by Pal (1958)

TABLE 1.—Results of DDT susceptibility tests on some *Anopheles* species from Thailand.

Species	Date tested	Locality	Stage tested	$LC_{50}$ *	95% Confidence limits of $LC_{50}$	$LC_{90}$	Slope
<i>Anopheles balabacensis</i>	May, 66	Laboratory colony	L	0.015	0.013-0.018	0.052	2.42
<i>An. maculatus</i>	Feb., 67	Kao Mai Kao, Chon Buri Province	A	0.65	0.51-0.84	1.35	3.86
		Kao Mai Kao, Chon Buri Province	L	0.010	0.005-0.021	0.023	3.63
<i>An. minimus</i>	Jan., 67	Botanical Gardens, Saraburi Province	A	0.31	0.25-0.37	0.49	6.41
		Botanical Gardens, Saraburi Province	L	0.016	0.0136-0.0198	0.036	3.82
<i>An. splendidus</i>	Feb., 67	Botanical Gardens, Saraburi Province	A	0.48	0.42-0.56	0.86	5.18
<i>An. tessellatus</i>	Dec., 66	Botanical Gardens, Saraburi Province	L	0.012	0.006-0.025	0.026	3.76
<i>An. vagus</i>	May, 66	Ia Bang Khen Dis- trict, Bangkok	A	0.99	0.86-1.16	2.82	2.82
	Apr., 67	Ib Bang Khen Dis- trict, Bangkok	A	1.34	1.18-1.51	3.71	2.89
	May, 66	II Mo Ban Takhop near Pak Thong Chai, Nakornra- jisima Province	A	0.97	0.81-1.16	3.01	2.61

\*  $LC$  values in ppm for larvae and in percent for adults.

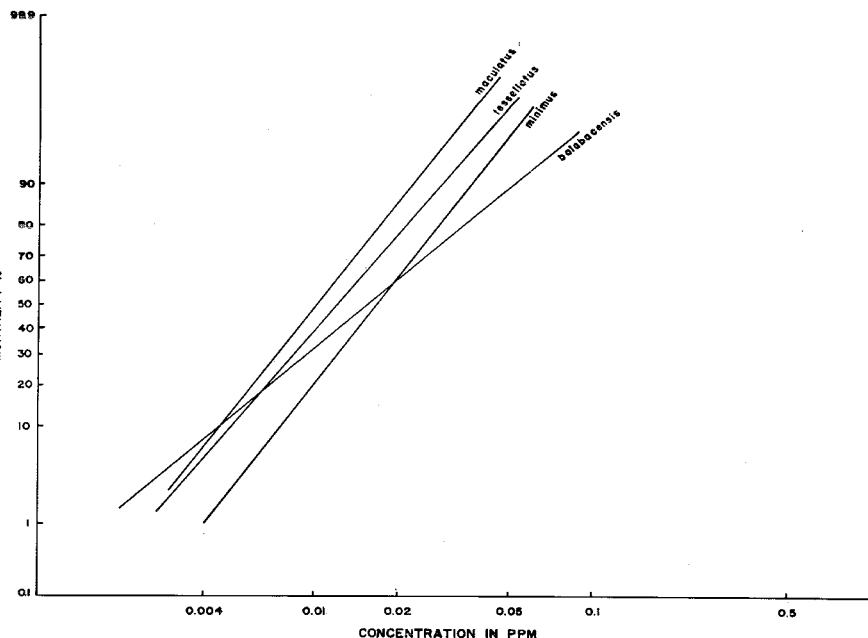


FIG. 2.—Dosage-mortality regression lines of DDT against larvae of some *Anopheles* species.

for adult *An. minimus* from an unsprayed area in Assam, India. The steep mortality regression line with a slope value of 4 also indicated that these adults were homogeneous with respect to the response to DDT (Fig. 3).

*Anopheles splendidus*.—Adults of this species, collected at the same location as those of *An. minimus*, were also tested for susceptibility to DDT. The  $LC_{50}$  value (0.48 percent) for adult *An. splendidus* from Thailand (Table 1) was lower than that obtained (0.631 percent) for the same species from an unsprayed area in Bihar, India (Pal, 1958).

*Anopheles tessellatus*.—Only larvae of this species were tested for susceptibility to DDT. The few blood-fed females collected at Saraburi were used to provide eggs for the testing of the larvae. The  $LC_{50}$  of 0.012 ppm and the steep regres-

sion line (Fig. 2) showed that larvae of this species are also susceptible to DDT.

*Anopheles vagus*.—Adults of this species, obtained from two different sources, were tested for their susceptibility to DDT. Females were collected while biting cattle at the Kasetsart University farm, in Bang Khen district of Bangkok and while biting man at Mo Ban Takop near Pak Thong Chai, Nakornrajisima Province. Almost identical  $LC_{50}$  values (0.97 and 0.99 percent) were obtained for this species at both locations. These values are about two times greater than that (0.56 percent) reported by Chellappah (WHO Bull., 1957) for this species in an unsprayed area in Singapore. The values obtained in our tests were also greater than those of the other susceptible anophelines tested (Table 1). The slopes of the regression lines indicated heterogeneous

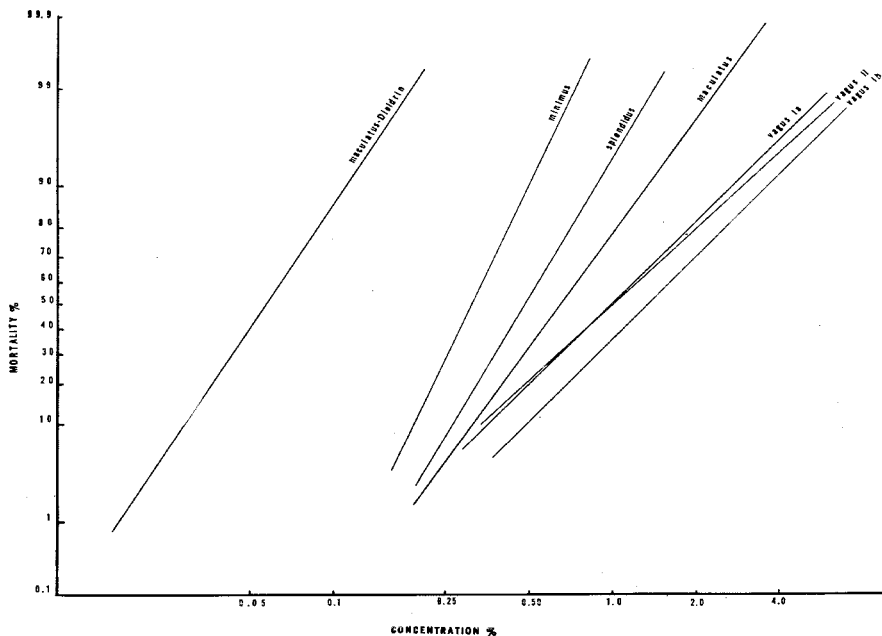


FIG. 3.—Dosage-mortality regression lines of DDT and dieldrin against adults of some *Anopheles* species

response to DDT in the *An. vagus* populations tested (Fig. 3).

Adults of *Anopheles vagus* from Bang Khen were also found to be resistant to dieldrin. When *An. vagus* adults were exposed to different concentrations of dieldrin for one hour, approximately 79 percent survived exposure to the highest (4 percent) concentration (Table 2). Although 57 percent survived exposure for

2 hours at 4 percent concentration, there were no survivors following exposure for 4 hours at this concentration. The appearance of dieldrin resistance in the *An. vagus* population was also confirmed by the "time in concentration technique" (French and Kitzmiller, 1963). Two random samples, each of fifty late fourth stage larvae, obtained from adults collected at Bang Khen, were exposed to a discrim-

TABLE 2.—Results of dieldrin susceptibility tests of adult *Anopheles vagus* from Bang Khen District, Bangkok, April, 1967.

Exposure time	Percent mortality at each concentration							Percent control mortality
	0.05%	0.1%	0.2%	0.4%	0.8%	1.6%	4%	
1 hour	8(120)	6(120)	17(120)	18(120)	21(120)	15(120)	21(120)	4(120)
2 hours	.....	.....	.....	.....	.....	.....	43(60)	3(40)
4 hours	.....	.....	.....	.....	.....	.....	100(60)	3(40)

\* Figures shown in parentheses represent the number of adults tested.

inating dosage of 8 ppm of dieldrin for 24 hours. Larval mortality was recorded at 15-minute intervals during the first 3 hours of exposure and at hourly intervals thereafter. The percentage mortality versus time was plotted on log probit paper (Fig. 4). The results obtained indicated

separating the susceptible from the resistant larvae. In sample A, 55 percent of the larvae were susceptible, 29 percent intermediate and 16 percent resistant; in sample B, 23 percent were susceptible, 50 percent intermediate and 27 percent resistant.

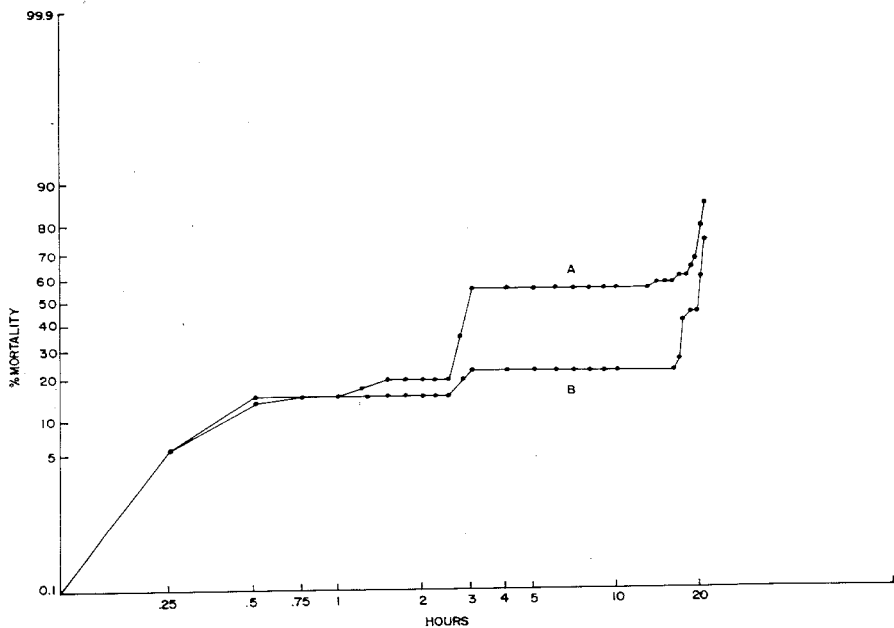


FIG. 4.—Mortality regression lines of dieldrin when tested against *Anopheles vagus* larvae by the "Time in concentration" technique.

the presence of three phenotypes for dieldrin resistance among *Anopheles vagus* larvae. Susceptible, resistant and intermediate larvae were distinguished by this technique in both samples (A and B) tested from the same population. The susceptible larvae of both samples were killed within 3 hours following exposure to the discriminating dosage of the toxicant, while the resistant individuals survived the 24 hours of exposure. The regression lines also showed distinct plateaus

The significance of the development of resistance to dieldrin and DDT by *Anopheles vagus* is difficult to evaluate at the present time since it is believed to play no role in the transmission of human malaria in Thailand. Sandosham (1959) reported that none of over 9000 wild-caught *An. vagus* dissected in Malaysia were infected, yet he reported that 55.2 percent of *An. vagus*, experimentally infected with malaria, were positive. *Anopheles vagus* is distributed widely over Thailand, and

although it is known to prefer cattle to man, this species has been reported biting man in large numbers on many occasions.

**SUMMARY.** Six species of *Anopheles* from four different localities in Thailand were tested for their susceptibility to DDT and/or dieldrin. Larvae and/or adults of all species tested were susceptible to those insecticides with the exception of *Anopheles vagus*, adults of which were resistant to dieldrin. Three phenotypes for dieldrin resistance in *An. vagus* larvae were distinguished. The appearance of resistance in *An. vagus* from the Kasetsart University experimental farm may be attributed to the widespread use of a variety of insecticides for experimental purposes.

**ACKNOWLEDGMENT.** The authors wish to express their appreciation to the efforts of Mr. Samarn Mancewongse and Mr. Inkam Inlao; without their assistance this study would not have been accomplished.

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## SEASONAL DISTRIBUTION OF TABANIDAE (DIPTERA) AT TEXAS HOLLOW, NEW YORK IN 1968

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During 1968, studies were carried out to test various collecting and trapping methods for Tabanidae. In the course of this work, a rather complete picture of the seasonal distribution of the Tabanidae of a limited area was secured. This information is given in Figure 1.

Texas Hollow is a north-south valley in Schuyler County, New York. Our studies were confined to approximately 100 acres in the Town of Hector about 1.3 miles southeast of the hamlet of Bennettsburg. The valley at this point is 0.4 of a mile wide. The altitude of the valley floor av-

erages about 1,140 feet and the dirt road giving access to the area is at an elevation of 1,230 feet. The hills defining the valley range from 1,600 to 1,700 feet. No collections were made above the level of the road.

The valley is drained by Cranberry Creek which flows north to Hector Falls Creek which flows westerly to Seneca Lake. The main physical features of the area studied consist of three ponds. One pond is formed by a small dam across Cranberry Creek which has flooded portions of a swamp. A second pond has