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INVESTIGATION OF THE SUSCEPTIBILITY OF *A. PHAROENSIS*

TO INSECTICIDES IN EGYPT - U.A.R.

by

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The susceptibility of *A. pharoensis* in the Egyptian Region, UAR, was first studied by workers of the Insect Control Section, Ministry of Public Health, Cairo, in 1957, 1958 and 1959, on samples collected from Marg, a locality situated at the north-eastern outskirts of Cairo.

The LC_{50} of 0.5% - 0.7% was obtained (using Busvine and Nash technique) by Gad and Kamel in 1957/1958, and later 1.2% (using the WHO standard test kit) was obtained by Gad in June 1959. For Dieldrin, an LC_{50} of 0.07% - 0.13% was obtained (using Busvine and Nash technique) by Shawarby and Kamel in 1958. The susceptibility studies made by Jolivet in Ethiopia in 1957/1958 showed an LC_{50} of 0.34% - 0.71% for DDT. The writers made their first contact with testing the susceptibility of *A. pharoensis* on 12 July 1959 in the course of establishing a background in an area newly selected for field training, about 10 km. north-west of Cairo. The area has never received any house spraying or larviciding. The results of the preliminary tests carried out between 12 July and 12 August, were surprising in that they showed a higher LC_{50} for DDT and a much higher tolerance to Dieldrin, in an unsprayed area. With DDT, the LC_{50} was found to be within the range of 1.8% - 2.6% and with Dieldrin no mortality could be obtained above 50% with the highest concentration available, 1.6%, and even with prolonged exposure up to 24 hours with the 4% concentration later available, mortality did not exceed 71.4%. The occurrence of such high tolerance in a population of an unsprayed area, lead the writers to think that this must have been created by some insecticidal pressure other than that of house spraying or larviciding. This query was readily answered by a history of prolonged and extensive agricultural pest control in which several chlorinated hydrocarbon insecticides were involved.

It was found necessary that testing should extend to Marg locality where lower LC_{50} levels have been reported previously by other workers as mentioned above. There again it was surprising to obtain from tests made by the writers about the end of August and the beginning of September 1959, an LC_{50} of about 2.5% for DDT but with Dieldrin the mortalities were much below 50% with all concentrations including 4% Dieldrin. Even prolonged exposure of 24 hours did not produce mortalities exceeding 76.5% on 4% Dieldrin concentration. These results were almost identical to those obtained from the Field Training Area on the western outskirts of Cairo. On investigating the previous insecticidal treatment, it was found that house spraying was restricted to certain private premises, but no general application of residual spraying has ever been undertaken in the area. DDT larviciding, on the other hand, was irregularly applied at some parts. The most important mass insecticidal treatment was that of the agricultural pest control, in which various chlorinated hydrocarbon insecticides were involved.

These findings directed the attention to the immediate need for mass susceptibility surveillance. A country-wide susceptibility survey, mainly in the Delta, under the supervision of the first writer, to be inaugurated before the termination of the mosquito season this year, was suggested by Dr. Farid, WHO Senior Regional Malaria Adviser. The first writer took the matter to the Minister of Health, (Egypt) UAR, who fully supported the idea of country-wide susceptibility testing and ordered for the provision of all possible facilities. The WHO Regional Office provided eight adult test kits for the country-wide susceptibility survey. Three medical officers delegated from the Ministry of Health, received intensive training by the first writer on susceptibility testing, for about two weeks, after which they were assigned for testing in various localities in the Delta. Hence, susceptibility tests could be performed in ten localities of six provinces during October 1959, under the supervision and direction of the first writer. The localities are : (Map of the Delta, Annex I).

Zagazig and Tal El Kebir, Sharkia Province, in the eastern part.

Kafr El Sheikh, Kafr El Sheikh Province, in the mid-northern part.

Shabshir, Gharabia Province,

Damanhour, Beheira Province.

Shebin El Kanater, Quaha, Barrages, Qualubia Province.

tests of which were started by the new medical officers during their training practice, about the end of September 1959, and completed later by some personnel from The Training Centre. Few tests could be made on samples obtained from the Munoufia Province and finally, a few tests could be accomplished at Ismailia in the Canal Zone, before the termination of the season. This is in addition to records initially obtained by both writers from the Field Training Area in Giza Province, and Marg locality in Qualubia Province.

Besides, Dr. Gad, the Entomologist of the Pre-Eradication team, carried out some tests in Fayoum Province, Tanta, Gharabia Province, Ras-El-Bar, Damiette Province and Mit Ghramra, Dakhalia Province. His records will be quoted in this paper.

For collecting information on land utilization and the history of agricultural pest control since 1950 from every area where susceptibility tests were to be made, a standard form was prepared by the first writer covering the basic knowledge to be obtained.

In order to standardize testing conditions and to eliminate mortality in the controls, the staff was trained and instructed on the minor details of the testing procedures covering collection, transportation, selection of specimens suitable for testing, care of mosquitos during period of observations etc., as will be shown later.

In the course of susceptibility testing investigations carried out or supervised by The Regional Malaria Eradication Training Centre, the total number of mosquitos tested was : 9570 in 500 batches for all DDT concentrations and 3177 in 169 batches for all Dieldrin concentrations. In the majority of tests, controls were maintained without any mortality as will be shown later.

The aim of this paper is to present the results of the susceptibility tests obtained so far from various parts of the Nile Delta Region of the U.A.R. It should be pointed out, however, that interpretation of results and views given in this paper in respect of susceptibility levels, are subject to further adjustment on the light of further revision based on statistical analysis.

TECHNIQUE & PROCEDURE:

The WHO standard adult test kit was used according to WHO instructions throughout the whole investigation. In order to standardize testing conditions and to minimize the unnatural mortalities in the controls, the first writer laid guiding notes specifically intended for newly trained personnel (Annex II). The notes are meant to be only for A. pharoensis testing in the conditions of the Egyptian Province of the UAR, but for other territories or other species they may be further adjusted. Therefore they should be treated as restricted.

Due to care exercised in mosquito collection, transportation, selection etc., mortality in the controls was much reduced. Thus, out of 179 control replicates in DDT tests and Dieldrin tests of one hour and two hours and Dieldrin tests of three, six and 24 hours, mortality was zero in 138 replicates, 1%-5.9% in 27 replicates, 6%-10% in only ten replicates and 13.3% in a single batch of 18 mosquitos used as control for 24 hours Dieldrin exposure.

page 4

Initially, Dieldrin tests covered three or four concentrations but later, particularly in the Delta survey, Dieldrin testing was restricted to 4%, the highest concentration tolerated. With regard to the total number of mosquitos tested on each DDT or Dieldrin concentration, detailed data is given in Table (1), page 5.

In all of the tests performed or supervised by The Training Centre, precautions given in the guiding notes were exercised. The stress was made on the selection of mosquitos for testing. As in WHO instructions, the unfed mosquitos should be, whenever possible, avoided. This was done in the field while mosquitos were being collected. Otherwise they were separated when selecting mosquitos during the pre-test period. The pre-gravid and gravid females, or more precisely those in stages VI and VII of Sella (1920) - Annex II - were similarly treated. In the majority of tests, opportunity did not offer a chance to test freshly fed females, strictly in Sella stage II. By the time collections were brought to the laboratory, mosquitos collected in the morning were mainly in Sella stage III and, in the afternoon a good majority had reached Sella stage IV and some usually had reached Sella stage V.

Reference should be made to some apparent variation in the values of the LC_{50} for DDT, graphically estimated initially when the data of the Field Training Area was compiled.

The first group of tests made on 12-16 July 1959 gave an LC_{50} of about 1.86% for DDT and the second group of tests made on 10-12 August 1959 gave an LC_{50} of about 2.6% for DDT. The first group was carried out in the forenoon at temperatures ranging between 27-29°C but the latter were made in the afternoon at temperatures ranging between 31.4-34°C. Later, in Marg area, a similar apparent difference between two groups of tests was obtained. One group was made in the forenoon on 3, 8, 10 September 1959 which gave an LC_{50} of 2% DDT, and the other was made 7, 8, 10 September 1959 in the afternoon which gave an LC_{50} of about 3% DDT.

As statistical analysis of the above differences could not be made in time, due caution was paid, however, as to standardize the time of the day at which exposures should be maintained, hence the afternoon was suggested.

The majority of tests of the country-wide susceptibility survey were carried out in the afternoon and the time at which exposures were made was always recorded.

While writing this paper, Mr. T. Sadek, Statistician of the Ministry of Health, Cairo, statistically analysed the foregoing data of forenoon and afternoon LC_{50} values and found no significant difference between the two in both Marg and the Field Training Area.

No chance was available to obtain further information on this point which needs a specially planned investigation.

Train Centre, in the Nile Delta

12 July - 31 October 1959

Exposure	D D T												D I E L D R I V																																			
	1%				2%				4%				Control				0.05%				0.1%				0.2%				0.4%				0.8%				1.6%				4%				Control			
	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T								
1 Hour	85	1724	114	2203	127	2418	165	3067	141	2679	2	29	2	30	5	107	16	267	12	200	14	250	50	982	21	394																						
2 Hours	-	-	-	-	-	-	8	158	4	83	-	-	-	-	-	-	-	-	-	-	2	45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
3 Hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
4 Hours	-	-	-	-	-	-	-	-	-	-	-	-	4	85	4	77	4	76	4	75	4	75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
24 Hours	-	-	-	-	-	-	-	-	-	-	-	-	8	144	9	182	10	161	10	215	10	182	10	182	11	202																						
Totals	85	1724	114	2203	127	2418	173	3225	145	2762	2	29	2	30	17	337	29	526	26	437	33	654	60	1164	38	701																						
Total Number of mosquitoes tested :		9570												3177																																		
Total Number of replicates :		500												169																																		

In this respect we may however refer to the work of Hadaway and Barlow (1956) who used the topical application technique for determining the susceptibility of Aedes aegypti and A. stephensi. The above authors obtained a significant difference in the value of LD50 from mosquitos exposed three hours after taking a blood meal and others which were exposed 24 hours after taking a blood meal. It was found that females dosed with DDT after three hours after taking a blood meal were slightly less susceptible than those in the unfed state, but that those which were tested 24 hours after obtaining a blood meal, when they were obviously half gravid, were more tolerant to the insecticide.

Carefully planned investigations for exploring the effect of such a factor, on results obtained from susceptibility tests in field conditions is suggested. In practice, on the other hand, it would be advantageous to record the time at which mosquitos were exposed and to define the condition of the mosquitos to be tested in terms of Sella classification.

In the following, results of susceptibility tests made in various parts of Lower Egypt are given.

Results of Susceptibility Tests in the Nile Delta region

Under the supervision of The Regional Malaria Eradication Training Centre,
Cairo, between 12 July - 31 October 1959

THE FIELD TRAINING AREA - GIZA PROVINCE

The Field Training Area (F.T.A.) occurs in Giza Province which occupies the southern-most part of the western Delta. The area is situated at about 10 km. north-west of Cairo and has been the main centre of various activities of the Regional Training Centre. Discovery of the Dieldrin resistance and DDT tolerance in A. pharoensis originated from the writers susceptibility studies in that area.

Tests of the Field Training Area were principally carried out by the writers assisted by three National Entomological Assistants, Mr. H. Bedin, Mr. I. Dardiri and Mr. A. Hassan. Tests carried out in the Delta were made by Dr. F. Abdul Wahab, Dr. Nabil Taha and Dr. Abdul Kader Ez El Arab, on training assignment to The Training Centre. They were assisted by Entomological Assistants and Insect Collectors from the Insect Control Section and the Malaria Section, Ministry of Public Health, Cairo, previously trained by The Regional Malaria Eradication Training Centre. Mr. T. Zaghoul, Junior Entomologist, assigned to the Training Centre, gave partial assistance in tests made in Qalubia Province and Mr. S. Samuel, Biologist in the Insect Control Section, gave partial assistance by performing the few tests in the Minoufia Province.

In the FTA, land is utilized in mixed crop cultivation such as cotton, corn, clover and vegetables but rice cultivation is quite patchy. The area has not received any house spraying or larviciding with residual insecticides. Agricultural pest control, which is mainly directed against the cotton leaf worm, Prodenia litura, (Fam: Noctuidae Lepidoptera), prior 1957, depended largely on a BHC-DDT dusting mixture commonly known as "cotton dust". This dusting powder contains 25% BHC (3% gamma), 10% DDT, 40% Sulphur and 25% filler. "Cotton dust" was widely substituted by Toxaphene from 1958 until present, as the latter toxicant was found to be consistently effective and its effect extends also to the cotton boll worm, Platyedra grossypiella, Saunders and Trias insulana, Bois. Besides, some other compounds such as Lindane emulsion, DDT water dispersible powder, have been employed in some patches and "cotton dust" is still preferred on certain vegetable crops.

The chemical treatment of cotton plants is usually applied annually in June and July at 10-15 day intervals and repeated 2-3 times according to the degree of infestation. With Toxaphene, about four applications are made against the cotton leaf worm in May and June and three applications may be made later. The period may extend to October, particularly to other vegetable plants to which the cotton leaf worm migrates when cotton advances towards ripening. The season of insecticidal pest control, therefore, coincides with that of activity of A. pharoensis.

In the Field Training Area, cotton cultivation usually covers 8,600 acres of which 70% are treated with insecticides. Ground nuts cover about 4,500 acres of which 80% are treated, and about 1,700 acres are covered by sweet potatoes, which are all well sprayed. Besides, vegetables are grown in sizeable patches and are invariably treated with insecticides. With regard to dosage, cotton dust is applied at a rate of 8-10 kg. per acre i.e. about 0.3 kg. GBH and 1 kg. technical DDT per acre. Toxaphene is applied at a rate of 3 litres of a 60% Toxaphene emulsifiable concentration per acre, i.e. 1.8 kg. technical Toxaphene per acre. Of the other mixtures, DDT (30 parts) with Lindane (9 parts) is also employed and actually preferred in the case of groundnuts and sweet potatoes. This mixture is applied at a rate of 2 litres per acre. Reference should be made to an uncommon type of insecticidal treatment, which was observed initially in the Field Training Area and later met with occasionally in the Delta, whereby the inhabitants apply "cotton dust" to walls, floors and beds in order to rid them of fleas and mosquitos.

The above treatments illustrate how the insect fauna in the area is being exposed to various insecticidal pressures. In what way this pressure acted on the mosquito fauna has not yet been completely studied. So far, only partial explanation may be drawn from important night observations made by the first writer in the FTA initially on 22 July 1959 and which was confirmed by subsequent night observations later made in August. Females of A. pharoensis as well as Culicini mosquitos, were found resting on cotton plants and on Hibiscus esculentus (okra) either in the unfed state

or in the freshly fed state or in later stages of blood digestion. Such A. pharoensis population disappeared with the sunrise from cotton and Hibiscus plants. Probably they rest on such plantation around human and animal habitations just for a temporary stance on their way to and from the favourable natural day resting places. Observations made simultaneously by the writers indicated the presence of A. pharoensis in appreciable density in rice fields through different house of the day time.

Recently fed A. pharoensis were repeatedly captured from rice plants during the early hours of the day together with mosquitos in subsequent stages of blood digestion, but the latter were more predominant at later hours of the day. The site of observation was at a radius of 100-500 metres from the nearest rice-fields, while cotton and Hibiscus plants were in close proximity to houses. From the circumstantial evidence, it can thus be postulated that since the main daytime resting places are not in access of feeding sites, the resting on cotton plants is just a temporary stance until further shift is made to the favourable resting places. Unfortunately the writers missed noting such temporary resting on freshly treated cotton plants; this will be taken care of in the next season as it would elucidate the point of contact made between A. pharoensis population and the chlorinated hydrocarbon insecticides used for agricultural pest control. The possibility that such insecticidal treatment, on contaminating the breeding places, enhanced selection through the larval population, is very remote. Mosquito breeding places are scarcely, if not utterly, formed within cotton fields, as irrigation of such crops is adjusted to defined intervals which are usually about once weekly in the initial stages of growth and then at more prolonged intervals with the progress of growth towards blooming and fruit formation. The land in cotton plantations is, usually rendered high and dry between irrigation intervals.

On the other hand, rice lands are almost always water ridden, hence rice fields are the principal breeding places of A. pharoensis in Egypt. Rice in the Egyptian territory is one of the crops that receives no insecticidal treatment. The question of contamination of breeding sites of rice fields during cotton treatment has not been investigated. Nevertheless, it is rather difficult to assume that in practice a large part of the breeding areas could be consistently contaminated by drifts to the extent that such high levels of tolerance has resulted.

Susceptibility tests which were carried out in the Field Training Area during the period from 12 July - 19 October have been compiled in Tables (2) and (3) - see pages 10 and 11.

The DDT tolerance in A. pharoensis was initially detected by the writers in the first group of tests made in the FTA from 12-16 July 1959, in the forenoon, which showed an LC50 of 1.8 % DDT confirmed later by the LC50 of 2.6% DDT obtained from the second group of tests, 19-22 July 1959, made in the same area in the afternoon. Also the presence of variable numbers of survivors on exposure to 4% DDT concentration was repeatedly observed in the above groups of tests. In these tests the number of

A. pharoensis that survived exposure to 4% DDT concentration was 54 out of a total of 534 mosquitos exposed to this concentration for one hour. In the above tests, complete kill by exposure to 4% DDT concentration was only observed in 5 replicates out of 31. Even with prolonged exposure to 4% there were two survivors out of 158 mosquitos exposed to this concentration. Results of tests made in September also confirmed the above findings.

The values of LC50 for DDT obtained initially from the Field Training Area by the writers from 12 July - 12 August 1959 and later, appeared much higher than those obtained from Marg locality in 1957-1958 and those which were obtained more recently in June 1959 by other workers, as will be mentioned later. After the DDT high tolerance was noted in A. pharoensis population of the FTA, a switch over to tests its susceptibility to Dieldrin deemed necessary and the results initially obtained about the end of July and later in August 1959, indicated that the highest concentration tolerated was 1.6% Dieldrin (see Table 4 - page 12).

The total number of mosquitos that survived various exposures to this concentration was invariably high, 86/115 on one hour exposure, 27/45 on two hours exposure, 33/69 on three hours, 27/75 on six hours and 64/144 on 24 hours exposure. When Dieldrin impregnated paper became available about the end of August 1959, it was found that the same population of A. pharoensis in the Field Training Area, tolerated the standard and even prolonged exposures to this concentration to the extent that the number of survivors was 76/96 on one hour exposure and 23/53 on 24 hours exposure. This abnormally high tolerance to Dieldrin was also demonstrated by failure of any concentration of Dieldrin, including the highest, 4%, to give a complete kill in any replicates on one hour or 24 hours exposures.

In fact mortality at 4% Dieldrin concentration did not exceed 35% on one hour exposure and 71.4% on 24 hours exposure. The average mortality obtained on one hour exposure to 4% Dieldrin concentration was 19.1% (corrected). The LC50 of Dieldrin must be much higher than 4%. On six hours exposure, a regression line could be drawn from which an LC50 of 1.25% Dieldrin could be estimated graphically. The only available record on the value of LC50 for A. pharoensis in Egypt was 0.07% - 0.13% Dieldrin which was obtained by previous workers from Marg locality in 1957 (by the Busvine and Nash technique). If the above value is taken as base-line, the highest concentration tolerated in the present investigation would be 30-57 times the value of the previous LC50. But then a question may arise why selection was much delayed in Marg whilst insecticidal pressure created by the agricultural pest control was on the scene there long before 1957.

On the other hand, by plotting mortality dosage curve on logarithmic-probability graph paper, no straight line could be created. There was increasing response in mortalities obtained with lower concentrations, 0.05% and 0.2% Dieldrin, giving a rising curve of mortality, but mortalities tended to level off after 0.2% concentration. This may suggest heterogeneity of the population and subsequently the presence of the gene of resistance in A. pharoensis population tested in the Field Training Area.

T-11 (2)

THE REGIONAL MILITARY MEDICATION
TRAINING CENTER - CAIRO

Summary of Results of DDT Susceptibility Tests
of the Field Training Area

July - October 1959 - 1 hour exposure.

Date	C.5%				1%				2%				4%				Control	Temperature Range	LC50 graphically
	P	D	%	ct.	R	T	D	%	ct.	R	T	D	%	ct.	R	T			
12-16 July	6	118	18	15.2	7	139	30	21.6	8	157	145	92.4	92.2	7	137	4	2.9	27 - 29	1.80 forenoon
				12.7				19.3					51.3						
19-22 July	-	-	-	-	-	-	-	-	-	10	129	87	67.4	4	55	0	0	31 - 34	
4-9 August	-	-	-	-	-	-	-	-	-	7	137	124	90.5	3	68	1	1.4	31	
10-12 Aug.	6	118	3	2.5	6	114	5	4.4	6	111	98	88.3		6	111	0	0	31.4-33	2.6 afternoon
26 August 8 September	-	-	-	-	6	114	4	3.5	7	138	23	16.7	16.1	7	134	1	0.7	28.2-32.1	2.65 afternoon
								2.8					87.7						
26 Sept. 29 Sept.	-	-	-	-	3	44	0	0	3	44	8	18.2	16.4	3	48	1	2.1		2 afternoon

TABLE (4)

PLANNING AND RESEARCH DIVISION
TRAINING CENTRE - CAIRO

Summary of DDT Susceptibility Tests of Marg Area

August - October 1959

Date	0.5%			1%			2%			4%			Control			Temperature Range	LC50 graphically					
	R	T	% M	R	T	% M	R	T	% M	R	T	% M	R	T	% M							
7-10 Sept.	-	-	-	4	77	9.1	6	105	29	27.6	6	112	95	6	109	0	0	26.4 - 33	2.50			
22-26 Sept.	10	199	1	0	10	196	13	3.8	10	191	82	41.2	24	463	431	92.9	14	273	8	2.9	26.2 - 29	2.17
14, 15 Oct.,	-	-	-	4	60	1	1.7	4	64	26	40.6	4	61	54	88.5	4	62	0	0	25.5 - 29	2.30	
Totals	10	199	1	18	333	21	20	360	137	34	636	580	24	444	8							

The resistance level of A. pharoensis to Dieldrin in the Field Training Area remained almost about the same throughout the whole period of investigation.

MARG LOCALITY - QALUBIA PROVINCE

Marg is a locality situated about 25 km. east of Cairo, which has been subject to repeated testing by previous workers in 1957, 1958 and 1959, as has been already advanced. Tests made by the writers in Marg were started on the 26 August 1959 and continued until 19 October. Mosquitos were collected from almost the same sites from which past collections were made.

Land is utilized in mixed crop and vegetable cultivation similar to that of the FTA in Giza Province but more rice is cultivated in Marg, hence A. pharoensis appears in relatively much higher density. Concerning previous insecticidal treatment, rice occupies in some parts about 1/4 - 1/3 of the cultivated area and no general house spraying has been undertaken in the area, only partial spraying was applied in 1958 to a few private houses around the sites of mosquito collection. Larviciding has been generally applied but not very regularly, with 5% DDT in Diesel oil. The agricultural pest control, as in the case of the Field Training Area, depended on the chlorinated hydrocarbon insecticides, but the choice of the insecticide varies according to owner experience. In some farms "cotton dust" has been used for the last ten years and is still preferred while in other farms it was replaced by Toxaphene three years ago.

Results obtained from tests made by the writers in Marg are shown in Table (4) indicating that the same phenomenon of DDT high tolerance represented by the LC50 level of 2%-2.5% DDT, also exists in Marg locality similar to what has been noted in the Field Training Area. Also here the highest concentration tolerated was 4% DDT as indicated by the total number of mosquitos that survived this concentration, 65 out of 636 mosquitos exposed. It was noted however that in the series of tests made between 26 August and 12 September, complete kill on 4% concentration was observed in two replicates out of eight whilst in the second series of tests made between 22 and 29 September, there was higher kill on the 4%, the number of replicates with 100% mortality being seven out of 22.

On the other hand, resistance to Dieldrin was demonstrated also in this locality. As in the case of the Field Training Area, there were many survivors on the 4% Dieldrin concentration. The highest mortality, which occurred by exposure to 4% Dieldrin concentration on one hour exposure, was 26.9% while on 24 hours exposure the highest mortality was 76.5% at the same concentration. This very low kill obtained with the highest concentration of Dieldrin was maintained all through the testing period as indicated by the mortality obtained at the end of the season, 16.9% on one hour exposure.

QUALUBIA PROVINCE

Qualubia Province represents the southern-most part of the Nile Delta. This province was suggested to the Government by Dr. M.A. Farid, Senior WHO Regional Malaria Adviser, for a pilot project to be started in 1960. Hence the susceptibility of A. pharoensis in this province at present, prior spraying, deemed important.

In addition to Marg locality, which is situated in the south-eastern end of the Qualubia Province, three localities, Shebin El Kanater, Quaha; and the Barrages were selected to give a complete representation of the area. Testing started by the Training Centre about the 27 September 1959.

SHEBIN EL KANATER:

Shebin El Kanater locality is situated 40 km. north-east of Cairo in the Qualubia Province. After a short preliminary survey, two farms were selected for mosquito capture on the outskirts of the locality and later a third farm was added. According to information gathered from local inhabitants, the area has not undergone any house spraying. The chemical control of agricultural pests is commonly practised for the control of the cotton leaf worm, Prodenia litura. Toxaphene has been in use for the last two years replacing "cotton dust" which was commonly used for five years previously. The use of "cotton dust" is now restricted to vegetables.

In Shebin El Kanater area, similar to Marg and the Field Training Area, mixed crop cultivation is observed but cotton outnumbered rice cultivation.

Results of susceptibility tests made in this locality are given in Table (6) below.

TABLE (6) Summary of results of DDT susceptibility tests of Shebin El Kanater Area, Qualubia Province, 27 September - 12 October 1959.

0.5%			1%			2%			4%			Control			LC50 graphically
12 replicates			12 replicates			12 replicates			13 replicates			13 replicates			
T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	
229	4	0.6	217	10	3.2	168	66	27.3	233	200	85.6	246	3	1.2	2.5
Temperature Range : 25.8 - 29.5°C															

As shown in Table (6), the high LC50 indicates a high level of tolerance similar to that previously obtained at the Field Training Area and Marg. The 4% DDT concentration failed to give complete kill in 11 replicates out of 13 replicates in which 233 mosquitos were exposed to this concentration. On the other hand, the number of mosquitos surviving exposure to concentration of 4% varied greatly from one mosquito in a batch of 20 to as many as six mosquitos in a batch of 17. Likewise, with the 2% DDT concentration, mortalities also varied greatly from 5.3% to 66.7%.

In some batches, while a very low kill was obtained by the 2% concentration, a high or complete kill was produced by 4% concentration. The reasons for such variations could not be properly understood at present, but may however suggest the heterogeneity of the population.

With Dieldrin, on the other hand, on one hour exposure, 49 mosquitos out of 54 survived the highest concentration, 4%. See Table (15).

QUAHA:

Quaha is situated in the central part of the Qualubia Province, at a distance of about 28 km. north of Cairo.

Land utilization is similar to that of Shebin El Kanater, being characteristic of areas near cities with mixed crop cultivation and some vegetables with patchy rice cultivation. From the farms where mosquitos were obtained, the acreage is utilized as follows: 25 acres rice, 30 acres cotton, 25 acres maize and 25 acres vegetables and clover.

The chemical control of agricultural pests is based on the use of "cotton dust" up to 1958 when Toxaphene was also introduced and is still in use up to the present. DDT spraying was only applied on a limited scale. In an industrial factory, from which some of the mosquitos tested were collected, according to local information, had been DDT sprayed during the last two years and lately in August 1959 with 5% water-suspension formulation. It is said that spraying was improperly carried out.

Summary of results of susceptibility tests of Quaha Area, are given below in Table (7).

TABLE (7) Summary of results of susceptibility tests of Quaha Area, 27 September - 14 October 1959.

0.5%			1%			2%			4%			Control			LC50 graph ically
4 replicates			4 replicates			5 replicates			6 replicates			6 replicates			
T	D	%M	T	D	%M	T	D	%M	T	D	%M	T	D	%M	
75	0	0	75	2	2.7	94	28	29.8	113	102	90	111	3	2.7	2.4
Corrected mrts:			0			27.8			89.7						
Temperature Range : 26.6 - 29.4°C															

From the above data it appears that the LC50 is almost of the same level as that obtained from the FTA, Marg and Shebin El Kanater localities of the same province. Again here, amongst mosquitos exposed there were survivors in 4% DDT concentration in all replicates, amounting to 11 mosquitos out of the total of 113 exposed.

Exposure for one hour to 4% Dieldrin gave also here very low kill, 37 mosquitos survived out of 40 exposed to this concentration for one hour. See Table (15).

BARRAGES:

The Barrages are also known as Kanater El Khairia and is situated in the western side of the Qalubia Province at a distance of about 25 km. north-west of Cairo. The collection of A. pharoensis was obtained from Gezerit El Sheir.

Land is utilized by a mixture of agriculture in the cultivation of barley and also there is an experimental fruit station. No rice is cultivated in this area and the main breeding places are seepage from the Nile during flood time, covering about 1.5 acres. The seepage usually disappears about the end of Spetember after the flood subsides. No house spraying has ever been done, only larviciding was maintained before 1957. For agricultural pest control, the Ministry of Agriculture used various insecticides for experimental purposes, but detailed information is still to be completed.

Summary of results of susceptibility tests carried out in this area are given below in Table (8).

TABLE (8) Summary of results of susceptibility tests of Barrages Area,
28 September and 17-20 October 1959

0.5%			1%			2%			4%			Control			LC50
2 replicates			2 replicates			3 replicates			3 replicates			3 replicates			
T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	
40	1	2.5	40	0	0	58	19	39.6	55	51	92.5	54	0	0	2
Temperature Range : 25 - 29°C															

From the above data, the IC50 obtained from Barrage population seemed to be more than 2% DDT concentration. Again here, mosquitos tested exhibited quite a good tolerance to lower dosage of the insecticide as indicated by the very low or no mortality observed with the 0.5% and 1% concentrations. With 2% concentration, mortalities varied from 15 to 47% and with 4% concentration there were survivors in all three batches tested, amounting to 4 out of a total of 55 exposed. Also here the 4% Dieldrin concentration failed to give high kill, 38 mosquitos survived one hour exposure to this concentration out of the 40 mosquitos exposed. See Table (15).

MUNOUFIA PROVINCE

The Munoufia Province is situated in the south-western part of the Delta, almost parallel to Sharkia Province. This area is well reputed for cotton and maize cultivation, with some vegetable growing also. Rice cultivation here is patchy. The information is not yet complete as regards previous insecticidal treatment but probably the same chemical control of agricultural pests is observed as in the localities of the Qalubia Province.

Unfortunately, testing for susceptibility in this province could not be started until the middle of October. The density of mosquitos therefore was found to be very low and consequently only a few replicates could be made. Summary of the results obtained is given below in Table (9).

TABLE (9) Summary of results of DDT susceptibility tests of Ashmoun - Munouf area - Munoufia Province, 14 - 21 October 1959.

1%			2%			4%			Control			LC50
1 replicate			2 replicates			4 replicates			4 replicates			
T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	
16	0	0	31	8	25.8	63	61	96.8	59	0	0	2%
Temperature Range :						24.6 - 28°C						

No valid conclusions can be drawn for the susceptibility level of A. pharoensis in Minoufia Province from such limited observations. Pending further investigations, it can be suggested that the value of the LC50 in this area is more than 2% DDT concentration, about similar to that observed in the Field Training Area and Qualubia Province. In the only batch of 18 mosquitos exposed to 4% Dieldrin concentration 17 mosquitos survived. See Table (15).

SHARKIA PROVINCE

Dr. F. Abdul Wahab from the Endemic Diseases Department of the Ministry of Public Health, on training assignment to The Regional Training Centre, was given charge of susceptibility testing at two localities in this province, under the supervision of, and after completion of his training with, the first writer. Sharkia Province represents the eastern side of the Nile Delta, north-east of Qualubia Province. The first area selected for testing occurs around Zagazig, the main town of the province, and another locality, Tal-El-Kebir, further north-east, was added later.

ZAGAZIG:

According to information collected by Dr. F. Abdul Wahab, land is utilized in mixed crop cultivation, mainly cotton and other crops. Rice is cultivated in not more than 5% of the total acreage of 3,890 out of the five localities. Neither house spraying or larviciding has been undertaken in the areas from which mosquitos were collected. The control of agricultural pests depended mainly on the use of "cotton dust" from 1953 to 1955 but since 1956 up to the present time, "cotton dust" has been widely replaced by Toxaphene. The latter, in some localities, was mixed with Lindane in an emulsion form as was mentioned earlier. The season of mass dusting or mass spraying against the cotton pests is mainly June-August against the cotton leaf worm and cotton boll worms, at fortnightly intervals. A. pharoensis was obtainable in appreciable density mostly from farms with rice cultivation. Summary of results of testing is given below in Table (10).

TABLE (10) Summary of results of DDT Susceptibility Tests of Zagazig
Area, 7 - 14 October, 1959.

0.5%			1%			2%			4%			Control			LC50 graph ically
8 replicates			8 replicates			9 replicates			9 replicates			9 replicates			
T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	
160	1	0	166	16	9.1	184	65	34.9	182	171	93.9	175	1	0.6	2.15

From the above data, the LC50 of 2.15% DDT concentration indicates also a high tolerance being present in the localities tested around Zagazig town. In this area, too, there were survivors only in two replicates out of nine replicates exposed to 4% DDT concentration. The total number of mosquitos which survived this concentration was 171 out of 182 exposed. There was also a wide range of variation on mosquitos on 2% concentration from 16.6% to 54.2%. There were also here survivors on 4% Dieldrin, about 121 mosquitos out of 127 exposed to this concentration for one hour.

TAL EL KEBIR:

This locality occurs at the north-eastern part of the Sharkia Province. According to information gathered by Dr. F. Abdul Wahab, who was responsible for testing the susceptibility also in this area, land in the vicinity of the farm from which mosquito collections were made is utilized as follows: 25 acres rice, 25 acres cotton and 15 acres mixed crops. This probably applies to most of the area of Tal El Kebir.

Chemical pest control of agricultural pests with "cotton dust" which was used at a rate of 4 kg. per acre, is about half the amount usually applied in other localities using the same mixture. It was used until 1959 when Toxaphene substituted "cotton dust". House spraying was only done in 1957 with 5% DDT water-suspension. The inhabitants at present throw "cotton dust" on walls, beds and floors. Summary of results obtained from this area is given below in Table (11).

TABLE (11) Summary of DDT Susceptibility Tests of Tal El Kebir Area

20 - 26 October 1959

0.5%			1%			2%			4%			Control			LC50 graphically
6 replicates			7 replicates			8 replicates			8 replicates			8 replicates			
T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	
128	12	7.2	140	39	26.4	160	94	57.7	158	158	100	161	4	2.4	1.7

The LC50 value obtained for DDT from this area likewise indicates the presence of DDT tolerance but of a relatively lower level. Also mosquitoes exposed to 4% DDT concentration for one hour were killed. Notwithstanding the possibility of a lower tolerance due to reduced agricultural pest control towards the end of the season, it may be also reasonable to suggest that mosquito population here, contrary to that of other areas, was not exposed to extensive agricultural chemical control for cotton cultivation in this area is comparatively limited. Also the treatment history obtained from the area indicates that the introduction of chlorinated hydrocarbon insecticides to agriculture five years ago and the dose per acre, was much lower than what was shown from other areas. Cotton dust, here, was almost always maintained at 4 kg. per acre, compared with 8-10 kg. per acre in other areas.

In regard to Dieldrin, on the other hand, 123 mosquitos exposed to the highest concentration, 4%, gave a mortality of 31.6% which is higher than that which has been obtained from other areas. See Table (15).

KAFR EL SHEIKH PROVINCE

Dr. Nabil Taha Nasr, on training assignment to the Training Centre from the Endemic Diseases Department, from 15 September, 1959.

Kafr El Sheikh Province occupies the farthest mid-northern part of the Nile Delta. The main town of this province is called Kafr El Sheikh and, according to information gathered by Dr. Nabil Taha from the farms from which mosquito collections were made around this main town, land is utilized in the growing of rice, cotton and other crops in the ratio 1 : 1 : 1.

No house spraying or larviciding has been made in places of mosquito collections. "Cotton dust" was commonly used from 1952 to 1955, then was substituted by 60% Toxaphene emulsifiable concentrate diluted twenty times of its strength and sprayed at a rate of 4 kg. to an acre at intervals of 15 days for about 5 or 6 times each season. The area was said to have been exposed to DDT aerial spraying in 1950. Summary of results obtained from this area is given below in Table (12).

TABLE (12) Summary of DDT Susceptibility Tests of Kafr El Sheikh Area,
7 - 14 October 1959

0.5%			1%			2%			4%			Control			LC50 graphically
8 replicates			9 replicates			9 replicates			11 replicates			9 replicates			
T	D	% M	T	D	% M	T	D	% M	T	D	% M	T	D	% M	
139	7	3	158	23	12.9	158	63	38.8	196	189	96.3	166	3	1.8	2-2.75
Temperature Range :						24 - 27°C									

The value of the LC50, 2% DDT, is estimated graphically from a regression line passing the highest three concentrations, 1%, 2% and 4%, treating the low mortality on 0.5% as odd. It has been noted however, that mortalities obtained with 0.5%, 1% and 2%, appear to form almost a straight line from which an LC50 value of 2.7% could be obtained by extrapolation. Pending further analysis, this probably suggests the presence of two populations.

Out of nine replicates exposed to 4% DDT concentration, there were 4 replicates only giving a complete kill. The total number of mosquitos that survived this concentration was 7 out of 196.

SILABSHIR - GHARABIA PROVINCE

This locality is situated 15 km. south of Mahalla El Koubra and was selected for testing following fruitless extensive search for mosquitos around Mahalla El Koubra.

According to information collected by Dr. N. Taha, who also took charge of testing in this area, under the supervision of the first writer, about 3,000 acres constitute the cultivated land around five farms from which mosquitos were collected. Land is utilized in rice, cotton and other crops at a ratio of 1 : 1 : 1. The area used "cotton dust" only in 1955 - 1957 but no other chemicals were used before that time. From 1958, Toxaphene also came to the scene here, replacing "cotton dust" which is not now used.

No house spraying or larviciding has ever been done in this area. Summary of results obtained from this area are given below in Table (13).

TABLE (13) Summary of Susceptibility Tests of Shabshir, Gharabia Province,
21 - 25 October 1959

0.5%			1%			2%			4%			Control			LC50 graphically
3 replicates			4 replicates			5 replicates			7 replicates			5 replicates			
T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	
57	0	0	71	1	0.4	95	26	27.2	132	123	93.1	96	1	1	2.45%
Temperature Range :						19 - 23.5°C									

The LC50 obtained from Shabshir is similar to the LC50 levels obtained from cotton planting areas. On the other hand, mosquitos that survived exposure to 4% Dieldrin were about 76 out of 77 mosquitos exposed to this concentration for one hour.

BEHEIRA PROVINCE

The Beheira Province occupies the farthest north-western section of the Nile Delta. Its main town is Damankhour. Around this locality, to the west, lies The Khazzam Land Reform estate, about 18,000 acres, where susceptibility tests were carried out in some villages. Land is utilized in the cultivation of various crops, of which rice is the main crop, as per the following ratio, rice 1/2 : cotton 1/3 : maize and other 1/6. It was reported that rice cultivation often extends to 2/3 of the acreage. The following is the information gathered in connection with the agricultural pest control.

1954	- Cotton Dust	(July - August)
1955	- Gammexane	(April - May)
	- Cotton Dust	(July - August)
1956-57	- Gammexane	(April - May)
	- Toxaphene and Ekatin (organo-phosphate)	(July - August)
1958	- Gammexane	(April - May)
	- Toxaphene, DDT & Lindane & Ekatin	(June - August)
1959	- Toxaphene, DDT emulsion Ekatin and Andrin	(April - August)

In addition to the above, the area has also been subject to variable insecticidal house spraying. BHC spraying was applied in houses of some villages, whilst DDT was used in another group of villages.

The two groups were sprayed about 2 months ago. Information on previous house spraying indicated that the area was sprayed with BHC since 1957. This year DDT was introduced for the first time in some villages.

Preliminary tests carried out covered mosquitos collected from unsprayed villages very close to the sprayed area situated about 2-3 km. away and also on mosquitos collected from villages sprayed with BHC about 8 weeks ago and from villages sprayed with DDT about the same time.

Summary of the results obtained from susceptibility testing in this area are given below in Table (14).

TABLE (14)

Summary of results of DDT Susceptibility Tests of Khazzan
Land Reform Area, 6-26 October 1959.

0.5%			1%			2%			4%			Control			LC50 graphically
12 replicates			13 replicates			13 replicates			12 replicates			13 replicates			
T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	
<u>Unsprayed, 7-24 October 1959</u>															
332	14	3.5	374	97	25.5	374	285	76	360	356	98.9	390	2	0.5	1.3%
Temperature Range : 22.5 - 27°C															
<u>BHC Sprayed, 12, 13 and 18 October 1959</u>															
39	4	10.3	54	13	24	55	48	87.3	50	50	100	48	0	0	1.27%
Temperature Range : 23 - 24.5°C															
<u>DDT Sprayed, 19-24 October 1959</u>															
86	2	2.3	102	32	31.4	106	85	80.2	101	100	99	103	0	0	1.32%
Temperature Range : 23 - 24.5°C															

Generally the LV50 value obtained from The Khazzan Land Reform Area is much lower than that obtained from other localities. Land utilization is markedly different from that of other localities tested. Khazzan Land Reform Area is characterized by extensive rice cultivation. As was mentioned earlier, rice does not usually receive any insecticidal treatment but the extensive agricultural pest control is principally directed towards cotton and other crops for combatting the cotton leaf worm and cotton boll worm. As might be expected in an area of extensive rice cultivation such as Khazzan, mosquitos are less exposed to insecticidal pressure arising from the agricultural pest control. Nevertheless, the LC50 value of about 1.32% DDT indicates a slight increase in tolerance due to partial exposure to agricultural insecticidal treatment.

Probably in an area of pure rice cultivation one might expect to find a much lower LC50 value. On the other hand, the agricultural treatment in Khazzan Land Reform seemed to have depended much on BHC, Toxaphene, groups and, to a certain extent, on organic phosphates rather than on DDT. Also house spraying was made with BHC and only DDT was applied this year. Cotton Dust (BHC & DDT) was initially used only in 1954-1955. In regard to tests made with mosquitos collected from the sprayed areas, the difference between the LC50 level obtained from villages sprayed with DDT and the LC50 of those villages sprayed with BHC was negligible. The unsprayed villages gave nearly the same level, probably because of their close proximity to the sprayed area. Due to the short time which has elapsed since the introduction of DDT in house spraying, probably it has not influenced so far an appreciable increase in the tolerance level to this insecticide. Further testing would be of much value in this area next season.

As regards Diieldrin, A. pharoensis in Khazzan Land Reform Area showed its resistance to this insecticide by the number of mosquitos that survived one hour exposure to 4% Diieldrin concentration, 104 out of the total of 113 mosquitos exposed.

THE CANAL ZONE

ISMAILIA:

Ismailia is a small town of about 50,000 inhabitants, situated on the Suéz Canal about mid-way between Port Said and Suez. Agriculture is quite patchy at the western side of the town. Main crops cultivated are groundnuts, sesame, maize and vegetables, with some fruit trees. Neither cotton nor rice is cultivated in this area. The use of chlorinated hydrocarbon insecticides for the control of crop pests is not commonly practised. On the other hand, from information collected by the second writer, house spraying was applied with DDT water suspension, on the outskirts of the town in 1958 and, during this year, BHC water-suspension was applied twice instead. DDT fogging, operated by Tifa, is frequently used in the town. Larviciding is also applied to swamps with Diesel oil to which DDT was added recently. From personal information it is noted that the area received intensive BHC spraying by the British troops about five years ago, for the purpose of fly control.

Time did not allow for intensive susceptibility testing but a few tests could be inaugurated before the end of October 1959, summary of which is given in Table &15).

TABLE (16) Summary of Susceptibility Tests of Ismailia, Canal Zone,
28 - 31 October 1959

1%			2%			4%			Control			LC50 graphically
2 replicates			4 replicates			4 replicates			4 replicates			
T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	T	D	% M ct.	
36	4	11.1	64	41	64	65	60	92.3	66	0	0	1.75
Temperature Range :						23.3 - 24.6°C						

From the above data, it appears that DDT tolerance is also present in A. pharoensis tested from Ismailia locality, but at a slightly lower level than what was observed in cotton planting areas. Perhaps this is due to the limited use of DDT in the Ismailia area.

The number of mosquitos that survived exposure to 4% DDT concentration was 5 out of 65 mosquitos exposed. On the other hand, BHC was frequently applied there probably enhanced selection for Dieldrin, as noted from mosquitos which survived exposure to the 4% concentration of the insecticides. See Table (15) - 28 out of 32 mosquitos exposed.

TABLE (15) Summary of results obtained with 4% Dieldrin on one hour exposure in various localities.

Locality and Date	No. of replicates	4% Dieldrin			Control		
		T	D	% M ct.	T	D	% M
E.T.A	5	96	20	19.7	216	3	1.4
Harg 26/9 - 19/10	7	136	25	16	As in DDT		2.7
↓ Snebin El Kanater 27/9 - 30/9	3	54	5	7.5	54	1	1.8
Quaha 28/9 - 29/9	2	40	3	5.3	44	1	2.3
Barrages 28/9	2	40	2	5	20	0	0
Munoufia 21/10	1	18	1	5.5	15	0	0
Kafr El Sheikh 6/10 - 14/10	7	126	14	10.3	As in DDT		0
Shabshir 21/10 - 23/10	4	77	1	0.1	As in DDT		0
Zagazig 7/10 - 14/10	6	127	6	4.0	As in DDT		0
Tal El Kebir 20/10 - 25/10	6	123	39	29.3	As in DDT		0
Damanhour 7/10 - 13/10	4	113	9	8.0	As in DDT		0
Ismailia 28/10 - 31/10	2	32	4	12.5	As in DDT		0

From the above data it appears that the highest mortality on 4% Dieldrin obtained from the ten localities situated in different parts of the Delta, was not in excess of 29.3% on one hour exposure.

To complete the picture of susceptibility of A. pharoensis in Lower Egypt, reference should be made to results obtained from other areas by Dr. M.A. Gad, the Entomologist of the Insect Control Section and of the Pre-Eradication Survey Team, Egypt, UAR. He kindly consented to quote his results here.

Date	Insecticidal treatment	LC50 DDT graphically	Exposure period	1.6% DIELDRIN Survivors Total		Control % M
<u>RAS EL BAR, DAMIETTE PROVINCE</u>						
14,15 July 13,14 Sept. 27-30 Sept.	BHC sprayed	0.93-1.2	1 hour 24 hours	27 19	34 37	1.9
<u>EL ANSHIET EL AWKAF, TANTA, GHARABIA PROVINCE</u>						
29,30 July 10,12 Sept.	unsprayed	2	1 hour	49	49	2.1
<u>FAYOUM</u>						
29,30 Aug.	unsprayed	1.9	1 hour 24 hours	12 8	15 25	2.1
<u>MIT GHAIH, DAKAHLIA PROVINCE</u>						
17,18 Oct.	unsprayed	2.5	-	-	-	-
<u>MARG, QUALUBIA PROVINCE</u>						
June 1959		1.2	-	-	-	-
August 1959		1.7	-	-	-	-
Sep-Oct. 59		1.5	-	-	-	-

SUMMARY AND RECOMMENDATIONS

- 1) A. pharoensis, the principal malaria vector in Egypt, was looked upon as a species susceptible to both DDT and Dieldrin. This was based on results of susceptibility tests on samples obtained from unsprayed locality, Marg, near Cairo in 1957 - 1958, when the LC50 for DDT was 0.5% - 0.7%, and more recently in June 1959 when an LC50 of 1.2% for DDT was obtained. The LC50 for Dieldrin in 1958 was 0.07% - 0.13%.
- 2) The first indication of Dieldrin resistance and increased DDT tolerance in A. pharoensis was reported by the writers from their susceptibility investigations during 12 July - 12 August 1959 in unsprayed locality near the Field Training Area, Giza Province, Cairo, when levels of LC50 as estimated graphically were 1.8% - 2.6% for DDT and about 1.25% Dieldrin on 6 hour exposure or more than 4% Dieldrin on 6 hour exposure.
- 3) When testing was extended about the end of August and the beginning of September 1959 to Marg locality, A. pharoensis taken from the same sites where previous records were made, results were almost parallel to those obtained from the FTA in July - August 1959, indicating pronounced resistance to Dieldrin and increased tolerance to DDT.
- 4) It was uncommon to meet such tolerance in unsprayed areas, but investigations showed that fauna in the areas under investigation were subject to various insecticidal pressure created by prolonged application of chlorinated hydrocarbon insecticides for the control of agricultural pests, mainly the cotton leaf worm, Prodenia litura F. In this control a dust mixture of 25% JHC (3% gamma), 10% DDT, 40% Sulphur and 25% diluent, was commonly used from 1950 until 1956 but was replaced later by Toxaphene emulsion which is still preferred in most localities.
- 5) The season of the above mentioned insecticidal treatment coincides with the breeding and activity season of A. pharoensis.
- 6) A. pharoensis females in various stages of blood digestion were repeatedly observed on cotton and Hibiscus esculentus plants during the night but direct observations of such resting habit on cotton treated plants was made.
- 7) A country-wide susceptibility surveillance was inaugurated in October 1959 covering ten localities representing 6 provinces in Lower Egypt. Results showed that, in unsprayed areas where cotton cultivation is prevalent, A. pharoensis have a high tolerance to DDT, represented by LC50 level of 2 - 2.6% concentration whilst in areas with an abundance of rice cultivation, the value of LC50 was much lower, of the order of 1.2 - 1.3% concentration.

The latter figure was also obtained from an area where DDT house spraying was applied once every two months before the test was made, and a similar level was also obtained from part of the above area which was sprayed with BHC more than once.

8) a well pronounced indication of Dieldrin resistance in A. pharoensis was drawn from the large numbers of mosquitos that survived the standard exposure to 4% Dieldrin concentration in all localities tested in Lower Egypt.

9) The foregoing results suggest that it is unlikely that the Egyptian Malaria Eradication Campaign can make use of any insecticide of the cyclodiem group. The hope now possibly remains with DDT.

Hence it is recommended that field, epidemiological, entomological and susceptibility investigations should be intensified in a large area with DDT high tolerance and to be sprayed with DDT before Egypt starts its attack phase.

10) Investigation is recommended for the study of the significance of variations in susceptibility results which might be obtained from exposing mosquitos in various stages of blood digestion.

11) A guide for Junior workers on susceptibility testing procedure has been prepared, and assisted to present investigators in minimizing the natural mortality in the controls, and ensured sound testing.

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TABLE (5)

THE REGIONAL MALARIA ERADICATION
TRAINING CENTRE- CAIRO

Summary of Dieldrin Susceptibility Tests of Marg

August - October 1959

		0.2%			0.4%			0.8%			1.6%			4%			Control			Temperature Range °C	LC50 graph- ically						
		P	T	D	R	T	D	R	T	D	R	T	D	R	T	D	R	T	D								
26 Aug. 12 Sept.	1 Hour	2	42	2	0.62	3	41	1	0	3	41	4	6.1	3	41	5	8.7	4	83	16	16.1	4	78	3	3.8	27-31.4	> 4
- 12 Sept.	24 Hours	2	39	9	22	2	40	12	28.9	2	39	14	34.9	7	129	81	62.2	4	73	1	1.4	27-31	< 4				
26 Sept.	1 Hour	-	-	-	-	-	-	-	-	-	-	-	-	2	39	7	17.9	1	19	0	0	28.4					
19 Oct.	1 Hour	-	-	-	-	-	-	-	-	-	-	-	-	1	14	2	14.3	1	14	0	0	25					

THE REGIONAL MALARIA ERADICATION
TRAINING CENTRE, CAIRO

GUIDING NOTES

1 November 1959

on

Susceptibility Tests

by

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Senior WHO Adviser
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These notes are intended to guide workers engaged in susceptibility investigations to the detailed procedure that should be followed to eliminate unnatural mortalities that might occur in exposed mosquitoes or in the controls. The main WHO instructions sent with the test kit should primarily be studied and followed carefully. These notes however may be only applicable to . pharoensis testing in the conditions of the Egyptian Province, UAR. They have not been revised by our HQ and are subject to further revision. They must therefore be treated as restricted.

1) Collection of mosquitos:

1. In order to obtain sufficient numbers of fed mosquitos for testing, collections are to be made in the early morning.
2. Avoid collecting mosquitos in stages Sella I, VI & VII; the test should be made on mosquitos preferably in stages Sella II, III & IV and certainly not later than Sella stage V. If possible, avoid also taking species other than that required for testing. This will save time unduly spent later in selection of the required species for testing.
3. Mosquitos may be collected by test tubes or by sucking tubes.
4. Not more than five mosquitos should be collected at a time in the field.
5. Utmost care must be exercised during collection to avoid exposure of mosquitos to strong suction or shaking.
6. Mosquitos collected should be put into paper cups kept moistened by placing a wet cotton wool pad on top of the net cover and not more than 35 mosquitos should be placed in each paper cup of half-pint capacity and preferably the number of mosquitos should be recorded on each paper cup. Avoid exposure of your collection to direct sunlight or to contamination with insecticides which may be found in villages or farms for agricultural or domestic use.
7. Record time during which collections were made.

2) Transportation:

1. Care should be exercised to keep paper cups containing mosquitos with wet cotton wool on top of net cover of each cup during transportation.

2. Avoid dropping or shaking boxes containing the paper cups.
3. Where vehicles are used, to avoid exposing the mosquitos in the boxes to vibrations, they should be carried on the hands.
4. Avoid exposure of the boxes to direct sunlight.

C) Maintenance of mosquitos until pre-exposure time:

1. Mosquitos should be kept in moistened paper cups in cardboard boxes in a cool, quiet place with no insecticidal contamination.

D) Selection of mosquitos for tests and pre-exposure:

1. From mosquito counts shown on paper cups, made during field collection, the total number of mosquitos can be known.
2. This total enables you to plan ahead how many mosquitos you may have per control and per each concentration. The number per control or per any concentration should not be less than 15 and not more than 25 mosquitos.
3. Again here, check that no Sella stage I, VI or VII are present and avoid taking species other than that which is required for testing.
4. Also avoid exposure of mosquitos to heavy suction or shaking.
5. To ensure proper selection and adequate and random distribution, pre-exposure tubes of all concentrations and those of controls, we may suggest filling all holding tubes simultaneously with mosquitos in batches of 2-3 starting from the control. On a sheet of paper register the number of mosquitos you add to each holding tube. This enables you to adjust adequate number of mosquitos for the control and for each concentration if possible recording the Sella stage when selecting mosquitos would be useful.

E) Exposure:

1. Mosquitos under exposure must be kept in a quiet place with a piece of wet cotton wool placed on top of the exposure tube, and cover the tube with a sheet of brown paper or similar.
2. When transporting mosquitos from exposure tubes to holding tubes, care must be taken to avoid damage to knock-down mosquitos lying on the slide if it is rapidly pulled out. The slide should be opened slowly and gradually and a small part of the hole can allow knocked-down specimens to drop to the holding tube.
3. Time at which the exposure was made should be recorded. For the sake of standardisation, exposure may be made for one hour anytime between 12 noon and 3 pm.

F) Observations:

1. Transfer holding tube to a cardboard box with wet cotton wool on top of each tube.
2. In arid climates and localities of relative humidity below 70%, cover the cardboard box with a wet towel and on top place a light plastic cover to ensure maintenance of mosquitos in adequately humid atmosphere during 24 hours observations.

G) Counting Mortalities:

1. Check again on the species in both dead and alive mosquitos.
2. Sometimes dead mosquitos assuming a standing position on the walls of observation tubes might be mistaken for alive. To prevent this, it is necessary to tap gently the wall of the tube in order to verify if the mosquito is alive.

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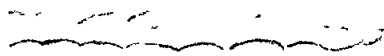
Food Digestion Stages

EM/ME-Tech.2/34

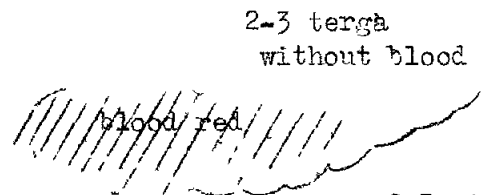
(1920) - (modified)

THE REGIONAL MALARIA ERADICATION
TRAINING CENTRE CAIRO

Sketch drawing, not from the original.



Stage I (unfed)



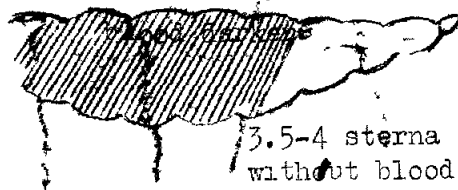
Stage II

2-3 terga
without blood

2-3 sterna
without

Stage III

3-3.5 terga
without blood



3.5-4 sterna
without blood

Stage IV

4 terga
without blood



3.5-4 sterna
without blood

Stage V

1-2 terga with blood



Blood at variable
number of sterna

Stage VI

Ovaries Stage V Characteristic



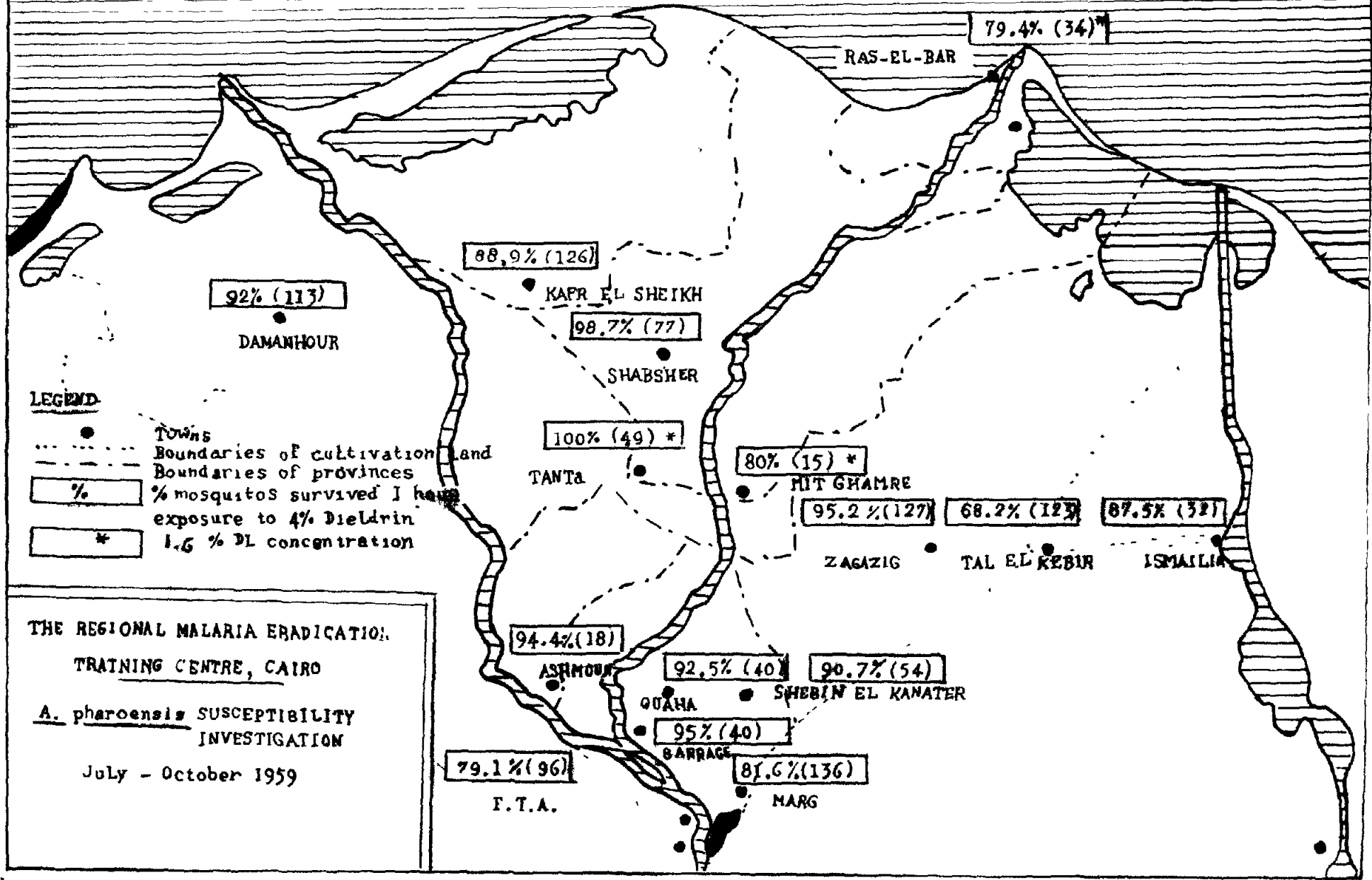
Dark strip of blood
at sterna

Stage VII



Ovaries fully developed
No more traces of blood

Map (2) DIELDRIN



LEGEND

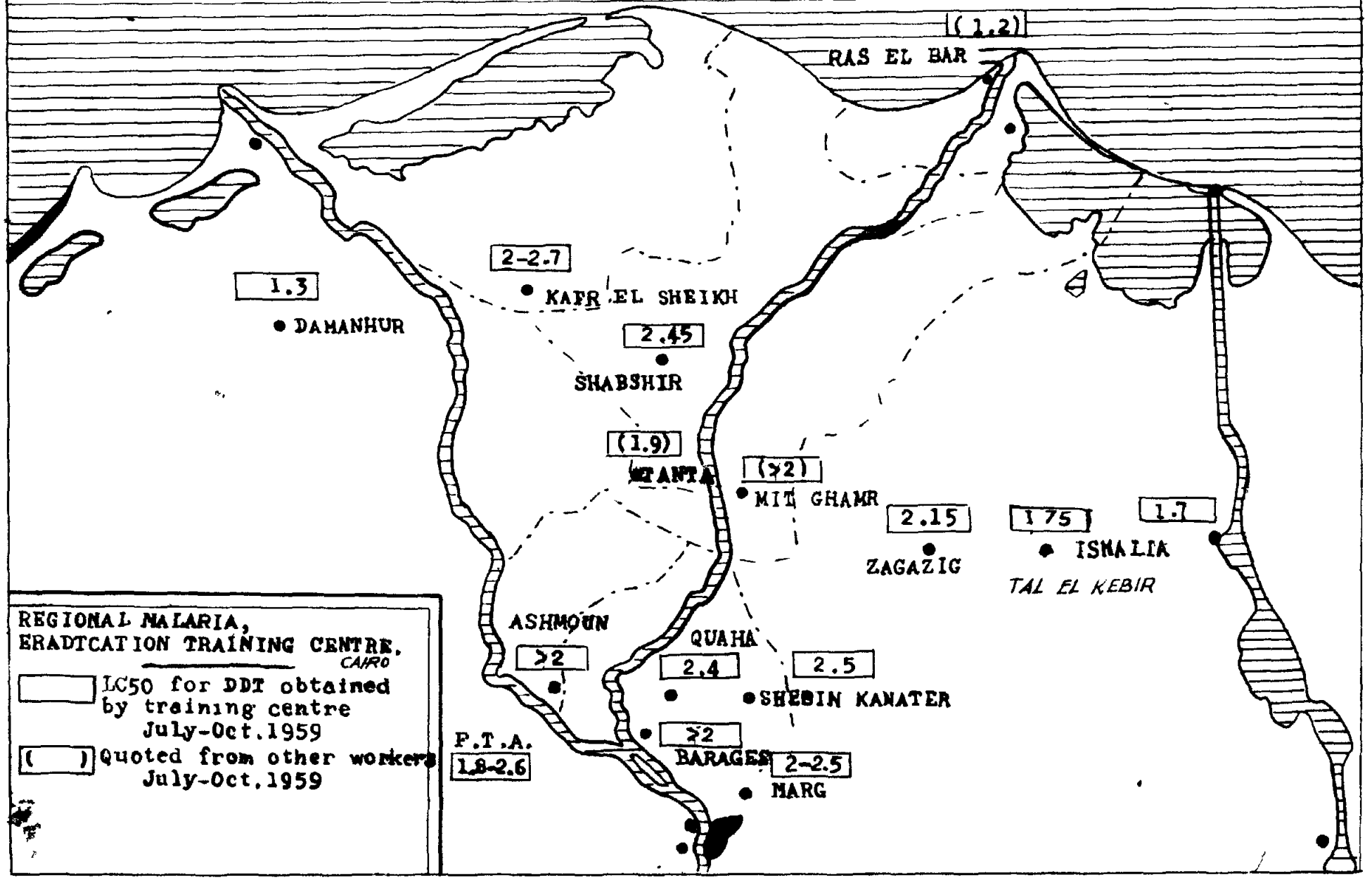
- TOWNS
- Boundaries of cultivation land
- - - Boundaries of provinces
- ☐ % % mosquitoes survived 1 hour exposure to 4% Dieldrin
- ☐ * 1.6 % DL concentration

THE REGIONAL MALARIA ERADICATION
 TRAINING CENTRE, CAIRO

A. pharoensis SUSCEPTIBILITY
 INVESTIGATION

July - October 1959

A. pharoensis DDT Susceptibility



REGIONAL MALARIA,
ERADICATION TRAINING CENTRE,
CAIRO

☐ LC50 for DDT obtained
by training centre
July-Oct. 1959

() Quoted from other workers
July-Oct. 1959