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IRRITABILITY OF SUSCEPTIBLE AND RESISTANT FIELD POPULATIONS
OF ANOPHELES CULICIFACIES IN MAHARASHTRA STATE, INDIA

by

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Irritability to DDT is probably one of the most important basic factors in bringing about the changes in the behaviouristic patterns of anopheline vectors of malaria in a country or state-wide programme of domiciliary DDT spraying which may result in control failures. The importance of irritability tests has been well emphasized by Brown (1958), de Zulueta (1959) and Muirhead-Thomson (1960). It is, therefore, needless to further stress the importance of obtaining base-line data on irritability of important mosquito vectors in any malaria eradication programme. The authors, therefore started preliminary studies on wild-caught Anopheles culicifacies of Poona district which are still susceptible to DDT in spite of several years of DDT spraying (from 1953 to 1960). The tentative WHO method was first adopted. But the method gave erratic results. Often the mosquitos exposed to control papers showed more irritation than those exposed to DDT papers. It was then considered that a better response might be obtained by experimenting at night as done by Brown (1958) on Anopheles albimanus in Panama. But again we were disappointed. By hit and miss methods we found that the erratic results during day-time were due to high temperatures prevailing at that time in the laboratory (33° to 35°C) and outside (38° to 40°C). We, therefore, made some modifications in the method of testing irritability: the final method evolved was found to be simple and easily performed under field conditions and has given fairly constant results for control mosquitos in different localities. The method finally adopted in collecting the data is described below.

Our method of testing irritability

The kit used is essentially the same as supplied by WHO for bio-assay tests and consists of:

1. Perspex funnel with a rim.
Size: Diameter: 9 cm (of base)
Rim: 1.5 cm
Height: 6 cm
2. Bent aspirator tube for removing mosquitos from the funnel at the end of experiment.
Size: 0.7 cm diameter
Angle: 45°
Length of bend: 4 cm
3. WHO holding tubes lined with clean paper for bringing mosquitos from the field.
4. WHO impregnated papers - as supplied in susceptibility kit.
5. Both-ends-open tubes.
Size: 1.5 cm x 15 cm for pre-conditioning mosquitos and transferring the same to exposure chamber.
6. Clips to hold the funnel against the glass panels.
7. Glass panels.
Size: 15.5 x 12.5 cm.

The mosquitos are caught by tubes open at both ends with cotton plug at one end and are identified in the field: only females with all legs, wings and other parts of body intact are selected and released in the holding tube. The holding tubes with mosquitos are carefully brought to the laboratory for testing. The irritability test is performed during the day in a room with light coming through the side window or door. The light intensity is approximately 1.5 lumens per square foot. WHO control or DDT-paper was kept on the glass panel, over which was fixed a perspex funnel by means of clips. The mosquitos were transferred from the holding tube to the tube open at both ends with one end plugged with cotton, allowing only one mosquito in each tube. Only one mosquito was released at a time in the funnel for

taking observations. Mosquitos in these tubes were cooled for 15 minutes by wrapping the tubes in moist lint, which brought down the temperature from 33° to 35°C, to 22° to 25°C in dry summer days. This pre-conditioning of mosquitos was found essential in hot summer days; the exposure chamber formed by the glass panel and the perspex funnel also being kept cool by fixing moist lint on the outside. During the monsoon the pre-conditioning of mosquitos was also done although this was not really essential as the room temperature was approximately 25°C. In this case pre-conditioning was carried out for the sake of uniformity and for allowing the mosquito to rest after its transfer from the holding tube to the open ended tube. During winter pre-conditioning would be necessary with warm water of about 25°C. The pre-conditioning was found to be very important in the test in order to obtain uniform response. The provision of artificial light as suggested in the WHO tentative method was found of little importance and was therefore omitted in these tests without affecting results.

After pre-conditioning for 15 minutes, only one mosquito was released for exposure to the control paper and one to the 2 per cent. DDT paper. Within 30 to 50 seconds of release mosquitos rested on the paper. The release holes in the funnels were plugged with cotton as otherwise mosquitos were liable to escape through them. Mosquitos were kept under observation for 15 minutes only, time being reckoned after first settling of mosquitos, which was about 30 to 50 seconds only. The period of three minutes allowed by Brown (1958) for settling in the case of A. albimanus was found to be too long for the present species used, and liable to vitiate the results. During this period of 15 minutes of exposure, the time for each flight and resting was noted down till the end of the exposure period. This observation enabled us to know the time required for the first flight as well as the total number of flights during 15 minutes and the duration of each flight.

The method described above has advantages over Brown's (loc. cit.) or de Zulueta's method (loc. cit.) as three types of observations are made on the same mosquito, viz., excitation time, number of flights and the pattern of flight, i.e., whether the flights are short or long ones. The exposure time was kept at 15 minutes and was found to be quite adequate for A. culicifacies, A. fluviatilis and A. stephensi. Laboratory bred Culex fatigans was not irritated even with a one hour exposure to two per cent. DDT

paper. Irritability tests have no significance in such species. Two per cent. DDT papers were chosen after preliminary trials with other concentrations which proved less suitable in bringing out differences in irritability more precisely. With this method about 20 mosquitos for control and 20 for DDT can be tested in one day by one technician and one assistant. With more assistants available more mosquitos could be tested in a day. The equipment required for this is so simple that the irritability test can easily be done under field conditions along with susceptibility tests.

After the exposure, the mosquito is removed with the angled aspirator and kept in the holding tube to observe mortality after 24 hours. The handling of exposed mosquitos with the angled aspirator was found to be too harsh for delicate species like A. culicifacies and resulted in high kills in control mosquitos (going up to 40 to 50 per cent.). Hence the mortality figures did not give any indication as to pick up of lethal or sub-lethal dosages and therefore these observations on mortality are not reported in this paper. Sometimes mosquitos were found to rest on the funnel wall; these were disturbed by bringing some object like a pencil near it and such a movement from the wall of the funnel to the impregnated paper was not reckoned as a flight.

Reflections, shadows, movements or draughts should be avoided in the experimental room as these may disturb the exposed mosquitos. The observer should sit about two feet away and should not write on the table on which exposure funnels are arranged as even the slight movement of the table may disturb the mosquitos exposed and may lead to erroneous results. The method described proved quite satisfactory under various field conditions. Further, we did not find any marked difference in irritability of fully engorged, half-gravid or fully gravid mosquitos. Unfed mosquitos were not tested, partly because extremely few were found in the morning catches, and partly because unfed mosquitos are liable to differ considerably from other mosquitos in irritability response. Hamon & Eyraud (1961), (Hamon, J. & Eyraud, M. (1961) Insecticide Circular No. 29, WHO) have found irritability being influenced by the age and difference in state of nutrition of mosquitos. We have, however, used only fully engorged females in the experiments reported below.

TABLE 1. CONSOLIDATED DATA OF IRRITABILITY TESTS PERFORMED ON DDT-SUSCEPTIBLE STRAIN OF A. CULICIFACIES OF MANJRI, DISTRICT POONA, AND DDT-RESISTANT STRAIN OF A. CULICIFACIES OF GADUTAR, DHULIA DISTRICT, AND DDT-SUSCEPTIBLE BUT DIELDRIN-RESISTANT STRAIN OF A. CULICIFACIES OF POTGAON, THANA DISTRICT

Locality (1)	District (2)	Susceptible or resistant to DDT (3)	Treatment (4)	Number of mosquitos exposed (5)	Number irritated in 15 minutes (6)	Per cent. irritated (7)	Total flights in 15 minutes (8)	Average no. of flights per mosquito (9)	Average excitation time for first flight in minutes (10)
Manjri	Poona	Susceptible	Control DDT 2%	54 54	6 51	11 94	9 179	0.17 3.31	12.40 5.57
Gadutar	Dhulia	Resistant	Control DDT 2%	59 76	10 47	17 62	15 98	0.25 1.29	13.53 9.82
Potgaon	Thana	Susceptible to DDT Resistant to dieldrin	Control DDT 2%	16	6	37*	8	0.50	11.96
				16	16	100	69	4.31	5.30

* High figure was probably due to some of the mosquitos not being fully fed.

TABLE 2. RESULTS OF IRRITABILITY TESTS ON DDT-RESISTANT AND SUSCEPTIBLE STRAINS OF A. CULICIFACIES WITH LINDANE AND DIELDRIN IN DIFFERENT LOCALITIES

Locality (1)	District (2)	Susceptible or resistant to DDT (3)	Treatment (4)	Number exposed (5)	Number irritated (6)	Per cent. irritated (7)	Total flights in 15 minutes (8)	Average no. of flights per mosquito (9)	Average excitation time in minutes (10)
Manjri	Poona	Susceptible	Control	54	6	11	9	0.17	12.40
			Lindane 0.1%	11	4	36	5	0.45	10.91
			Dieldrin 0.4%	17	2	12	2	0.12	14.14
Gadutar	Dhulia	Resistant	Control	59	10	17	15	0.25	13.53
			Lindane 0.1%	51	9	18	13	0.25	13.39
			Dieldrin 0.4%	30	8	27	13	0.43	9.77
Potgaon	Thana	DDT suscep- tible but resistant to dieldrin)	Control	16	6	37	8	0.54	11.96
			Dieldrin 0.4%	16	7	44	10	0.63	12.53

Results of irritability tests on the three strains of A. culicifacies with regard to DDT are summarized in Table 1. Tests with lindane and dieldrin are summarized in Table 2. As there was no significant difference in the duration of flight of the above strains, data are not included.

It will be seen from Table 1 that the DDT-resistant strain of A. culicifacies from Dhulia is significantly less irritable than either of the remaining two DDT-susceptible strains of Poona and Thana districts. The percentage of mosquitos irritated is 62 in the Dhulia strain while it is 94 and 100 respectively in the Poona and Thana strains. Similarly the average number of flights per mosquito in 15 minutes of exposure to DDT is also less in Dhulia, viz. 1.29, than in Poona, viz. 3.31, or in Thana, viz. 4.31. Also the average time required to excite the mosquitos is much longer in the Dhulia strain, viz. 9.82 minutes, than in the Poona strain, 5.57 minutes, or in the Thana strain, 5.3 minutes. All these three criteria therefore point to the resistant strain of Dhulia being significantly less irritant than either the Poona or Thana strains. These also point out that there is no significant difference in irritability of the two susceptible strains of Poona and Thana.

Table 2 shows that the differences in the irritability data collected on Poona, Thana and Dhulia strains with lindane 0.1 per cent. and dieldrin 0.4 per cent. are negligible as compared to control papers in these places indicating that irritability produced by these insecticides at these concentrations and exposure period is negligible. However, lindane 0.1 per cent. has in one instance shown higher irritability in the Poona strain as 36 per cent. of the mosquitos were irritated as compared to 11 per cent. in the control.

But the number of mosquitos tested was very small in Poona with lindane 0.1 per cent. (11 mosquitos only). Hence the difference in irritability from that of the control may not be taken as reliable. Lindane and dieldrin have shown to excite mosquitos far less than DDT.

Summary

The WHO tentative method of testing irritability of mosquitos to DDT has been modified so as to make it suitable for field trials and to provide - in one and the same experiment - three types of observations on the same mosquito, viz. (i) number of take-offs per mosquito in 15 minutes, (ii) excitation time of first take-off and (iii) pattern of flights (whether flights are short or long ones).

With the modified method a statistically significant difference has been found in irritability to DDT of DDT-resistant A. culicifacies of Dhulia and DDT-susceptible A. culicifacies of Poona and Thana districts, Maharashtra State, India. The DDT-resistant strain of A. culicifacies is found to be significantly less irritable to DDT than either of the DDT-susceptible strains of A. culicifacies from Poona or Thana districts. Preliminary trials on irritability to lindane and dieldrin have also been carried out on A. culicifacies and these insecticides are found to be less irritant than DDT.

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