STUDIES ON THE EFFECT OF SUBLETHAL DOSES OF INSECTICIDES ON *Tribolium castaneum* Herbst

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THESIS

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C E R T I F I C A T E

This is to certify that the thesis herewith submitted contains the results of bonafide research work carried out by Shri O.T. Madhavan, under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

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INTRODUCTION

INTRODUCTION

Under field conditions of insecticide applications, the insects come in contact with different doses of the insecticide. These doses may be lethal or sublethal. Insects picking up lethal doses die, while those getting sublethal doses survive. The number of survivals will vary according to the intensity of the insecticidal pressure.

It is a matter of common observation in insecticidal bioassay tests that when a population of insects is subjected to different ascending grades of insecticide pressures, there will be survivals at the various levels eventhough their number may be progressively decreasing. Progressively decreasing doses of insecticides will be present on the plants on the consecutive days following insecticide application.

Though there is evidence to show that sub-lethal doses of insecticides affect the biological and physiological activities of insects, the precise action of the sublethal doses on these activities have not been fully understood. Objective studies on the effect of sublethal doses on insects are very few and these include those of Wene (1947), Tenhet (1947), Beard (1952), Kuenen (1958), Loschiravo (1960), Saini (1966), Osherova (1967)

Srivastava & Fine (1967). The results reported by these workers are however quite contradictory. Further, no effort has been made so far to understand the influence of increasing or decreasing sub-mortal insecticide pressures on the various life processes of insects. The investigations presented hereunder have hence been undertaken with a view to study the effect of sublethal doses of five insecticides of different modes of action on the development, survival and fecundity of <u>Tribolium castaneum</u>. Every insecticide has been used at graded and decreasing levels of sublethal doses. The studies have shown that the larval duration, growth of the larva and fecundity of the adults are significantly affected by the variations in the sublethal doses.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The earliest study on the effect of sub-lethal doses of insecticides to insects appears to be that of Wene (1947). He found that the larva of Epilachna when fed on leaves treated with cryolite ceased to feed before they injested a lethal dose and suffered retarded development. In order to ascertain whether this retardation was due to inadequate feeding or due to effect of poison, larvae that had recently moulted were subjected to starvation and to sub-lethal doses containing 90 per cent sodium fluoaluminate on bean leaves. Duration of the 3rd instar was increased from 2.6 days from continuous feeding on untreated leaves to 3.4 and 4.3 days by starvation for one and two days respectively. The larval duration was altered to 4.8 and 5.8 days respectively when fed on treated leaves for 1 and 2 days, before being transferred to untreated ones. There was no mortality among larvae fed continuously on clean leaves or starved for one day. But the percentage of the larvae that survived when starved for two days or fed for one or two days on treated leaves fell to 76.8, 47.3 and 44.1 respectively. Duration of the 4th instar was increased from 5.3 to 6.5 and 9.3 days by starvation for one day and three days respectively and to 8.1 and 10.3 days by feeding on treated leaves for one and two days respectively. Corresponding reduction in percentages that survived were from 97.1 to 94.1, 79.1, 61.4 and 24.7. Five 3rd instar larvae consumed 80 per cent of the area of the leaf in 24 hours, but five that have been starved for one day ate 80 per cent of 2 leaves. Five others ate less than 2 per cent of leaf treated with chemical in the same period and about the same amount of an untreated leaf on the following day. In addition to killing some larvae, chemicals thus reduced the injurious effects of the survivors and therefore might be more effective than mortality records indicated.

Beard (1952) treated the adults of the large milkweed bug and the larvae of the greater wax moth with a single dose equivalent to the LD 50 of a toxicant and tested the susceptibility of the survivors, one week later to the same toxicant or other toxicants. It was found that in the case of toxicants the survivors instead of being more resistant and less variable in susceptibility than un-treated insects, as expected, were equal in susceptibility and variability or in several cases, even more susceptible and more variable, than the normal population. The degree of selective pressure of pyrethrum had no effect on the susceptibility to nicotine. By administering two unit doses at different times, in comparison with two unit doses at the same time, arsenic appeared to be eliminated by both insects. Nicotine appeared to be eliminated by waxmoth larvae, but was additive in the bug. Both DDT and pyrethrum showed some sensitising activity and in general were more additive. The pattern of susceptibility of chemically selected insects being essentially the same as that of unselected insects even in the absence of additive effects, suggests a dynamic type of variation among individuals. Dynamic variation could help to explain the conditions under which resistance might develop.

Moore (1933) found that exposure of red scale to low concentration of HCN, before fumigation, reduced the kill in some cases, in another case, pre-treatment had no effect, or even increased the final kill. Campbell (1926) found that individual silk worms, receiving a number of sub-lethal doses of neutral sodium arsenate solution administered orally, during larval development, did not acquire tolerance for arsenic. Early sub-lethal doses were eliminated as there was no evidence of cumulative action. Kono (1949) reported that prefumigation of rice weevil with low doses of carbon-di-sulphide decreased susceptibility to the chemical. House flies pre-treated with synergists were more susceptible to pyrethrum than those not pre-treated. Hoffman (1957) found that house flies surviving six exposures during a three day-period to surface treated with DDT were killed much more easily than unexposed flies, when it is exposed to a higher dose of DDT, suggesting that a toxic by-product accumulated in the flies. Milkweed bug pretreated with nicotine were more susceptible to pyrethrum than bugs not pretreated. Bugs pretreated with pyrethrum were less susceptible to nicotine than bugs not treated.

Tenhet (1945) observed that when large number of cigarette beetles were exposed to sublethal doses of pyrethrum spray, the surviving beetles deposited only about half as many eggs as beetles not exposed to such spray. Failure to deposit eggs may be due to partial paralysis of genetalia.

Tattersfield and Kerridge (1955) found that when CO_2 was administered in sublethal concentration to adults of Drosophila melanogaster, susceptible to the toxic effect of this gas, it increased their resistance. This effect did not appear to be permanent. Successive applications did not increase the resistance. Topical application of DDT in sublethal doses resulted in a reduction in the number of eggs laid or in the rate of oviposition.

Beard (1956) treated milk weed bug and wax moth larvae with sublethal doses of nicotine, arsenic, DDT and pyrethrum. In no case, did the treated individual become more resistant, though in some cases, they became more susceptible, possibly as a result of carry-over of the compound, or lack of complete recovery. Similar results were reported for DDT in housefly by Hoffman et. al. (1950). Burns (1931) grew Drosophila larva in a medium with a sublethal level of DDT or BHC and tested the adults for resistance. Comparisons were made with both DDT-resistant and susceptible strains, but in neither case, was there any significant difference between those reared in treated medium and control. There is still a possibility that continued sublethal treatments over many generations might have an effect. But Luers (1956) grew Drosophila for more than 50 generations in a medium containing sublethal concentrations of DDT and obtained no detectable enhancement of resistance. Thus nonselective doses do not appear to increase resistance.

Knutson (1958) showed that sublethal exposure of *Drosophila* to dieldrin increased the fecundity and life-span of the flies. The greater number of the total progeny of the dieldrin-exposed flies resulted apparently, because these flies lived longer than control flies and therefore had a longer time in which to reproduce.

Kuenen (1958) in his treatment, exposed *Calandra* to very low doses of DDT, mixed with wheat in which it was reared (0.1, 0.125, 0.25 mg/100 grams of wheat flour). Weevils exposed to 0.1 and 0.125 mg produced about 20 per cent more off-spring than the unexposed weevils. At 0.25 mg DDT, reproduction was even higher per living female in the first five weeks. But mortality was high and the total number of offsprings was much lower than in the other cultures. It was suggested that less susceptible an arthropod species was to DDT, the greater was the possibility of its stimulations to reproduction.

Brown (1958) exposed adult house flies to sublethal doses of diazinon, dieldrin or DDT daily and these flies became progressively more susceptible to them. Pre-exposure of either normal or resistant strain to the insecticide increased their susceptibility to DDT. There is no acquired tolerance afforded by completely sublethal doses. *Drosophila* reared for 50 generations in a sublethal concentration of DDT, did not show any decrease in its susceptibility to DDT.

Lakoey (1958) found that when the 4th instar larvae of Colorado potato beetle were dusted with DDT there was no effect on the subsequent duration of development of the survivors, but the mortality rate of the resulting adults were increased, whether they were fed or not and females laid more eggs than did untreated beetles.

Hadway (1959) demonstrated DDT detoxification in normal flies by showing that daily sublethal doses given to the flies were only half cumulative. Loschiavo (1960) exposed _Tribolium_ adults to low doses of 1, 1.5 or 2 milligrammes ethelenedibromide per litre for 5 hours at 80°F and then transferred them to a culture medium. The females proved more susceptible than males and the survivors laid few eggs during the following 10 days, whether mated with treated males or untreated males. The percentage of eggs hatched was reduced when both the parents were treated with 1.52 mg/litre, but viable eggs gave rise to larvae that developed normally. When eggs were fumigated similarly those treated when 4 days old, failed to hatch, whereas one day old eggs were unaffected by 0.5 mg but reduced in viability by 1 mg ethylenedibromide/litre. Development after hatching was normal. Fumigation of pupae with 0.5 to 2 mg/litre showed that the susceptibility increased with age and was greater for females than for males. Many showed abnormal darkenings of the cuticle and abnormal development at emergence. There was no evidence of a strain of _Tribolium_ resistant to the fumigant.

Osherova (1964) studied the effect of treatments with demeton in 2 concentrations of 0.015 and 0.001 per cent on the survival, development and fecundity of <u>Tetranychus telarius</u> on bean plants in successive generations. Counts of the mites were made three days after spraying the bean plant with the insecticides and pairs of female and male deutonymphs were then transferred to untreated plants and observed daily from the time the female began to oviposit until the appearence of first adult of the new generations. When 0.015 per cent demeton was applied, in the 1st, 2nd, 4th, 7th and 9th generations, treated female progeny lived for an average of 7.1 days as against 9.6 days when the parents were untreated, and laid an average of 27.5 eggs as against 46.9 in control. With 0.001 per cent demeton, the 1st, 5th and 7th generations, female progenies lived for an average of 9.6 days as also the progeny of the parents which were untreated; the eggs laid were 48.5 in treated and 48.1 in untreated. The remainder of the mites were transferred to untreated plants and allowed to develop until the next spraying. With 0.015 per cent the female of 1st, 2nd, 4th, 7th, 9th and 11th generations lived for an average of 7.3 days as compared with 9 days for no treatments; they laid 25 eggs each as compared with 37.1 in control. With 0.001 per cent 1st, 3rd, 5th and 7th generations lived for 9.3 days as compared with 9.4 days for untreated generations and laid 40.6 and 29.8 eggs respectively. When the parent generations were sprayed with 0.005 per cent the immature stage lasted 11.3 and 11.6 days in the first and second filial generations respectively as compared with 10.8 and 10.7 days respectively for no treatment; with 0.001 per cent, the larval stages lasted 10.6 and 8.5 days as compared with 11 and 9.9 days respectively. It is concluded that the after-effects of treatments were unfavourable to the mites when the concentrations were high but favourable when they were low.

Saini (1966) in the laboratory investigation to study the effect of DDT and dicofol on reproduction of red mite, the red mites were treated either by contact for 15 minutes with filter paper soaked in solution of the material in acetone or to bean leaves sprayed with emulsion of DDT in xylene. After the treatment, the mites were transferred to the untreated plants or the excised leaves floating in a nutrient solution in petridishes. Although there was a decline of 21.7 per cent in the number of offspring produced in one test, when immature mites were treated with 0.1 per cent DDT which caused considerable mortality, there was increased number of offspring in other tests, but the rate of reproduction was not significantly higher than those of untreated mites and the results were similar with both DDT and dicofol at all concentrations tested (0.05 to 0.1 per cent). Indirect effect of DDT was studied by treating the primary leaves of bean plants and using the untreated trifoliate leaves for determining the mite-reproduction and nitrogen content. With one exception, when 12 per cent more offspring were produced by mites on plants treated with 0.05 per cent

DDT, than that produced by mites on untreated plants there was no significant difference between the number of progenies; but the females of F1 generation matured more rapidly on leaves from treated plants. The optimum nitrogen content for population increase was 3.8 per cent. Effect of nitrogen and sugars on population were studied by floating untreated trifoliate leaves on solution of 0.004 mg calcium nitrate, 0.5 per cent glucose or sucrose or all these together. Presence of nitrogen and sucrose resulted in increase in reproduction of 13.3 per cent and 15.6 per cent respectively and with all the three components, the increase was 20 per cent. Glucose alone, reduced reproduction by 6.2 per cent.

Beard (1965) reported that flies exposed to sublethal doses of DDT gave rise to offspring which increased resistance to DDT. This increase in resistance was due to the selection of resistant genotypes rather than induction. Treatments interfered with normal reproduction and this serves to select in favour of resistant genotypes.

Srivastava and Fine (1967) dosed 20 virgin males and females of house flies with a sublethal dose of DDT 0.25 mg/fly and sexes kept in seperate cages for 24-hour observation periods. Then they were mated together in 20 single families. Sublethal doses did not affect mating abilities of flies. Slight reduction in reproduction capacities and percentage hatch in DDT line may reflect pathological ovogenisis. Sublethal doses can lead to the development of low level of resistance. Such treatments did not maintain resistance beyond seven generations. Selection with DDT causing partial mortality demonstrates that it is more effective in rapid development of high levels of resistance than sublethal doses of DDT.

MATERIALS AND METHODS

MATERIALS AND METHODS

Materials

Test insect: *Tribolium castaneum* H. (Tenebrionidae) was used as the test insect.

Rearing medium: Wheat flour sieved through a sieve of 80 mesh was used as the rearing medium of the test insect.

Insecticides: The following insecticides were used.

DDT: 2, 2-bis-(p-chlorophenyl)-1,1,1-trichloroethane. A technical material containing 77 per cent pp isomer supplied by M/s. Bharat Pulversing Mills Private Limited, Hexamer House, Bombay.

Endrin: 1,2,3,4, -10, 10-hexachloro-6,7, epoxy-1, 4,4a,5,6,7, 8,8a, octahydro-1, 4-endo, endo, 5,8 dimethanonaphthalene. A technical material supplied by M/s. Central Laboratory, Tata Fison Limited, Bombay.

Sevin: 1, naphthyl-N-methyl carbamate. A 100 per cent technical material manufactured by M/s. Union Carbide, India Limited.

Thiodan: 1,2,3,4,7,7-hexachlorobicycle (2:2:1)-heptan-5,6-bisoxy methylene sulphate. A 100 per cent technical grade supplied by M/s. E.I.D. Parry Limited, Ranipet.

Parathion: 0,0, diethyl-O-P-nitrophenyl thionophosphate A 100 per cent technical grade supplied by M/s. Bayer Agrocheme Ltd., Bombay.

Other materials: These included petri-dishes, 10 c.m. diameter, pippettes of 1 ml, 10 ml measuring jar, glass rods.

Methods

Rearing of *Tribolium castaneum* larvae for the experiments:

Adults of *Tribolium* were put in wheat flour sieved through 80 mesh sieve. This flour was sieved at intervals of 2 to 3 days to collect the eggs laid by the beetles. The eggs could be collected on the sieve as they could not pass through 80 mesh sieve. This was continued till no more eggs could be obtained. The eggs thus collected were put in clean petri-dishes at 100 eggs per dish and allowed to hatch, under laboratory conditions. The first instar larvae thus obtained were used in the experiments and they were used within 24 hours of hatching.

Preparation of wheat flour media containing different concentrations of insecticides:

The different wheat flour media containing the sublethal doses of the insecticides were prepared by impregnating the wheat flour with the insecticides from their solutions in acetone. Ten grams of the flour were used for each replication and this was mixed with 9 ml of acetone solution containing the required quantity of the insecticide. The flour was taken in a petri-dish, the insecticide solution poured on it and briskly mixed up with a glass rod. Mixing was continued till the medium returned to the powdery consistancy from the paste-like form. The dishes containing the impregnated flour were then incubated at 100°F for 12 hours to enable complete evaporation of the acetone.

The details of the preparation of the different insecticides concentrations are given below:-

DDT

Stock solutions:

A. 1 gm of DDT technical + 10 ml of acetone = 0.1 gm DDT/1 ml solution

B. 1 ml of A + 9 ml of acetone = 0.01 gm DDT/1 ml solution

C. 1 ml of B + 9 ml of acetone = 0.001 gm DDT/1 ml solution

D. 1 ml of C + 9 ml of acetone = 0.0001 gm DDT/1 ml solution

Preparation of dilutions of insecticidal media:

9 ml of acetone + 10 gm of wheat flour = 0 ppm

0.1 ml of C + 8.9 ml of acetone + 10 gm of wheat flour = 10 ppm

0.2 ml of C + 8.8 ml of acetone + 10 gm of wheat flour = 20 ppm

0.4 ml of C + 8.6 ml of acetone + 10 gm of wheat flour = 40 ppm

0.8 ml of C + 8.2 ml of acetone + 10 gm of wheat flour = 80 ppm

Endrin

Stock solutions

A. 1 gm of Endrin technical + 10 ml of acetone = 0.1 gm Endrin/1 ml solution

B. 1 ml of A + 9 ml of acetone = 0.01 gm Endrin/1 ml solution

C. 1 ml of B + 9 ml of acetone = 0.001 gm Endrin/1 ml solution

Preparation of dilutions of the insecticidal media:

9 ml of acetone + 10 gm of wheat flour	= 0 ppm
0.1 ml of C + 8.9 ml of acetone + 10 gm of wheat flour	= 10 ppm
0.2 ml of C + 8.8 ml of acetone + 10 gm of wheat flour	= 20 ppm
0.4 ml of C + 8.6 ml of acetone + 10 gm of wheat flour	= 40 ppm

Sevin

Stock solutions:

A. 1 gm of technical sevin + 10 ml of acetone = 0.1 gm of sevin/1 ml solution

B. 1 ml of A + 9 ml of acetone = 0.01 gm of sevin/1 ml solution

C. 1 ml of B + 9 ml of acetone = 0.001 gm sevin/1 ml solution

Preparation of dilutions of the insecticidal media:

9 ml of acetone + 10 gm of wheat flour	= 0 ppm
0.1 ml of C + 8.9 ml of acetone + 10 gm of wheat flour	= 10 ppm
0.2 ml of C + 8.8 ml of acetone + 10 gm of wheat flour	= 20 ppm
0.4 ml of C + 8.6 ml of acetone + 10 gm of wheat flour	= 40 ppm
0.8 ml of C + 8.2 ml of acetone + 10 gm of wheat flour	= 80 ppm
1.6 ml of C + 7.4 ml of acetone + 10 gm of wheat flour	=160 ppm

Thiodan

Stock solutions:

A. 1 gm of technical thiodan + 10 ml of acetone = 0.1 gm of thiodan/1 ml of solution

B. 1 ml of A + 9 ml of acetone = 0.01 gm of thiodan/1 ml of solution

C. 1 ml of B + 9 ml of acetone = 0.001 gm of thiodan/1 ml of solution.

Preparation of dilutions of the insecticidal media:

9 ml of acetone + 10 gm of wheat flour	= 0 ppm
0.1 ml of C + 8.9 ml of acetone + 10 gm of wheat flour	= 10 ppm
0.2 ml of C + 8.8 ml of acetone + 10 gm of wheat flour	= 20 ppm
0.4 ml of C + 8.6 ml of acetone + 10 gm of wheat flour	= 40 ppm
0.8 ml of C + 8.2 ml of acetone + 10 gm of wheat flour	= 80 ppm
1.6 ml of C + 7.4 ml of acetone + 10 gm of wheat flour	=160 ppm
3.2 ml of C + 5.8 ml of acetone + 10 gm of wheat flour	=320 ppm

Parathion

Stock solutions:

A. 1 gm of technical parathion + 10 ml of acetone = 0.1 gm of parathion/1 ml of solution

B. 1 ml of A + 10 ml of acetone = 0.01 gm of parathion/1 ml of solution

C. 1 ml of B + 10 ml of acetone = 0.001 gm of parathion/1 ml of solution

D. 1 ml of C + 10 ml of acetone = 0.0001 gm of parathion/1 ml of solution

Preparation of dilutions of the insecticidal media:

9 ml of acetone + 10 gm of wheat flour	= 0 ppm
0.25 ml of D + 8.75 ml of acetone + 10 gm of wheat flour	= 0.25 ppm
0.5 ml of D + 8.5 ml of acetone + 10 gm of wheat flour	= 0.5 ppm
1 ml of D + 8 ml of acetone + 10 gm of wheat flour	= 1 ppm
2 ml of D + 7 ml of acetone + 10 gm of wheat flour	= 2 ppm

Exposing the larvae to sublethal doses of the insecticides:

Fixed number of first instar larvae was put into different media containing sublethal doses of the insecticides contained in petri-dishes and closed with their lids.

Assessment of results:

The following effects of the sublethal doses of the insecticides on the insect were observed.

a) Larval mortality

b) Larval growth

c) Larval duration

d) Pupal mortality

e) Pupal duration

f) Fecundity of adult female

g) Sex-ratio

h) Viability of eggs laid.

These were observed by examining the insects under the treatments, every 3 days. To assess the larval growth, a few random larvae were measured from each replication at each time of the observations. Fecundity of beetles was assessed by sieving out the eggs from the medium at regular intervals.

DETAILS OF EXPERIMENTS AND RESULTS

DETAILS OF EXPERIMENTS AND RESULTS

Experiment-1

Effect of sublethal doses of DDT in feeding medium on the survival, growth and fecundity of *Tribolium castaneum*

Details of Experiment:

Test insect: First instar larvae of *Tribolium castaneum* raised as described under 'Methods' were used for the experiment.

Treatments:

a) Wheat flour medium containing 10 ppm DDT

b) Wheat flour medium containing 20 ppm DDT

c) Wheat flour medium containing 40 ppm DDT

d) Wheat flour medium containing 80 ppm DDT

e) Wheat flour medium containing no DDT(control)

Number of replications in each treatment = 3

Number of insects in each replication =20

Procedure: The preparation of the media containing the different dilutions of the insecticides, exposure of larvae to the treatments, and assesment of results were done as detailed under 'Methods'.

Results:

Results of the experiments are set out in Tables 1 and 2 in Figures 1 to 3. It may be seen from Table 1 and Fig.2 that the larval duration is less on larvae fed on feeding media containing 10 and 20 ppm than in the larvae fed on the pure medium. The larval duration, however, on the whole, increases when the concentration of the insecticide is raised to 40 and 80 ppm. In general it will be observed that the larval duration increases steadily in proportion to the increase in the dose of the insecticide in the feeding medium. Larval mortality also shows a positive relationship with the insecticide doses. Pupal duration and pupal mortality do not show any relationship with the insecticide doses.

Table 2 and Fig. 1 show that the growth of the larvae decreases with increase in doses of DDT and is minimum in the medium containing 80 ppm. This suppression of growth is more pronounced in the early stages of the larvae.

Fig. 3 represents the relative fecundity of beetles reared out on different DDT containing sublethal media. It is observed that the fecundity increases considerably (from 35.8 to 130 and 132.3 eggs per female) as the sublethal dose of DDT increases from 10 to 20 or 40 ppm. A sublethal dose of 10 ppm does not affect the fecundity materialy. The sex ratio and viability of the eggs laid do not appear to be influenced by the sublethal doses of DDT in any definite pattern (Table-1).

Table-1

Effect of sublethal doses of DDT on the development, fecundity, sex ratio and viability of eggs of *T. castaneum*

Insecticide concentration (ppm)	Average larval duration (days)	Larval mortality (per cent)	Average pupal duration (days)	Pupal mortality (per cent)	Average number of eggs laid per female	Sex ratio M:F	Viability of eggs (per cent)
0	37.6	20.0	7.00	4.1	37.3	1:2.3	64.6
10	33.5	33.3	7.25	..	35.8	1:3.0	63.2
20	34.0	60.0	7.50	..	132.3	1:2.4	67.1
40	39.6	83.3	7.00	10.0	130.0	1:2.0	52.7
80	47.0	95.0	7.30	..	..	3:0.0	.. The surviving three beetles were all males

Table-2

Growth rate of larvae of *T.castaneum* bred in media containing different concentrations of DDT

Concentration in ppm	Average length (in mm) of larvae at different intervals (days)								
	4	7	11	14	18	22	26	31	40
0	2.0	3.0	3.26	3.46	4.32	4.60	5.32	5.6	6.0
10	2.0	3.0	3.10	3.29	4.15	4.50	4.50	5.4	..
20	2.0	2.5	3.01	3.26	3.65	4.50	4.73	5.8	..
40	1.5	2.5	2.75	3.15	3.25	3.60	4.10	5.5	..
80	1.5	2.5	2.75	2.91	3.08	3.33	3.75	4.5	5.2

Graphical relationship between dosage of DDT in feeding medium and length of larvae of *Tribolium castaneum*

Fig. 2 Histogram showing the relationship between doses of DDT in feeding medium and larval duration of *T. castaneum*

Fig. 3 Histogram showing the relationship between doses of DDT in feeding medium and fecundity of *T. castaneum.*

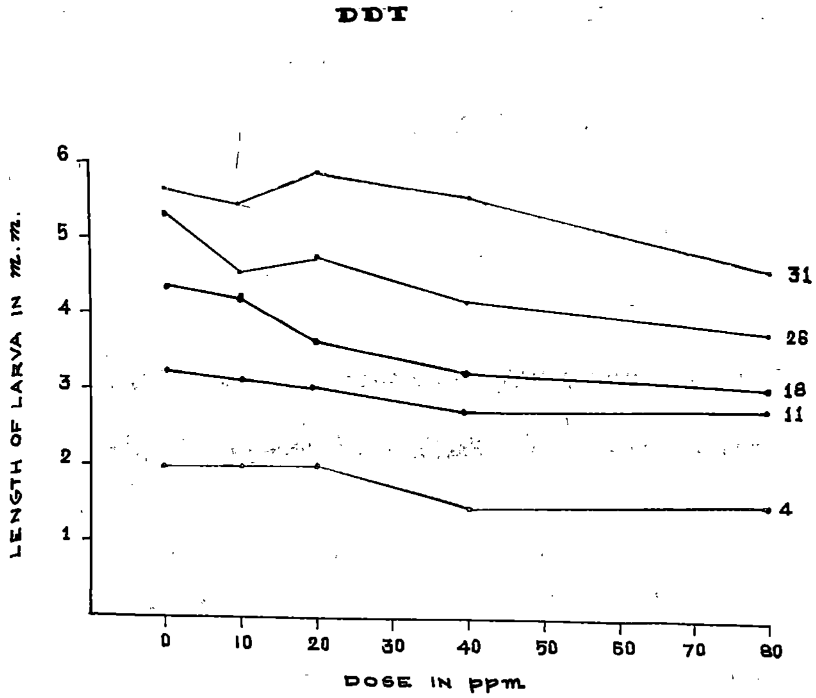


FIG 1

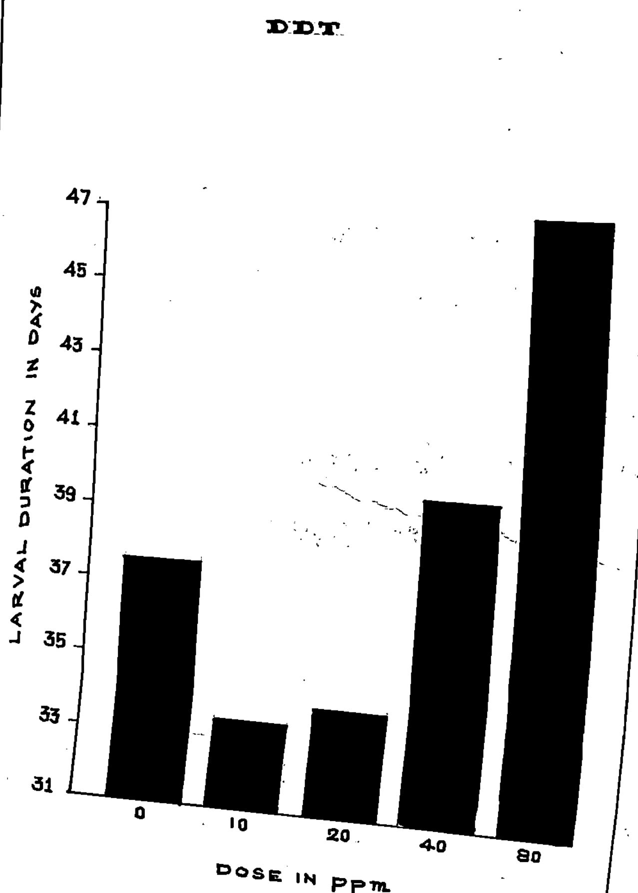


FIG 2

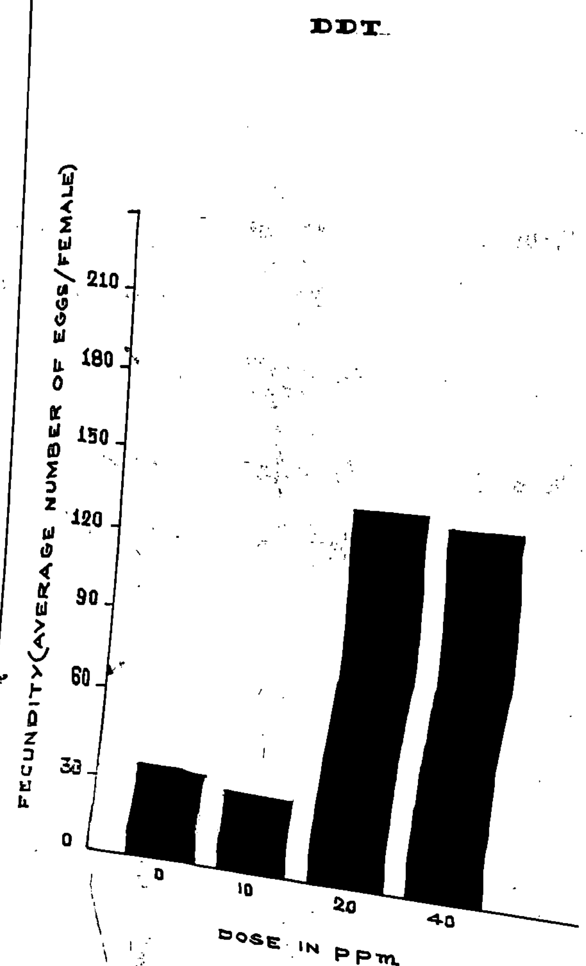


FIG 3

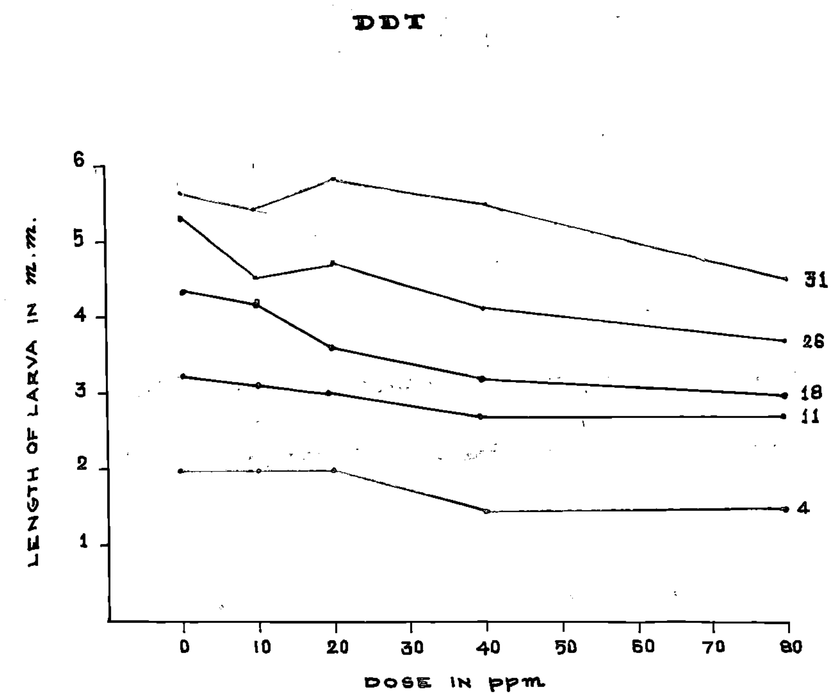


Fig 1

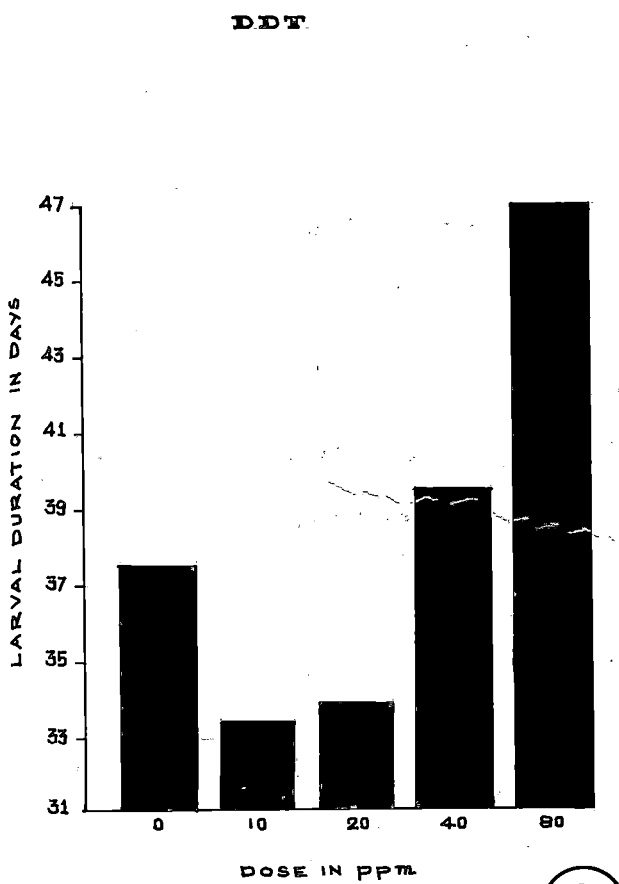


Fig 2

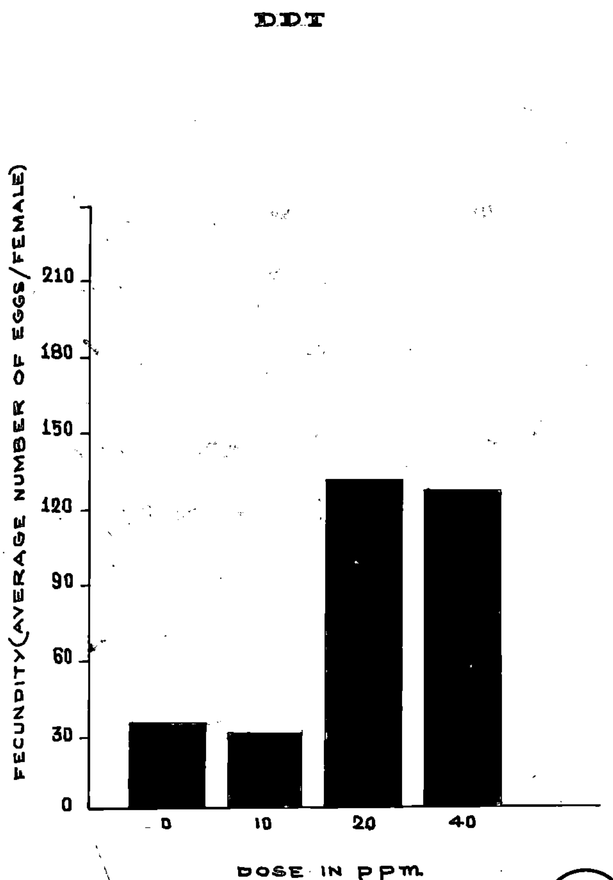


Fig 3

Experiment-2

Effect of sublethal doses of endrin in feeding medium on the survival, growth and fecundity of *T. castaneum*

Details of experiment:

Treatments:

a) Wheat flour medium containing 10 ppm endrin

b) Wheat flour medium containing 20 ppm endrin

c) Wheat flour medium containing 40 ppm endrin

d) Wheat flour medium containing no endrin (control)

Rest of the details as in Experiment-1.

Results:

Results of the experiments are given in Tables-3 and 4 and Figures 4, 5 and 6. It may be seen from Table 3 and Figure 5 that the larval duration increases steadily with the increase in the dosage of the chemical; it is least in pure medium and is maximum in the medium containing 20 ppm. There is a slight fall in the larval period in the medium containing 40 ppm of endrin. Larval mortality shows a positive relationship with insecticide doses. Pupal duration and pupal mortality do not show any relationship.

Fig. 6 gives a comparison of the average fecundity of the beetles reared in different sublethal doses of endrin. Fecundity increases considerably, when the sublethal dose of endrin

Table-3

Effect of sublethal doses of endrin on the development, sex ratio and viability of eggs of *T. castaneum*

Insecticide concentration (ppm)	Average larval duration (days)	Larval mortality (per cent)	Average pupal duration (days)	Pupal mortality (per cent)	Average number of eggs laid per female	Sex ratio M:F	Viability of eggs (per cent)
0	30.9	16.6	7.3	2.0	42	1:1.0	60.8
10	50.2	28.3	7.1	2.3	76	1:1.4	71.1
20	51.8	48.3	7.1	3.2	127	1.1:1.0	64.8
40	47.4	91.6	7.4	..	234	1.5:1.0	48.0

Table-4

Growth rate of larvae of *T. castaneum* bred in medium containing different concentrations of endrin

Concentration in ppm	Average length (in mm) of larvae at different intervals (days)								
	7	13	17	22	25	29	34	37	42
0	3.5	4.0	5.0	5.8	6.0	..	..	..	..
10	2.0	2.6	3.9	4.3	4.7	4.7	5.0	5.0	5.9
20	2.0	2.3	3.6	4.2	4.3	4.5	4.8	5.0	5.5
40	2.0	2.0	2.4	3.3	3.4	3.6	3.9	4.8	5.0

Fig. 6 Histogram showing the relationship between doses of endrin in feeding medium and fecundity of *T.castaneum*

Fig. 5 Histogram showing the relationship between doses of endrin in feeding medium and larval duration of *T.castaneum*

Fig. 4 Graphical relationship between dosage of endrin in feeding medium and length of larvae of *Tribolium castaneum*.

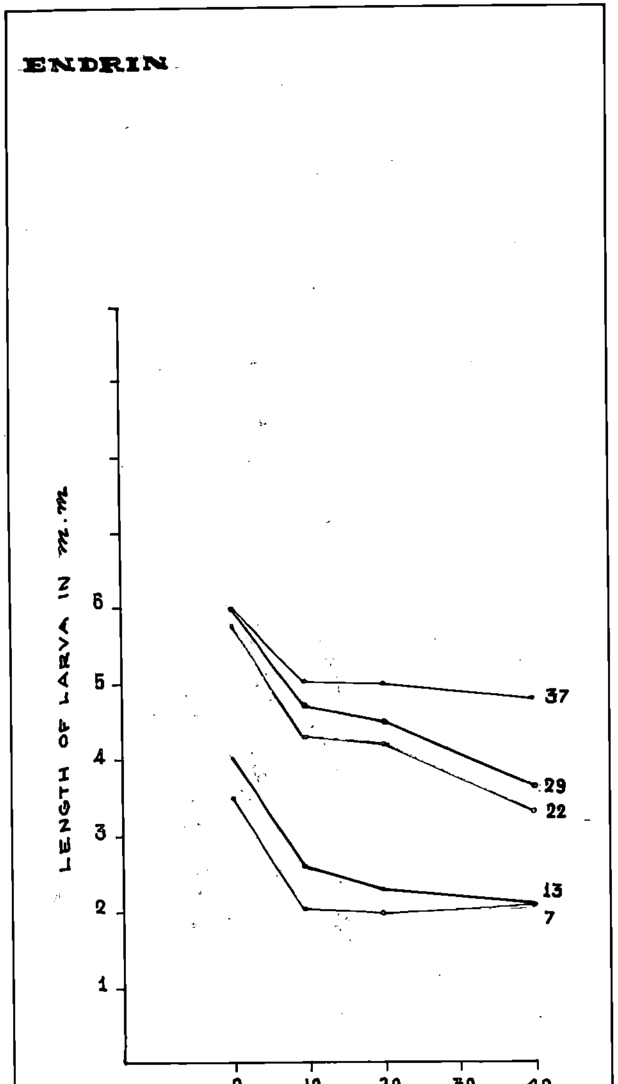


FIG 4

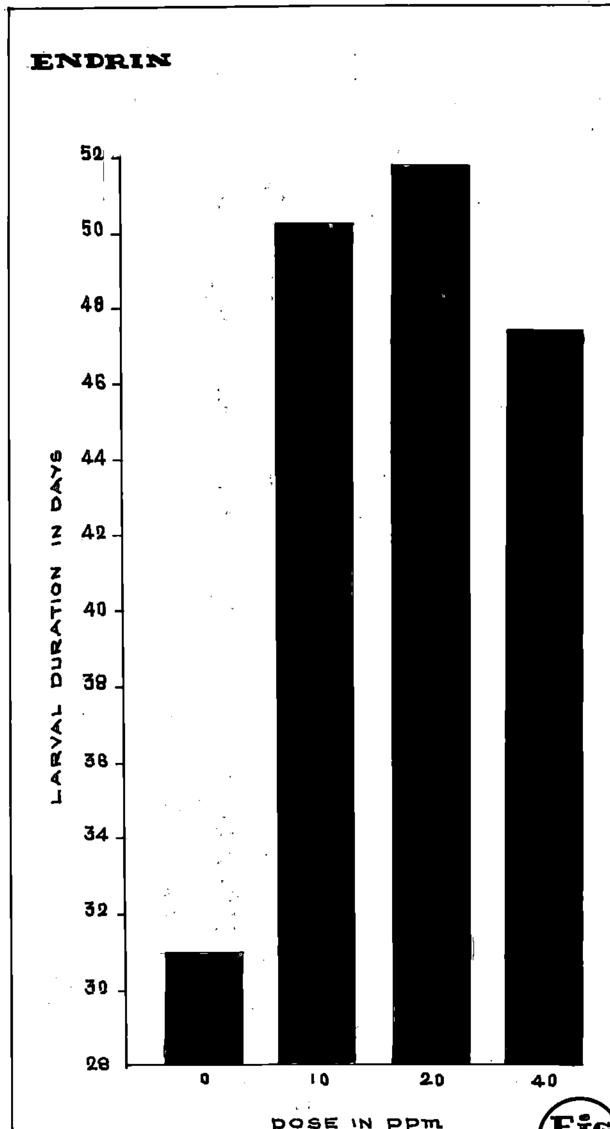


FIG 5

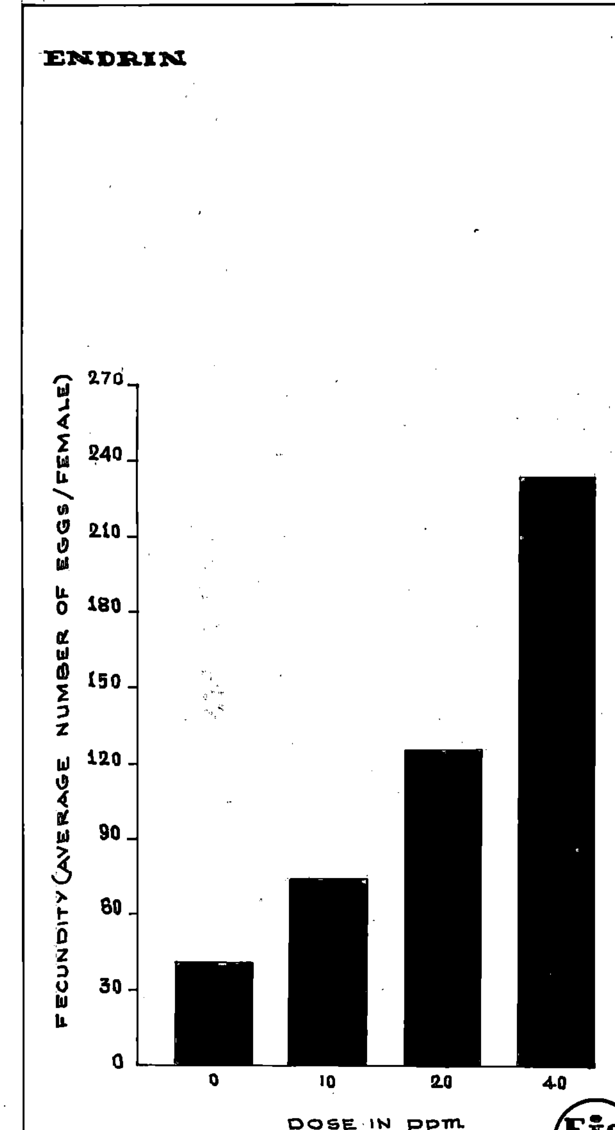


FIG 6

is increased to 20 and 40 ppm the fecundity of the beetles in the latter medium being of about 3 times more than that of the beetles reared in the pure medium. The sex ratio and the viability of eggs appear to be not affected by the sublethal doses of the toxicant. Table 4 and Fig. 4 show that the larval growth decreases with increase in dosage of the chemical and that this effect is marked in the early growth period; the growth is only half in 40 ppm when compared to that of the larvae in the pure medium on 13th and 17th days.

Experiment-3

Effect of sublethal doses of sevin in the feeding medium on the survival, growth and fecundity *T.castaneum*.

Details of experiment:

Treatments:

a) Wheat flour containing 10 ppm sevin.

b) Wheat flour containing 20 ppm sevin

c) Wheat flour containing 40 ppm sevin

d) Wheat flour containing 80 ppm sevin

e) Wheat flour containing 160 ppm sevin

f) Wheat flour containing no sevin (control)

Rest of the details as in Experiment-1.

Results:

Results of the experiment are given in Table-5 and 6 and Figures 7, 8 and 9. It may be seen from Table 5 and Fig. 8 that the larval duration is less in larvae fed on feeding medium containing 10 ppm of endrin [Sevin] than the larvae fed on pure medium. But, on the whole, larval duration increases when the concentration is raised from 20 to 160 ppm and this increase is, in general, proportional to the increase in the concentration of the insecticide within the medium. Larval mortality also shows a positive relationship with insecticide doses. Pupal duration does not show any significant difference and so is the case with pupal mortality.

Fig. 9 represents the relative fecundity of beetles reared out in different sublethal media of sevin. It is observed that the fecundity is maximum under 10 ppm of sevin. From 20 ppm upto 80 ppm it steadily increases; but is reduced considerably under 160 ppm. Even then it is found to be far above the level of 0 ppm. So, on the whole, it is evident that sublethal doses favour fecundity.

Sex ratio and viability of eggs appear to be not influenced by the sublethal doses of sevin.

Table-6 and Fig.7 show that the growth of larvae is slow when the concentration is increased and is only half in 160 ppm when compared to that of larvae in the pure medium on 8th and 20th days.

Table-5

Effect of sublethal doses of sevin on the development, fecundity, sex ratio and viability of eggs of *T. castaneum*

Insecticide concentration (ppm)	Average larval duration (days)	Larval mortality (per cent)	Average pupal duration (days)	Pupal mortality (per cent)	Average number of eggs laid per female	Sex ratio M:F	Viability of eggs (per cent)
0	34.7	18.3	6.9	..	60.8	1.0:1.0	58.9
10	33.5	18.3	7.2	2.0	144.4	1.1:1.0	63.4
20	36.0	10.0	6.9	1.8	122.9	1.5:1.0	70.2
40	41.5	35.0	7.2	2.5	131.6	3.2:1.0	65.6
80	42.5	38.2	7.3	..	142.2	1.0:1.3	70.3
160	46.9	71.6	7.7	..	104.4	1.0:2.4	58.8

Table-6

Growth rate of larvae of *T.castaneum* bred in medium containing different concentrations of sevin

Concentration in ppm	Average length (in mm) of larvae at different intervals (days)						
	8	12	20	23	29	34	40
0	3.0	3.4	5.0	5.5	6.0	..	..
10	2.5	3.3	4.8	5.4	5.7	..	..
20	2.5	3.3	4.8	5.5	5.6	..	..
40	2.0	2.8	3.0	4.5	5.1	5.7	..
80	1.5	2.4	3.4	4.2	5.0	5.6	..
160	1.5	2.1	2.5	3.0	4.0	4.9	5.7

Fig. 7 Graphical relationship between dosage of sevin in feeding medium and length of larvae of *Tribolium castaneum*.

Fig. 8 Histogram showing the relationship between doses of sevin in feeding medium and larval duration of T. castaneum.

Fig. 9 Histogram showing the relationship between doses of sevin in feeding medium and fecundity of *T. castaneum*.

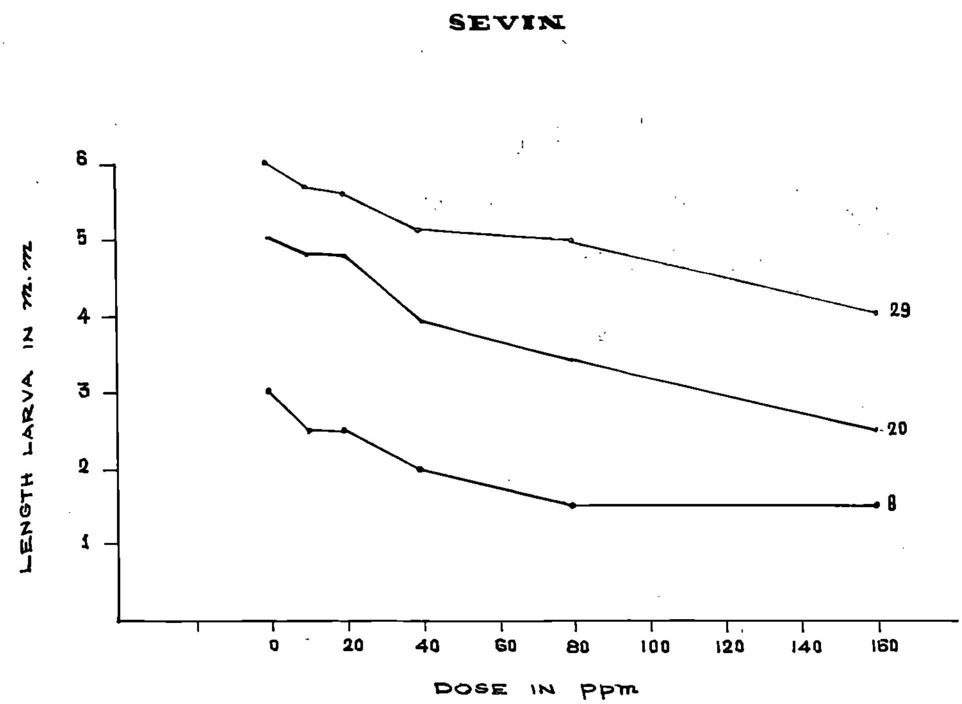


FIG 7

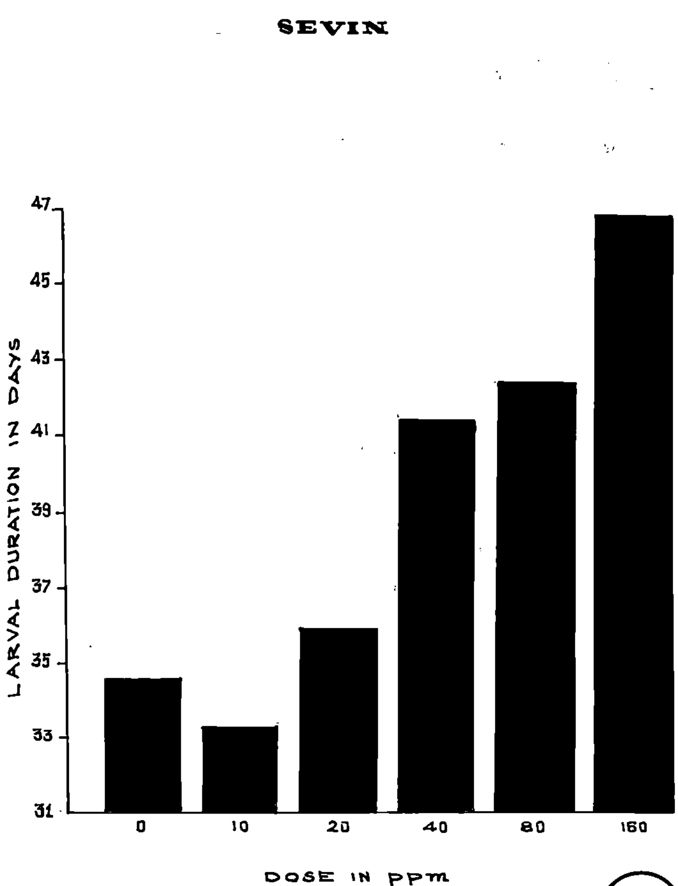


FIG 8

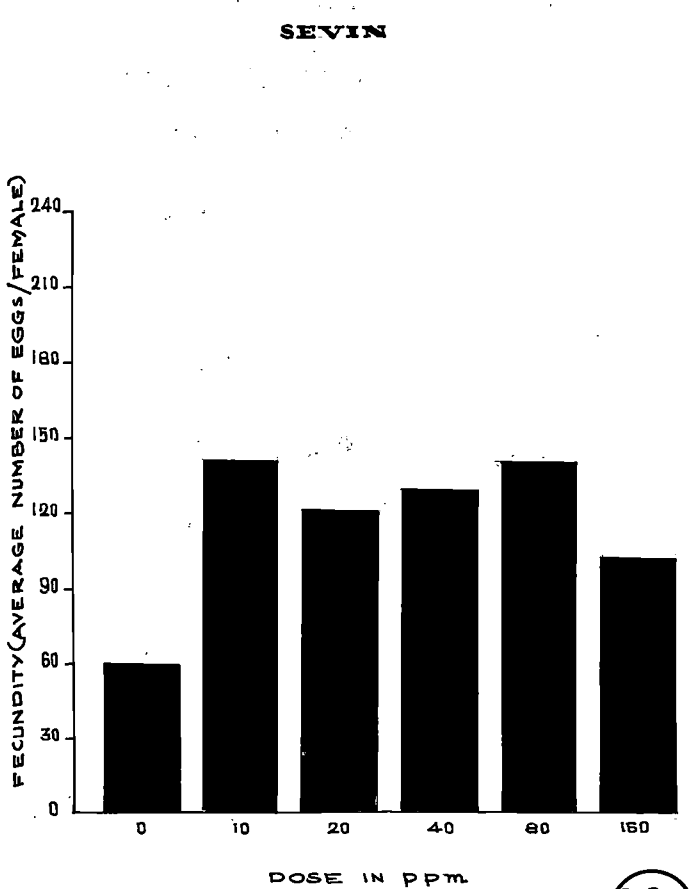


FIG 9

Experiment-4

Effect of sublethal doses of thiodan in feeding medium on the survival, growth and fecundity of *T.castaneum*

Details of experiment:

Treatments:

a) Wheat flour medium containing 10 ppm thiodan

b) Wheat flour medium containing 20 ppm thiodan

c) Wheat flour medium containing 40 ppm thiodan

d) Wheat flour medium containing 80 ppm thiodan

e) Wheat flour medium containing 160 ppm thiodan

f) Wheat flour medium containing 320 ppm thiodan

g) Wheat flour medium containing no thiodan (control).

Rest of the details as in experiment-1.

Results:

Results are given in Tables-7 and 8 and Figures 10, 11 and 12. Table 7 and Fig. 11 show that the larval duration is not significantly affected by the change in the concentration of thiodan in the medium. The larval mortality shows a steady increase with increase in the dosage. There is no significant difference in the pupal duration. But the pupal mortality is found to be more in medium containing 80 ppm and 160 ppm.

Fig. 12 represents the relative fecundity of beetles reared out in different sublethal media of thiodan. It is observed that the number of eggs laid per female increase steadily in

Table-7

Effect of sublethal doses of thiodan on the development, fecundity, sex ratio and viability of eggs of *Tribolium castaneum*

Insecticide concentration (ppm)	Average larval duration (days)	Larval mortality (per cent)	Average pupal duration (days)	Pupal mortality (per cent)	Average number of eggs laid per female	Sex ratio (M:F)	Viability of eggs (per cent)
0	42.5	15.0	7.5	..	46.8	1:1.5	57.6
10	39.4	30.0	7.7	2.3	42.5	1:1.0	55.2
20	38.1	21.6	7.7	..	54.7	1:1.8	57.0
40	38.4	38.2	7.7	2.7	91.2	1:1.3	62.2
80	45.7	51.6	7.4	20.7	154.8	1:1.5	60.4
160	40.1	63.3	7.2	13.6	289.0	1:2.0	69.6
320	46.1	93.3	7.0	..	373.0	1:1.0	63.4

Table-8

Growth rate of larvae of *T.castaneum* bred in medium containing different concentration of thiodan

Concentration (ppm)	Average length (in mm) of larvae at different intervals (days)						
	7	15	19	24	28	34	43
0	3.5	4.1	4.6	4.9	5.4	5.6	6.0
10	3.2	4.1	4.4	4.8	5.3	5.6	6.0
20	3.0	3.5	3.6	4.5	4.7	5.3	5.7
40	2.9	3.5	3.5	4.1	4.5	5.2	5.6
80	2.3	2.5	3.4	4.0	4.3	5.0	5.3
160	2.1	2.3	3.0	3.9	4.2	4.8	5.0
320	2.1	2.1	2.5	3.2	3.5	4.2	4.5

Fig. 10 Graphical relationship between dosage of thiodan in feeding medium and length of larvae of *Tribolium castaneum*

Fig. 11 Histogram showing the relationship between doses of thiodan in feeding medium and larval duration of *Tribolium castaneum*.

Fig. 12 Histogram showing the relationship between doses of thiodan in feeding medium and fecundity of *T.castaneum*.

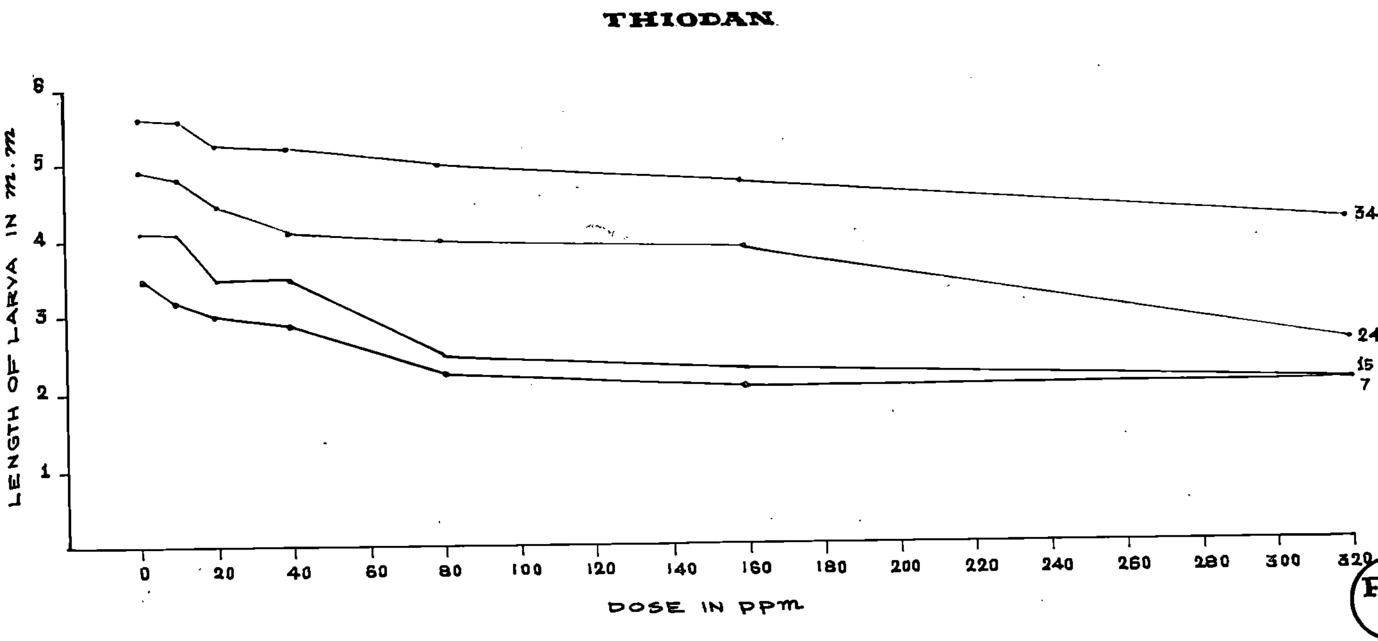


Fig 10

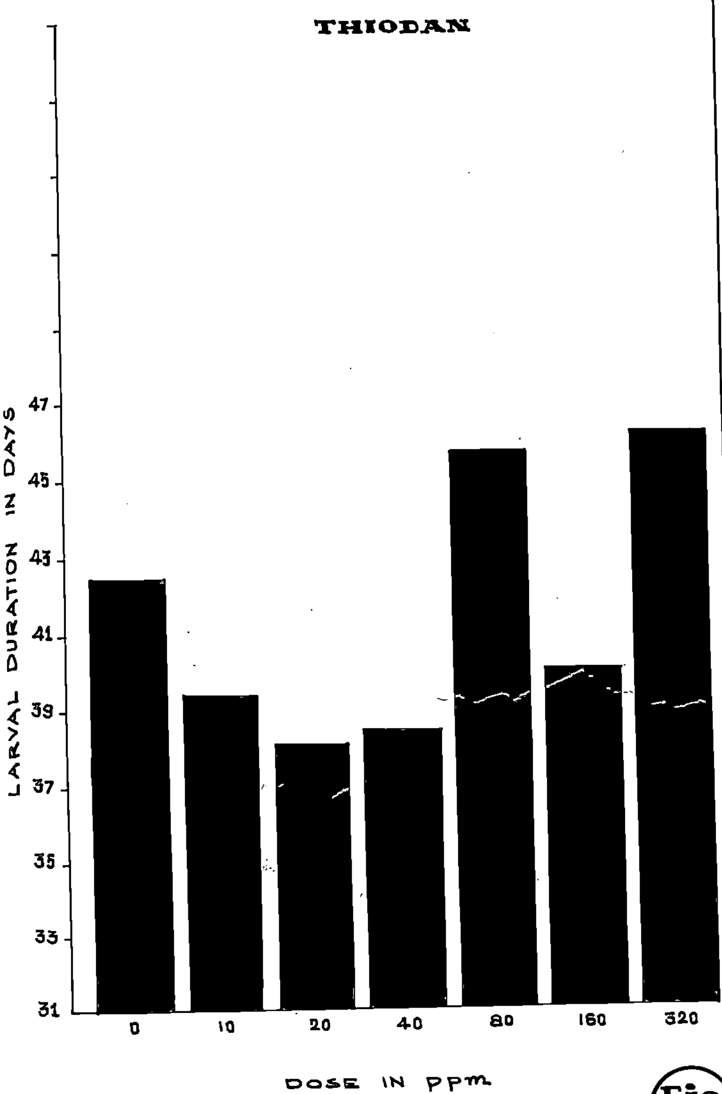


Fig 11

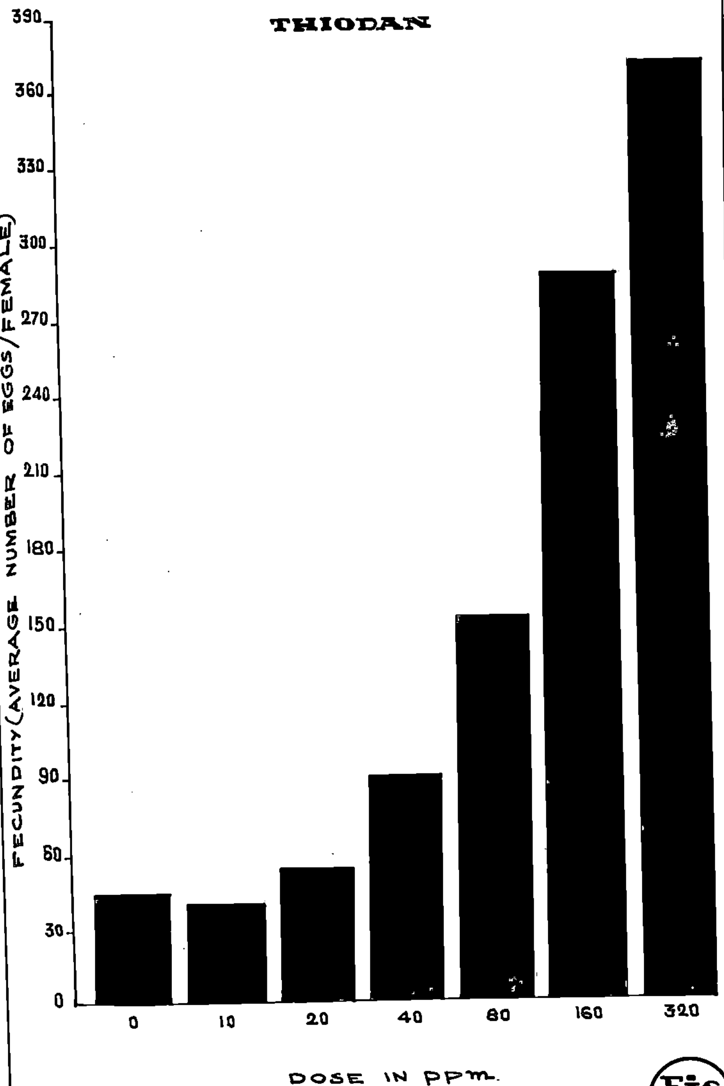


Fig 12

proportion to the increase in the sublethal contents of thiodan in the medium. The maximum number of eggs laid by a female is 373 in the medium containing 320 ppm thiodan as against 46.8 eggs laid by a female reared in pure medium.

Table 8 and Fig. 10 show that the growth of larva is slow when concentration is increased.

Sex ratio and viability of eggs appears to be not influenced by the sublethal doses of thiodan.

Experiment-5

Effect of sublethal doses of parathion in feeding medium on survival, growth and fecundity of *T.castaneum*

Treatments:

a) Wheat flour medium containing 0.25 ppm of parathion

b) Wheat flour medium containing 0.50 ppm of parathion

c) Wheat flour medium containing 1.00 ppm of parathion

d) Wheat flour medium containing 2.00 ppm of parathion

e) Wheat flour medium containing no parathion (control)

Rest of the details as in experiment-1.

Results:

Results are represented in Tables-9 and 10 and Figures 13, 14 and 15.

Table 9 and Fig. 14 show that the larval duration is steadily increasing with increase in concentration of the insecticide. This increase is from 36.7 days in control to 52.2 days in medium containing 2 ppm parathion. Larval mortality is also on the increase although in the case of 0.50 ppm and 1 ppm the mortality shows decrease. Pupal duration does not show any significance, but the pupal mortality is the maximum in the medium containing 1 ppm of parathion.

Fig. 15 represents the relative fecundity of beetles reared out in different sublethal media of parathion. It is observed that the fecundity of beetles is 210 per female in medium containing 0.25 ppm of parathion as against 42 per female in pure medium. There is evident decrease in the fecundity as the parathion contents in the medium is increased from 0.25 to 2 ppm. In all these cases, however the fecundity remains much higher than the control.

Sex ratio and viability of eggs appear to be not influenced by the sublethal doses of parathion.

Table 10 and Fig. 13 show that the growth of larvae is slow when the concentration is increased.

Table-9

Effect of sublethal doses of parathion on the development, fecundity, sex ratio and viability of eggs of *Tribolium castaneum*

Insecticide concentration (ppm)	Average larval duration (days)	Larval mortality (per cent)	Average pupal duration (days)	Pupal mortality (per cent)	Average number of eggs laid per female	Sex ratio (M:F)	Viability of eggs (per cent)
0	36.7	11.6	7.1	1.8	42	1:1.6	73.5
0.25	38.4	28.3	7.2	11.6	210	1:1.0	62.7
0.50	30.0	26.6	7.2	..	149	1:1.3	69.6
1.00	39.0	16.6	7.3	14.0	133	1:1.2	73.8
2.00	52.2	75.0	7.2	6.6	104	1:1.0	52.7

Table-10

Growth rate of larvae of *T.castaneum* bred in medium containing different concentration of parathion

Concentration in ppm	Average length (in mm) of larvae at different intervals (days)				
	8	14	20	32	38
0.00	2.6	3.6	4.6	5.7	6.0
0.25	2.7	3.4	4.5	5.6	6.0
0.50	2.7	3.3	4.4	5.2	5.6
1.00	2.6	3.2	3.8	4.7	5.3
2.00	2.2	2.5	3.0	4.2	5.0

Fig. 13 Graphical relationship between dosage of parathion in feeding medium and length of larvae of *Tribolium castaneum*.

Fig. 14 Histogram showing the relationship between doses of parathion in feeding medium and larval duration of *T. castaneum*.

Fig. 15 Histogram showing the relationship between doses of parathion in feeding medium and fecundity of *T. castaneum*.

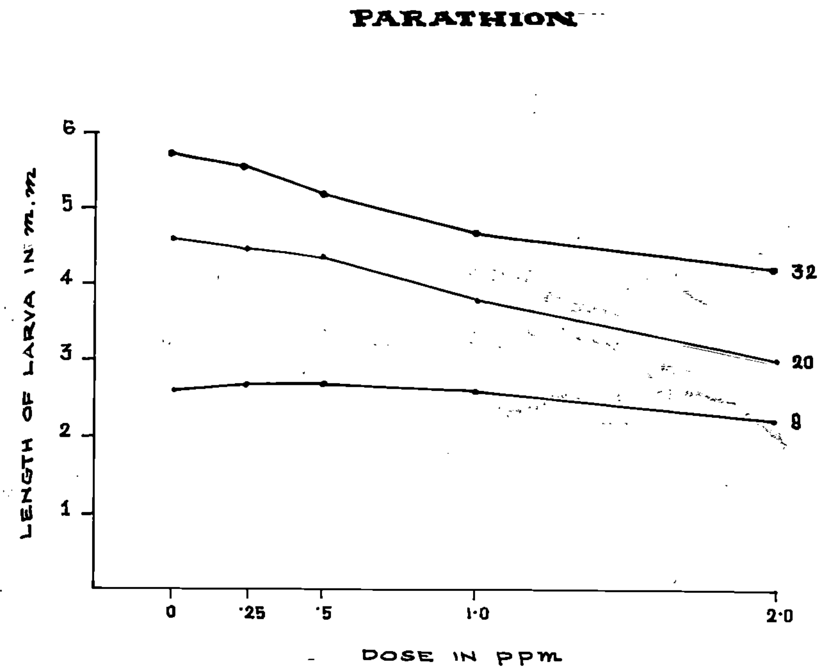


FIG 13

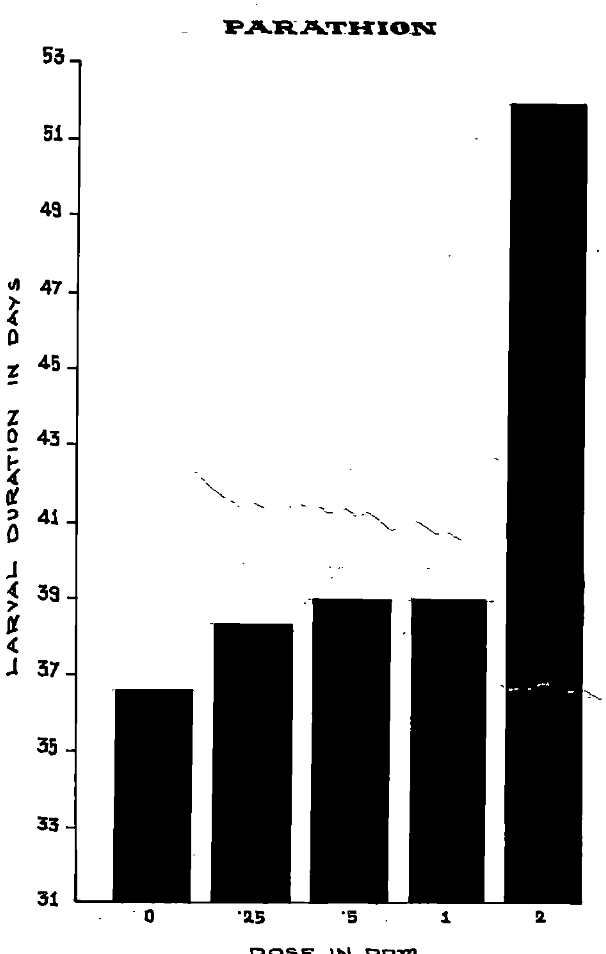


FIG 14

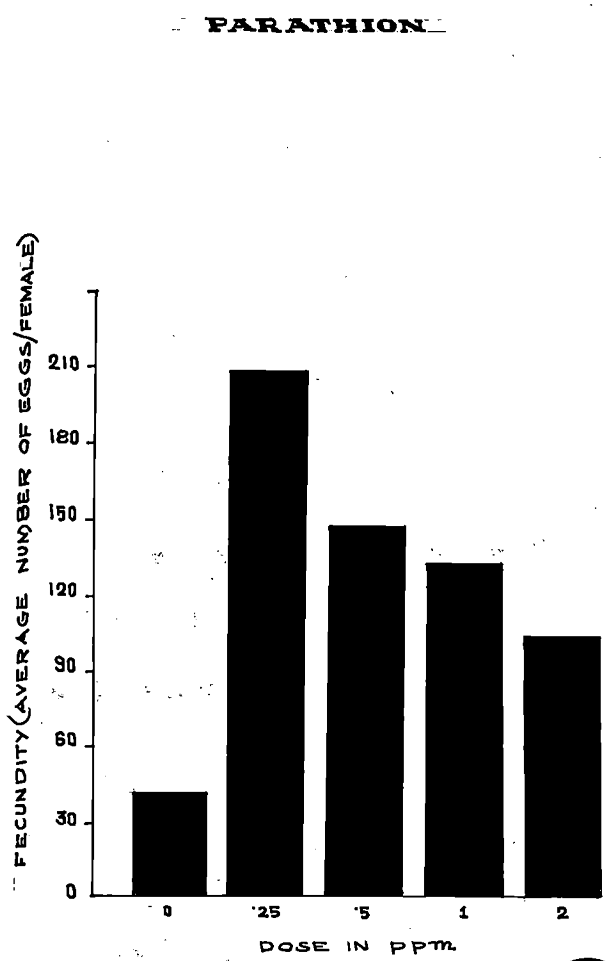


FIG 15

DISCUSSION

DISCUSSION

Results of the investigations presented above will show that the developmental period of the larvae of *Tribolium castaneum* is prolonged as a result of the stress on them imposed by the sublethal doses of insecticides. The increase in the larval duration is seen to be directly proportional to the increase in the concentration of the toxicant within the sublethal range. The insecticides which show these effects clearly are DDT, endrin, sevin and parathion. In the case of thiodan, however, no well defined effect on the larval duration is manifested. Wene (1947) and Bhatia and Pradhan (1968) also have made similar observations. The latters' observation is on *Tribolium* itself wherein they have observed that DDT at a sublethal dose (on a resistant strain) increases the larval period considerably.

The growth of the larva of *T. castaneum* also is drastically affected by the sublethal doses of the insecticides tried under the present investigations. All the insecticides including thiodan under the submortal conditions cause a reduction in the growth of the larvae and this reduction is proportionate to the graded concentration of the toxicant present in the medium. The inhibition of growth of the larvae appears more spectacular during the early part of the larval existence.

The suppression of the growth of the larvae can happen in two ways, by the inhibition of the hormonal system (like the juvenile hormones) governing the growth processes or by the reduction in the feeding of the larva. Which of these two processes is affected by the insecticides can be conclusively understood only by further experimentation.

The different insecticides under study have different modes of action. Parathion and sevin act upon the cholinesterase system of hormones while the others act differently. All these insecticides, however, appear to exert the same action on the growth and development processes of the larva under sublethal doses.

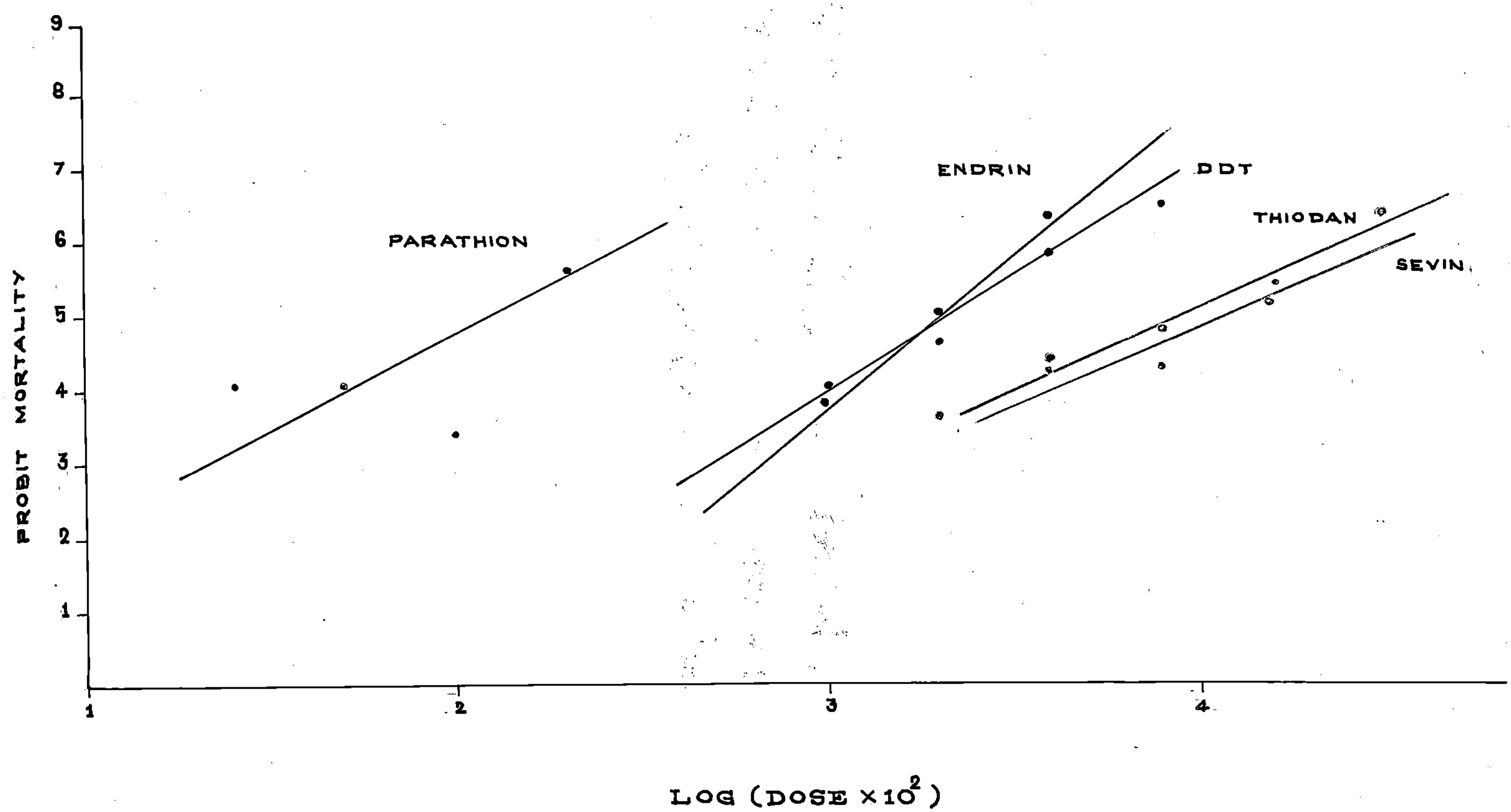
The most spectacular and noteworthy effect manifested by the sublethal doses of the toxicants is on the adult beetles' fecundity. The fecundity of the beetle subjected to the sublethal levels of the insecticides shows a great increase over that of the untreated beetles. Different patterns of this increase in relation to the different sublethal doses are evident with the various insecticides. Thus in the case of DDT, endrin and thiodan the number of eggs laid per female beetle increases as the sublethal dose in the medium increases. With parathion the greatest increase in the fecundity is evidenced in the lowest sublethal dose and it decreases as the toxicant dose is increased. In the case of sevin no definite relationship between increase in fecundity and concentration changes is evident.

The sublethal doses of all the insecticides under test, irrespective of their mode of action, thus appear to stimulate the reproductive processes of the adult beetle. It may be that the hormonal systems governing the reproductive processes and egg production are stimulated by the toxicants at sublethal concentrations.

The results of previous studies reported in literature on the effect of sublethal concentration of insecticides on insect fecundity are contradictory. Thus Tattersfield and Kerridge (1955) found that topical application of DDT to Drosophila at sublethal doses resulted in the reduction of its oviposition rate. Similar observations of the reduction in oviposition was observed in houseflies with DDT by Srivastava and Fine (1967). On the other hand Knutson (1938) observed that dieldrin at submortal doses increased the fecundity and life span of Drosophila. Lakocy (1958) found that the Colorado potato beetle laid more eggs when its 4th instar larvae were dusted with DDT; but the treatment had no effect on the subsequent duration of development of the survivors.

Fig. 16 represents a comparison of the toxicity of the five insecticides under test to Tribolium larvae when exposed to feeding media containing different graded concentrations of sublethal doses of the insecticides. It may be seen that parathion is far more toxic to the larva than the others. Among the rest, DDT and endrin are nearly equitoxic and more toxic than sevin and thiodan which by themselves have the same toxicity.

Fig. 16 Log dose-probit mortality relationship between different sublethal doses of insecticides in the feeding medium and *Tribolium* larvae.



PROBIT MORTALITY
9
8
7
6
5
4
3
2
1
1
2
3
4
PARATHION
ENDRIN
DDT
THIODAN
SEVIN
LOG (DOSE ×10^2)

FIG 16

It is also noteworthy to observe that none of the insecticides under study at their sublethal levels affect the fertility of the insect; no sterilising effect is evident. Thus the viability of the eggs laid by the beetles reared out and confined in sublethal media of the insecticides is not affected in any way. The sex ratio of the beetle also is not affected by the sublethal doses of the toxicants.

SUMMARY

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SUMMARY

The effect of sublethal doses of DDT, endrin, sevin, thiodan and parathion on the larval duration growth and survival, pupal duration and survival, adult fecundity and sex ratio and viability of the eggs, of Tribolium castaneum has been ascertained by rearing it in wheat flour media containing 4 to 5 graded sublethal concentrations of each insecticide.

The developmental period of the larvae of T.castaneum is prolonged as a result of the pressure of sublethal doses of the insecticides contained in the medium; this prolongation is directly proportional to the increase in the concentration of the toxicant within the sublethal range and it is more pronounced in the case of DDT, endrin, sevin and parathion, than thiodan.

The sublethal doses of the insecticides cause reduction in the growth of the larvae and this reduction is proportionate to the graded concentration of the toxicant present in the medium and the inhibition of growth is more spectacular during early part of larval existance than in its later stages.

Fecundity of the beetle subjected to sublethal levels of insecticides shows a great increase over that of untreated beetles. These increases have different patterns with reference to different sublethal levels.

Probit mortality relations between the sublethal doses of insecticides and <u>Tribolium</u> larvae show that parathion is far more toxic to the larvae than the others.

None of the insecticides affects the fertility of the insect, showing that the sterilizing effect is almost nil. Sex ratio of the beetle is also not affected by the sublethal doses of the toxicants.

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