EFFECTS OF CERTAIN EXTRINSIC AND INTRINSIC FACTORS ON THE SUSCEPTIBILITY OF LARVAE OF *CULEX PIPIENS FATIGANS* WIED[1] TO DDT[2]

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INTRODUCTION. The importance of standardising age, size and stage of nutrition of an insect, and temperature before and during insecticidal tests has been stressed by Busvine (1957). He found it to be particularly important in mosquito larvae which develop rapidly with consequent changes in their physiological state. Thevasagayam (1957), Mulla (1961) and Paulini and De Sousa (1962) have shown that susceptibility of mosquito larvae to chemicals varies at different stages in the life history.

During the course of a general study, susceptibility tests carried out on different days on early fourth instar larvae of *C. p. fatigans* from an advanced laboratory stock colony derived from Penang, and on those from various wild populations, showed large differences in larval mortality to a given concentration of DDT. There was also some difference among replicates run on the same day. Some of the possible causes for this are variations due to differences in sex, instar, age, nutritional state, differences in testing temperature and day-to-day changes in the composition of the tap water in which larvae were tested. Therefore, a series of experiments was made to test whether the variability was due to these factors or to a character of the species. Since information on the susceptibility of various instars of Malayan *C. p. fatigans* to DDT was also lacking, tests were made with different instars.

MATERIALS AND METHODS. Larvae were obtained from a highly susceptible colony which was originally established with a large number of adults caught from Lamir (Thomas, 1962a). Of various colonies studied during the period of the investigations, this one showed the least degree of fluctuation in larval susceptibility to DDT. Maintenance of adult colonies, rearing and testing of larvae and analysis of data were similar to those already described (Thomas, 1962b).

The effect of temperature on the susceptibility of larvae to DDT was studied by testing early fourth instar larvae simultaneously in an air conditioned room where the temperature was either 24° ± 1° or 25° ± 1° C. and in a room where the temperature range was 24° C. to 29° C. Three sets of experiments were done in tap water and in distilled water to find out how much the variation in larval susceptibility was due to day-to-day changes in the composition of tap water in which larvae were tested.

The DDT was in crystalline form; from this, a 2.5 percent stock solution was made up in analar acetone. This was diluted with acetone to give the appropriate concentrations of the insecticide.

The tests were made in 450 ml. glazed porcelain bowls containing 250 ml. of tap water. The test solutions were prepared by adding 1 ml. of the appropriate strength of insecticide solution under the surface of the water in the bowls and stirring vigorously. Controls were prepared by adding 1 ml. of analar acetone to the water. Twenty larvae were used per bowl and they were allowed to remain

[1] = *Culex pipiens quinquefasciatus* Say; see 'A Synoptic Catalog of the Mosquitoes of the World,' by Alan Stone, K. L. Knight and Helle Starcke.—(Editor).

[2] The work reported here, carried out at the Institute for Medical Research, Kuala Lumpur, was done in the course of studies towards a Ph.D. degree in the Department of Zoology, University of Malaya.

in the test solution for 24 hours without food.

No study was made of the fate of DDT in suspensions; there may have been some loss by co-distillation or heterogenous dispersion as reported in the literature.

The relative susceptibility of various instars to DDT was studied by testing the early first, second, third and fourth instar larvae. In addition, two lots of early fourth instar larvae which included about 10 and 20 percent respectively, of late third instar larvae were also tested. Whenever early fourth instar larvae were picked up from large laboratory cultures for tests, a small and varying proportion (5—15 percent) of late third instar larvae was invariably present. The last series of experiments was therefore, done to find out how much variation in DDT-susceptibility can be caused by the presence of such a proportion of third instar larvae along with early fourth instars in test batches.

The first instar larvae used in these experiments were newly hatched and unfed, but all other stages were fed and were obtained from the normal rearing dishes. The percentage of late third instar among early fourth instar larvae was calculated by counting the larval skins in test solutions and controls after 24-hour exposure period. The mortality among the controls of second, third and fourth instar larvae during 24 hours of starvation was never more than 1 or 2 in a total of 200 larvae. The mortality among controls for first instar larvae was between 2 to 3 percent. When there was any mortality among controls, the results were corrected by using Abbott's formula (Abbott, 1925).

RESULTS. Effect of Temperature. Tables were prepared giving the mortality rates for various replicates at various concentrations and the LC_{50} values obtained for early fourth instar larvae. Figure 1 illustrates the dosage-mortality regression lines obtained from these data. The LC_{50} values were 0.066 p.p.m. (0.063—0.069) at $24° \pm 1°$ C, compared with 0.070 p.p.m. (0.067—0.073) obtained at $25° \pm 1°$ C. and 0.078 p.p.m. (0.074—0.82) when the temperature ranged between 24° and 29° C. In all experiments, the tests done at lower temperatures gave slightly lower LC_{50} values, indicating that the larvae of *C. p. fatigans* were more susceptible to DDT at lower temperatures. However, the larvae tested at the two, more or less constant temperatures also showed considerable fluctuations in the mortality rate for any given dose and these fluctuations were of the same magnitude under all of these temperatures. It is, therefore, clear that although day-to-day changes in temperature during the test period can cause differences in susceptibility levels, these alone are not sufficient to account for the variability obtained in various experiments on the larvae.

EFFECTS OF TAP WATER. The results of the experiments on the effects of tap water show that the LC_{50} levels were always significantly higher when tests were made in distilled water. There was much variability in larval mortality at any concentration in the various replicates of these two sets of experiments. The range of susceptibility levels of the larvae, as shown by the LC_{50} values, tested in distilled water, was just as great as that tested in tap water. The LC_{50} of larvae tested in tap water during these experiments were 0.070, 0.066 and 0.052 p.p.m., compared with 0.078, 0.084 and 0.063 p.p.m. for those tested in distilled water. The LC_{50} values recorded in the first two experiments run in tap water were not significantly different from each other as the 95 percent confidence limits overlap. Similarly, those obtained in the first two experiments conducted in distilled water were also not different from each other. In the third experiment, however, the LC_{50} values recorded in tests run in tap and distilled water were significantly lower than the corresponding values in the two earlier experiments. These results show that although the larvae are slightly more tolerant in distilled water, the fluctuations noticed in the range of larval mortality at any

concentration of DDT were not entirely due to day-to-day changes in composition of the tap water used for larval tests.

RELATIVE SUSCEPTIBILITY OF VARIOUS INSTARS. The percentage mortality at various concentrations of DDT for different instar larvae of *C. p. fatigans* and their LC_{50} values were calculated and the dosage mortality regression lines are presented in Figure 2. Early first instar larvae were found to be the most susceptible to DDT with an LC_{50} value of 0.006 p.p.m. They were about 10 times more susceptible to DDT than the early fourth instar larvae which had an LC_{50} of 0.066 p.p.m. The early second instar larvae were only slightly more tolerant than the first instar with an LC_{50} value of 0.008 p.p.m. The early third instar larvae were much more tolerant than the earlier instars and had an LC_{50} value of 0.028 p.p.m. The LC_{50} values for cultures of early fourth instar larvae with about 20 and 10 percent late third instar

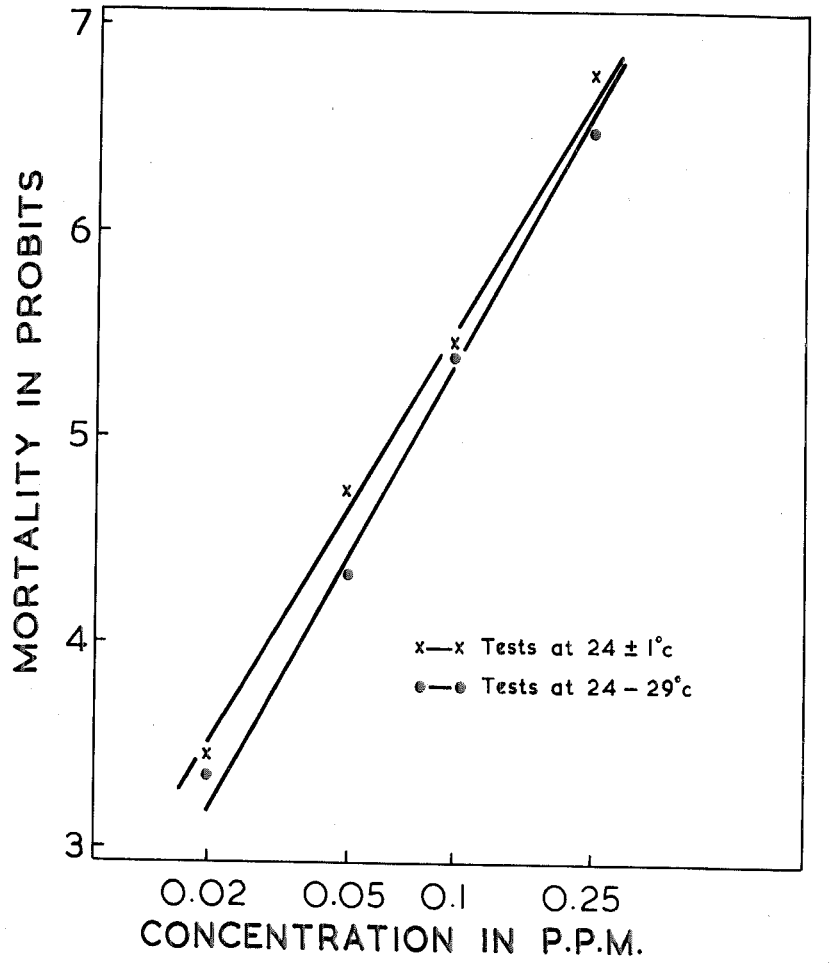

FIG. 1.

larvae were 0.059 and 0.068 p.p.m. respectively; these values were more than twice the LC_{50} value obtained for early third instar larvae alone. These results showed that when about 20 percent third instar larvae were present along with fourth instar larvae in tests, the susceptibility level was significantly lower than that of fourth instar larvae alone; but if about 10 percent third instar larvae only were present, the LC_{50} was not significantly different.

DISCUSSION. The possible factors that cause variation in the experiments are extrinsic or intrinsic ones. The first consists of factors such as nature of breeding medium, the density of the larvae population prior to tests, the volume, surface area and the depth of test solution. All these factors were strictly standardised in these experiments. Other more important factors belonging to this category are the changes in the composition of the tap water in which larvae were tested and the changes in temperature during the tests.

In these experiments, the larvae which were tested over a wider range of temperature of 24°—29° C. always gave significantly higher LC_{50} values than those at a slightly lower and more constant temperatures of 24°±1° C. and 25°± 1° C. This is in line with the findings of Guthrie (1950), Hoffman and Lindquist (1949), Lindquist *et al.* (1945, 1946), Richard and Cutkomp (1946) and Fan *et al.* (1948) with DDT in various insects. Busvine (1957) suggested that this may be due to increased storage capacity of lipids and perhaps, accelerated elimination of poison at high temperatures overcoming the increased penetration and distribution of the poison. It should be stressed that, in the present study, although the larvae were found to be more tolerant when tested at high

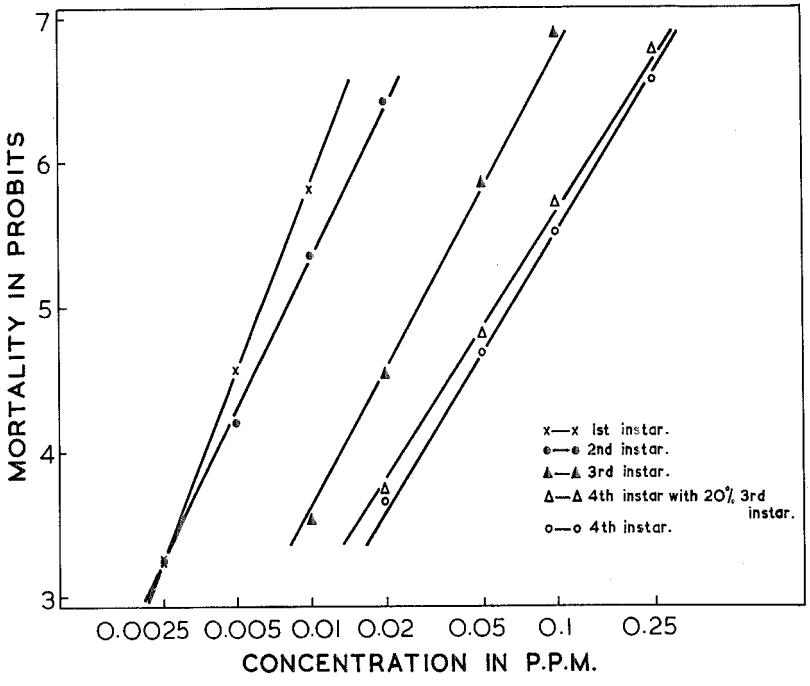

FIG. 2.

temperatures, the tests conducted under all temperatures gave variable mortality rates at any given concentration.

The larvae gave higher LC_{50} values when tested in tap water; but the difference in tolerance revealed in these two series of tests was not large. In addition, when the larvae were tested on different days in distilled water, these also gave fluctuating LC_{50} values, like those tested in the tap water. It was concluded, therefore, that although changes in these factors during tests could produce some variability, these, by themselves, are not sufficient to account for the great range in susceptibility noticed in various tests. This is in agreement with the results obtained on adults of *Ae. aegypti* with dieldrin (Bransby-Williams, 1959).

The other factors that may cause variation are the difference in sex ratio and proportion of the third instar larvae present in various test batches. The sex ratio cannot be controlled when larvae are used for tests; but when large numbers of larvae, distributed in a number of replicates, are tested the sexes are supposed to be equally distributed. About 10 percent third instar larvae among early fourth instar larvae in test batches did not cause any appreciable change in susceptibility levels, while about 20 percent of third instar among them lowered the susceptibility level significantly. Under the standardised rearing and testing conditions, the possibility of having about 20 percent of third instar larvae in test batches was very remote; and even if this proportion of late third instar larvae were present in tests, these could not have been responsible for the large variations in results recorded in different tests.

Another possible cause for difference in susceptibility levels between batches of larvae was considered to be the age of the adults at the time of laying the eggs, from which the larvae for tests were obtained. The tests conducted on any one day, on larvae derived from individual rafts laid by adults of same age, of a laboratory colony, gave greatly varying results (unpublished data). The age of the adults at the time of egg laying also, therefore, did not seem to have any effect on the susceptibility of the larvae. It should be concluded that although changes in these factors during tests can cause some variability of the larvae, the sum total of these is not sufficient to account for the great range in susceptibility recorded in various tests. The variability then may be a character of the larvae of *C. p. fatigans*.

Tests on different instars showed that the first, second and third instar larvae were more susceptible to DDT than the fourth instar larvae. This is contradictory to the results reported on *C. quinquefasciatus* by Mulla (1961). He found that, the first and the fourth instars were equally susceptible to DDT, but that the first instar larvae were more susceptible to nine other insecticides he tested. Similar higher resistance of first instar larvae has earlier been reported in *Aedes aegypti* to heptachlor suspensions (Burchfield *et al.*, 1953). However, it is usual to find the earlier instars to be more susceptible; Paulini and De Sousa (1962) found that susceptibility of larvae of *C. p. fatigans* decreased with age, the first instar being 2 to 3 times more susceptible to eight insecticides including DDT than the fourth instar. Thevasagayam (1957) found the third instar larvae to be more susceptible to parathion and endrin than the fourth instars. He did not test the various stages against DDT. Difference in liability of penetration, greater sensitivity associated with higher metabolism, more numerous sense organs and/or greater buffering power of fat have been suggested as the possible causes for the differences in resistance levels between various stages in the life cycle of an insect (Busvine, 1957).

SUMMARY. The effects of various factors such as changes in testing temperature and composition of tap water in which larvae were tested were studied on larvae of *C. p. fatigans* reared under standardised conditions. Larvae tested at room temperature which ranged from

24°–29° C. gave higher LC_{50} values than those tested at lower and more constant temperatures of 24°±1° C. and 25°± 1° C. Similarly, the larvae tested in distilled water gave higher LC_{50} values than those tested in tap water. However, the results show that although changes in these factors during the test period can cause differences in the DDT-susceptibility levels of the larvae, these, by themselves, are not sufficient to account for the variability present in various experiments on the larvae of the colonies of *C. p. fatigans*.

The tolerance of the larvae to DDT increased with age; the fourth instar larvae were found to be about 10, 8 and 2½ times more tolerant than the early first, second and third instar larvae respectively. The presence of 10 percent of late third instar larvae among early fourth instar larvae in tests did not cause any significant changes in the tolerance of the larvae, but when 20 percent third instar larvae were present the LC_{50} value was significantly lower.

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