STUDIES ON THE TOXICITY OF SOME INSECTICIDES TO GRUBS OF *Epilachna vigintioctopunctata* FABRICIUS

BY

R. JAYAKUMARI B. Sc.

THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN AGRICULTURE (ENTOMOLOGY) OF THE UNIVERSITY OF KERALA

DIVISION OF ENTOMOLOGY
AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE
VELLAYANI, TRIVANDRUM

1967

CERTIFICATE

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

(C.K.N.Nair),
Principal & Additional
Director of Agriculture
(Research)

(M.R.G.K.Nair),
Professor of Entomology.

Agricultural College &
Research Institute,
Vellayani, Trivandrum,
August, 1967.

ACKNOWLEDGEMENTS

The author desires to record her deep sense of gratitude to Dr. M.R. Gopalakrishnan Nair, M.Sc., Assoc. I.A.R.I., Ph.D., F.E.S.I., Professor of Entomology, Agricultural College and Research Institute, Vellayani, Trivandrum, for the valuable guidance and unspared pains given for the successful conduct and completion of these investigations.

The author is highly grateful to Dr. C.K.N. Nair, M.Sc., Ph.D. (Cornell), D.R.I.P (Oak Ridge), Principal and Additional Director of Agriculture (Research) for kindly providing facilities for carrying out the investigations.

Sincere thanks are also due to Shri. K.V. Mammen, M.Sc.(Ag.), Lecturer in Entomology, for the help rendered during the execution of this work and the preparation of the thesis.

The author wishes to express her sincere gratitude to Shri.E.J. Thomas, M.Sc., M.S. (Iowa), for his valuable help in the statistical analysis and interpretation of the data.

(JAYA KUMARI. R)

C O N T E N T S

INTRODUCTION

INTRODUCTION

The Epilachna beetle, *Epilachna vigintioctopunctata* is a serious pest of solanaceous and cucurbitaceous vegetable crops. Though both the adults and the grubs of the beetle damage the leaves of these crops the grubs are far more destructive than the adults, they being persistent pests on the plants. It is often the grubs which are encountered on the plants and these remain on the lower surface of the leaves scraping the green matter. The insecticide applications are usually aimed more against the grubs than against the adults.

That the different instars of an insect show different susceptibilities to insecticidal action is well known (see the Review of Literature). But information on the relative susceptibility of the different instars of crops pests to contact toxicants is meagre. The only studies done in this field in India are those of Mukerjee (1953) on *Diatarexia oleracea*, *Tenebrio molitor* and *Periplaneta americana* and Pradhan and Bindra (1953, 1957) on *Schistocerca gregaria*. Hence in the present investigations studies have been made to understand the susceptibility of the different instars of the grub of *E. vigintioctopunctata* to the toxic action of some insecticides. Apart from the scientific interest of these studies involving the stage susceptibility as a factor of insecticidal action, the results of the studies were considered to be of value in the practical field of the control of the pest.

The grubs of E. vigintioctopunctata habit the under surface of the leaves and this may reduce the effectiveness of insecticide applications as the insecticides fall more on the upper surface than on the lower surface. The grub feeds in such a way that the upper epidermis of the leaf is left intact and hence the insecticide has to penetrate the leaf tissues for reaching the insect. It is known how far the different insecticides can thus penetrate the leaves. A few trials have been undertaken to s tudy this also.

The literature relating to the relative susceptibility of different instars of insects to insecticidal action has been reviewed.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

A perusal of literature reveals that the different developmental stages of insects vary considerably in their susceptibility to insecticides. This has been found to be the case with all insecticides irrespective of their modes of actions, viz. stomach poisons contact poisons and fumigants.

Jaubert (1927) observed that the adults and larvae of Galleria mellonella were killed by using chloropicrin, but that the eggs could be killed only by using a mixture of chloropicrin and carbontetrachloride, in the ratio of 1:9 where carbontetrachloride might be acting as a fat solvent.

Pepper and Hastings (1944) found that application of pyrethrum dusts and sprays were very effective against the first and second instar larvae of Lozostege sticticalis L., but less so against the fifth. Analysis of the exoskeletons of larvae in the third, fourth and fifth instars showed a decrease in fat contents of the exoskeleton from 11.7 to 3.9 and 0.2 per cent in the successive instars, accompanied by an increase in the percentage of protein and to a less extent of chitin and ash.

Eaton and Davies (1951) studying the acaricidal activity of 90 synthetic organic compounds found that considerable difference were found between the winter eggs, the summer eggs and the adult females of the fruit-tree red-spider mite, Paratetranychus pilosus (C. and F.) in their susceptibility to the acaricides. But both the compounds azoxy benzene and n-dedocyl thiocyanate were appreciably toxic to all the stages.

Dennis (1952) carried out experiments to study the comparative effect of mixtures of the fumigants carbon-tetrachloride and carbondisulphide (80:20) and carbon-tetrachloride, carbondisulphide and sulfurdioxide (78:20:2) against Calandra oryzae (L) and Tribolium confusum Duv. in empty and wheat filled containers. The addition of SO_2 thus increased the toxicity of the mixture to both species.

Parkin (1953) found that in general the larvae of Lasioderma serricorne (F) and Trogoderma granarium Everts were more resistant than their adults to 50% DDT dust in talc.

Mukerjee (1953) observed that DDT at 0.075 per cent w/v failed to prevent the development of the embryos of Diatarexia oleracea, death occurring only when the fully developed embryo began to gnaw its way out of the shell. Pyrethrins on the other hand prevented embryonic development if applied at sufficiently high concentrations. With both insecticides, resistance of eggs decreased with age, but that of the larvae of _D. oleracea_ and _Tenebrio molitor_ and nymphs of _Periplaneta americana_ increased, the resistance being markedly higher in the later instars. Neither DDT nor pyrethrins prevented development of the pupae even at the highest concentrations used (0.5 and 2.5 per cent. w/v respectively). Both, however, prevented normal emergence of _T. molitor_ and pyrethrins that of _D. oleracea_. In each case, pupae of middle age were more resistant than younger or older ones. Adults of _T. molitor_ aged 4 weeks were more resistant to both insecticides than those aged 1 or 8 weeks, but adults of _P. americana_ were more resistant when 1-4 weeks than when 12-24 weeks old.

Pradhan and Bindra (1953) studied the relationship between changes in susceptibility and various factors relating to the successive instars of the desert locust. The increased resistance observed in the successive instars of the pest was considered to be due to the increase in the thickness of the cuticle, or its compactness or both.

These changes affected the entry of insecticides in the different instars.

David and Gardiner (1954) tested the effect of schradan, paraoxon, bis (dimethyl-amino) fluorophosphine oxide and sodium fluoroacetate (the last two being referred to as the oxide and acetate respectively) against eggs and larvae of Pieris brassicae (L.) on cabbage of brussels sprouts in the green house. Schradan proved to have very little toxic action on eggs or larvae, but the other three compounds showed both contact toxicity in dips and systemic action when taken up by the roots from solution and from soil. The order of decreasing toxicity was paraoxon, acetate, oxide, schradan. Paraoxon proved more toxic to the third-instar larvae than to aphids and though it did not prevent the eggs from developing, it killed the larvae in the act of hatching.

Ebeling and Pence (1955) determined the LD 50's of sixteen acaricides used as wettable powders, emulsifiable concentrates, or in both formulations, against the two-spotted spidermite on bean leaves. The susceptibility of the different developmental stages of the mite varied with the different groups of acaricides. With aramite and the organic phosphates (parathion, malathion, EPN, diazinon and demeton) the LD 50's for the larvae were higher than those for the adults. In the case of chlorobenzilate, DMC, R-242 and compound 876, the LD 50's for larvae and adults were similar. With neotran, ovotran compound 923, karathane, DN-111 and DN-289, the larvae were found to be more susceptible than the adults. Among the latter group, neotran, ovotran and compound 923 showed lower LD 50's against the eggs than against the adults. With all other acaricides, however, the eggs were by far the most resistant stage. The ratio of the LD 50's for eggs to those for adults were particularly high with organic phosphates and aramite, but this was due in part to the extremely low concentrations required to kill the adults.

Bioassy studies on desert locust by Pradhan and Bindra (1957) revealed that the median lethal concentrations were about doubled for each successive instar from the first to fifth, the older stage being more resistant than the younger ones. Detailed studies on the morphology and physiology of the cuticle indicated that effectiveness varied directly with the surface area per unit body weight and the percentage of wax in the exuviae and inversely with the thickness or compactness of the cuticle. The index estimated by dividing the average weight of exuvium by its average surface area increased from the first to the fifth instar, but the probable thickness of the wax layer did not show any regular gradient. The percentage of total protein and also that of certain cuticular content slowly increased from the first to the fifth instar. The chitin and water soluble content did not show any regular gradient. The surface area per unit body weight was the highest (4.72) in the first instar and decreased slowly to 1.04 in the fifth instar.

Langenbuch (1958) have an account of the differing susceptibility of third and fourth-instar larvae of Leptinotarsa decemlineata to DDT, when larvae were allowed to feed on leaf sandwiches containing DDT in order to avoid its contact action, or exposed after starvation to a DDT solution on filter paper in petri dishes, the older larvae proved much less susceptible to the poison than the younger ones.

Adkisson (1959) observed the relative susceptibility of the different life stages of the rice weevil Calandra oryzae to certain fumigants. Assessment of the LC 50's for carbontetrachloride indicated that the eggs, fourth instar larvae and pupae were the most and the free adults the least resistant and that the third instar larvae were less resistant than the newly emerged adults that had not yet left the grains. The slopes of the curves indicated that the first and second larval instars were the most sensitive to changes in concentrations. The LC 50's for ethylenedichloride showed that the third instar larvae were the most resistant, followed by the eggs and the first and second instar larvae, which among themselves were equally susceptible. The adults, pupae, fourth instar larvae and the newly emerged adults were the least resistant, with no differences between them. The slopes of the curves suggested that the eggs and the larvae in the first, second and third instars, were more sensitive to changes in concentration than the later stages. Ethylene dichloride was considerably more toxic than carbon tetrachloride to all stages of the weevil.

Krohne and Lindgren (1959) conducted trials with laboratory reared eggs, larvae pupae and adults of <u>Calendra oryzae</u>. Samples of wheat containing the different stages of the insect were fumigated at different rates for two hours at 80°F, the resulting LD 95's showed that the pupae were less and the larvae more susceptible than the adults to acrylonitrile, carbonbisulphide, chloropicrin, ethylene dibromide, hydrogen cyanide and methyl bromide. The eggs appeared to be relatively more susceptible to acrylonitrile, ethylene dibromide and hydrogen cyanide, than to carbonbisulphide and relatively more resistant to methyl bromide. Ethylene dichloride was much less effective than the other fumigants. From these results they found that at LD 95, acrylonitrile and ethylene dibromide were the most toxic to all stages of C. oryzae, followed by hydrogen cyanide, methyl bromide and chloropicrin; carbonbisulphide and ethylene dichloride were the least toxic.

Pedersen (1961) studied the susceptibility of different stages of rice weevil in wheat of various moisture content to a mixture of carbon tetrachloride and methallyl chloride (4:1) when exposed for 48 hours at 80° F. The dosage necessary for a given kill of any stages increased as the moisture content of the wheat was increased. Comparison of the LC 50's calculated from the mortality after 10 days showed that the third instar larvae was less and the pupa and adult more susceptible than the egg and the first instar larva at 12.5 per cent moisture content, whereas susceptibility increased progressively from the third instar to the first instar, pupa egg and adult at 14 per cent. The LC 90's were estimated graphically and they showed that the first instar, pupa and egg were more susceptible than the third instar and less so than the adult at 12.5 per cent moisture content and that susceptibility increased progressively from the third instar to the pupa, first instar, egg and adult at 14 per cent.

Benschoter (1961) showed that when naked eggs, larvae and pupa of Anastrepa ludens (Lw.) were fumigated with ethylene chlorobromide (1-bromo-2 chloroethane) at 73.5 to 78°F at different concentrations, the eggs were less susceptible to increased dosage than the other stages, so that the order of susceptibility varied at different mortality levels. At LD 99, the larvae were the most susceptible followed by the adult, egg and pupa.

Abou El Ghar (1964) conducted studies to test the effect of certain insecticides against cotton leaf worm Prodenia litura F. with a view to assess its ovicidal action, response of the different instars to the tested insecticides, knockdown effects on the different instars and timing of application in the field. He found that all the tested insecticides had a weak ovicidal action. The effect of insecticides on different instars indicated that older instars were generally more resistant to the insecticides than the newly hatched larvae. Of the tested insecticides, Dipterex was outstanding for its strong knockdown action against young and old instars. The timing of application of the insecticides was found to be most effective at the initial stage of development of the larvae.

MATERIALS AND METHODS

MATERIALS AND METHODS

Materials

Insecticides: Details of the insecticides used in the studies are given below:

DDT: A technical grade containing about 77% of the active ingredient, supplied by M/s Bharat Pulversing Mills (Private) Limited, Bombay and 20% E.C. supplied by M/s Mysore Insecticides, Madras.

Malathion: A technical grade and a 50% E.C. supplied by M/s Cyanamid India Limited, Bombay.

Parathion: A 100% technical grade supplied by M/s Bayer (India) Limited, Bombay.

Sevin: A commercial water wettable powder containing 50% of the active ingredient manufactured by Union Carbide, India Limited.

BHC: A technical grade supplied by M/s Tata Fison (Limited) Bombay.

Diazinon: A commercial emulsifiable concentrate called Basudin containing 20% of the active ingredient manufactured by Tata Fison Limited, Bombay

Phosphamidon: A commercial water miscible concentrate containing 100% of the active ingredient called dimecron, manufactured by CIBA Pesticides, India Limited, Bombay.

Thiometon: A commercial emulsifiable concentrate containing 100% of the active ingredient called ekatin, supplied by Sandoz (India) Limited, Bombay.

Formothion: A commercial emulsifiable concentrate containing 25% of the active ingredient called anthio supplied by Sandoz (India) Limited, Bombay.

Dipterex: A commercial water wettable powder containing 80% of the active ingredient, manufactured by Bayer (India) Limited, Bombay.

Endrin: A technical grade supplied by Tata Fison (Limited) Bombay.

Emulsifier: Triton x 100 and Triton x 172 supplied by Indofil Chemicals Limited, Bombay were used as emulsifiers in the preparation of emulsions from Technical materials.

Benzene: Benzene supplied by M/s Quality Product, Bombay-2, was used as a solvent in the preparation of emulsions from technical materials.

Glass wares used:

(a) A pipette of 1 c.c. capacity.

(b) Measuring cylinders of 10 c.c. and 100 c.c. capacities.

(c) Glass stoppered bottles.

(d) Petri dishes (9.5 cm. diameter)

(e) Chimneys of length 15 cm. and diameter 5 cm. open at both ends.

(f) Rectangular jars - 26 x 16 x 9 cms.

(g) Circular troughs - 26 x 10.5 cms.

Test insects: First, second and third instar larvae of *E. vigintioctopunctata* required for the experiment were reared in the laboratory on fresh leaves of bitter gourd. Two days old larvae were used in all the experiments.

Methods.

Rearing of *E. vigintioctopunctata*: Adults of the *Epilachna* beetles were reared in rectangular jars and circular troughs and fed on fresh leaves of bitter gourd.

When the adults laid eggs they were collected and transferred into chimneys placed on petri dishes and closed with muslin cloth. Grubs emerging each day were kept in separate chimneys with the date of emergence noted. Each day the cages were examined to ascertain the instar of the grubs and the dates of moults were noted.

Preparation of insecticide formulations:

For studies on contact toxicity to grubs of *E. vigintioctopunctata*:

All the insecticides were used as sprays in these studies. The spray fluids were used as emulsions formulated in the laboratory from technical grades except in the case of sevin which was used as suspension prepared from wettable powder.

Five graded concentrations of each insecticide were used for the experiment. In the case of technical materials, solution in benzene was prepared in each case, from which lower concentrations of emulsions were obtained by mixing with water containing 0.625 per cent of the emulsifier, trition x 100 or triton x 172. The proportions of the solutions, emulsifier and water in the final formulation were so adjusted that the percentage of benzene and emulsifier in all the dilutions were kept constant at 5 per cent and 0.625 per cent respectively. In the case of sevin the wettable powder was suspended in water to give the highest concentration and this suspension was further diluted with water to give the required lower concentrations. Details of the proportions of the different constituents used for the preparation of the different concentrations of each insecticide are given below:

1. <u>DDT (Tech.)</u>

0.4 gm. DDT (Tech.) + 10 c.c. Benzene	4% solution	(S)
1 c.c. S + 19 c.c. E.W.	0.2 emulsion	(T_1)
½ c.c. S + ½ c.c. benzene + 19 c.c.E.W.	0.1% "	(T_2)
¼ c.c. S + ¾ c.c. benzene + 19 c.c.E.W.	0.05 "	(T_3)
¼ c.c. S + 1¾ c.c.benzene + 38 c.c.E.W.	0.025% "	(T_4)
¼ c.c. S + 3¾ c.c.benzene + 76 c.c.E.W	0.0125% "	(T_5)

Control:

1 c.c. benzene + 19 c.c. E.W.	5% benzene emulsion	(T_0)

(E.W. = Emulsifying water ctontaining 0.625% Triton x 100)

2. **Malathion** (Tech.)

0.2 gm. Malathion (Tech.)+10 c.c. benzene	2% soln.	(S)
1 c.c. S + 19 c.c. E.W.	0.1% emulsion	(T_1)
½ c.c. S + ½ c.c. benzene + 19 c.c.E.W.	0.05% emulsion	(T_2)
¼ c.c. S + ¾ c.c. benzene + 19 c.c.E.W.	0.025% "	(T_3)
¼ c.c. S + 1¾ c.c.benzene + 38 c.c.E.W.	0.0125% "	(T_4)
¼ c.c. S + 3¾ c.c.benzene + 76 c.c.E.W.	0.00625% "	(T_5)

Control:

1 c.c. benzene + 19 c.c.E.W.	5% benzene emulsion	(T_0)

3. **Parathion** (Tech.)

0.1 gm. Parathion (Tech.) + 10 c.c. benzene	1% soln.	(S)
2 c.c. S + 3 c.c. benzene + 95 c.c. E.W.	0.02% emulsion	(T_1)
1 c.c. S + 4 c.c. benzene + 95 c.c. E.W.	0.01% "	(T_2)
½ c.c. S + 4½ c.c. benzene + 95 c.c.E.W.	0.005% "	(T_3)
¼ c.c. S + 4¾ c.c. benzene + 95 c.c.E.W.	0.0025% "	(T_4)

¼ c.c. S + 9¾ c.c. benzene + 190 c.c.E.W.	0.00125% "	(T_5)
¼ c.c. S + 19¾ c.c. benzene + 380 c.c.E.W.	0.000625% "	(T_6)
¼ c.c. S + 38¾ c.c. benzene + 760 c.c.E.W.	0.0003125% "	(T_7)

Control:

1 c.c. benzene + 19 c.c. E.W.	5% benzene emulsion	(T_0)

4. Sevin (w.p.)50%

1 gm. sevin (50% w.p) + 999 c.c. water	0.05% suspension	(S_1)
10 c.c. S_1 + 10 c.c. water	0.025% "	(S_2)
10 c.c. S_2 + 10 c.c. water	0.0125% "	(T_1)
10 c.c. T_1 + 10 c.c. water	0.00625% "	(T_2)
10 c.c. T_2 + 10 c.c. water	0.003125% "	(T_3)
10 c.c. T_3 + 10 c.c. water	0.0015625% "	(T_4)
10 c.c. T_4 + 10 c.c. water	0.00078125% "	(T_5)
10 c.c. T_5 + 10 c.c. water	0.000390625% "	(T_6)

5. BHC (Tech.)

0.4 gm. BHC (Tech.) + 10 c.c. benzene	4% solution	(S)

1. c.c. S + 19 c.c.E.W.	0.2% emulsion	(T_1)
½ c.c. S + ½ c.c. benzene + 19 c.c. E.W.	0.1% "	(T_2)
¼ c.c. S + ¾ c.c. benzene + 19 c.c. E.W.	0.05% "	(T_3)
¼ c.c. S + 1¾ c.c. benzene + 38 c.c.E.W.	0.025% "	(T_4)
¼ c.c. S + 3¾ c.c. benzene + 76 c.c.E.W.	0.0125% "	(T_5)

Control:

1 c.c. benzene + 19 c.c.E.W.	5% benzene emulsion	(T_0)

For studies on stomach toxicity:

1. **Malathion:**

1 c.c. Malathion 50% E.C. + 499 c.c. water 0.1% emulsion

2. **DDT:**

1 c.c. DDT 25% E.C. + 124 c.c. water 0.2% emulsion.

3. **Diazinon:**

1 c.c. Basudin 50% E.C. + 499 c.c. water 0.1% emulsion.

4. **Phosphamidon:**

0.5 c.c. Phosphamidon 100% E.C. + 1249.5 c.c. water 0.04% emulsion.

5. **Sevin:**

1 gm. sevin (50% w.p.) + 499 c.c. water 0.05% suspension (S_1)

10 c.c. S_1 + 10 c.c. water	0.025% suspension	(S_2)
10 c.c. S_2 + 10 c.c. water	0.0125% "	

6 Parathion: (Tech.)

0.1 gm. Parathion (Tech.) + 10 c.c. benzene	1% soln.	(S)
1 c.c. S + 19 c.c. E.W.	0.05% emulsion.	

7. BHC (Tech.):

0.4 gm. BHC (Tech.) + 10 c.c. benzene	4% soln	(S)
1 c.c. S + 19 c.c. E.W.	0.2% emulsion	

8. Thiometion:

1 c.c. Thiometon 100% E.C. + 999 c.c. water.	0.1% emulsion

9. Formothion:

1 c.c. formothion 25% E.C. + 999 c.c. water	0.025% emulsion

10. Dipterex:

0.5 gm. Dipterex (80% w.p) + 399.5.c.c. water	0.1% suspension

11. Endrin:

0.3 gm. Endrin (Tech.) + 10 c.c. benzene	3% soln.	(S)
1 c.c. S + 4 c.c. benzene + 95 c.c.E.W	0.03% emulsion.	

Spraying the larvae: The larvae to be sprayed were placed on blotting paper in a clean petri dish. They were then sprayed under Potter's tower using 1 c.c. of the emulsion per spraying. After the spray fluid had dried up the grubs were transferred to fresh petri dishes and supplied with fresh food. A control was kept in each case which was sprayed with water only in the case of wettable powder. In the case of technical grades the control was a 5 per cent benzene emulsion. Each treatment including control was replicated thrice.

Experimental conditions: All the experiments were conducted in the laboratory under laboratory conditions of temperature and humidity. The temperature and humidity during the experimental period were noted.

Assessment of the effect of the various insecticides: The lethal effect of the insecticides on the grubs was assessed by counting the number of dead grubs observed at the end of 24 hours and 48 hours after spraying.

Determination of stomach toxicity of insecticides to grubs of *E. vigintioctopunctata*:

For this, fresh bitter gourd leaves were collected with the leaf stalks intact and they were prevented from withering by keeping them with their leaf stalks dipped in water. Each insecticide emulsion or suspension, as the case may be, was sprayed on the upper surface of these fresh bitter gourd leaves, each with 1 c.c. of the spray fluid, the spraying being done under a Potter's tower. The leaf was placed on a blotting paper before being sprayed, to prevent any spray droplets running under the leaf and contaminating the lower surface of the leaf. Soon after spraying, the leaf was kept exposed to air for the spray fluid to dry up. The leaf was then kept with its leaf stalk dipped in water for one hour. The sprayed leaf was then spread out in a petri dish with its lower surface (unsprayed surface) facing up. The leaf was thus glued on to the petri dish with gum. Ten second instar grubs were then placed on each leaf and the dish closed with its lid. Mortality counts were taken 24 hours after the grubs were allowed to feed on the leaf.

This method is considered to have eliminated the contact action of the insecticides on the grubs as the insecticides are confined to the upper surface of the leaves only, with which surface the grubs do not come in contact. At the same time the insecticides which might have pentrated the epidermis and reached the bulk tissue are taken directly into the stomach of the grubs when they fed upon this bulk tissue. Thus the grubs in this experiment show only the stomach effect of the insecticides.

EXPERIMENTAL DETAILS AND RESULTS

EXPERIMENTAL DETAILS AND RESULTS

Experiment I

Dosage mortality relationship between DDT and the different larval instars of *Epilachna vigintioctopunctata*

Experimental details

Date of experiment:	1st instar larva	29--11--1966
	2nd instar larva	8--11--1966
	3rd instar larva	15--11--1966
Doses of DDT used:	0.0125, 0.025, 0.05, 0.1 and 0.2 per cent.	
Control:	unsprayed.	
Test insects:	First, Second and Third instar larvae of *Epilachna* sp. were used. See under 'Material' for further details.	
Number of replications:	3	
Number of insects in each replication:	10	
Temperature during experiment:	86.6°F - 78.6°F	
Humidity during experiment:	80.1%	

Procedure: The larvae were sprayed with the different concentrations under the Potter's Tower and supplied with fresh food material. Results were assessed by observing the mortality among the larvae at 24 and 48 hours after treatment. For full details see under 'Methods'.

Results: Results are given in Table 1 and represented in Fig. 1 and 2.

The figures represent the results on the log dosage probit mortality scale. It may be seen that at 24 hours the third instar larva appears as more susceptible to DDT than the first and second instars. Among the latter two, the 1st instar is more susceptible than the 2nd. At 48 hours, however, the relative susceptibility is highest in the 3rd instar followed by 2nd and 1st instars.

Experiment II

Dosage mortality relationship between Malathion and the different larval instars of *E. vigintioctopunctata*.

Experimental details

Date of experiment	1st instar	14--12--1966
	2nd instar	21--12--1966
	3rd instar	21--12--1966

Doses of malathion used: 0.00625, 0.0125, 0.025, 0.05 and 0.1 per cent.

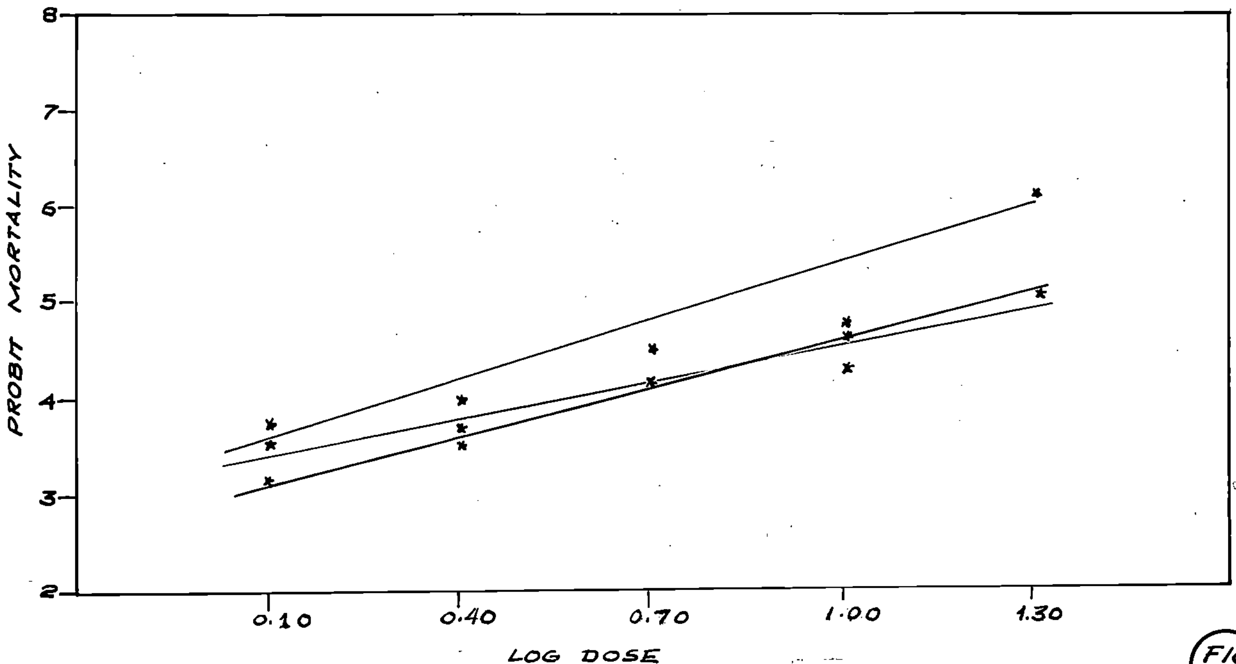
Table 1

Dosage mortality relationship between DDT and different larval instars of *E. vigintioctopunctata*

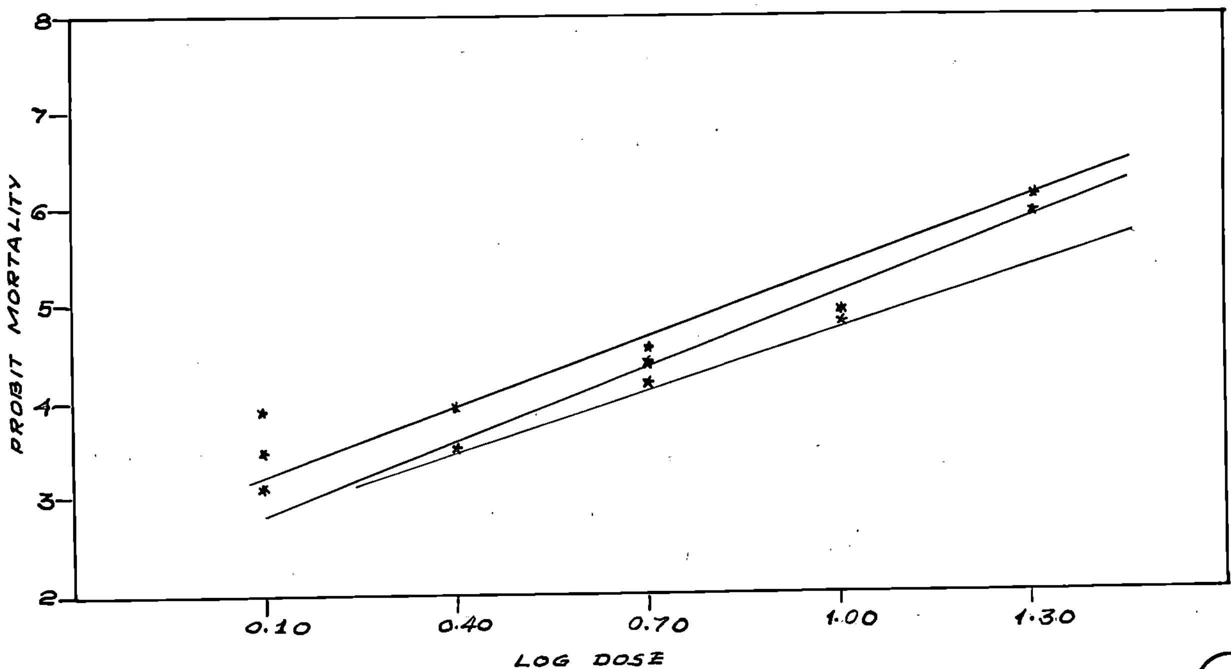
Dose %	Log dose	Instars	Mortality at 24 hours %	Mortality at 24 hours Probit	Mortality at 48 hours %	Mortality at 48 hours Probit
0.2	1.30	1	100	..	100	..
		2	53.3	5.08	80	5.84
		3	86.7	6.11	86.7	6.11
0.1	1.00	1	40	4.75	40	4.75
		2	23.3	4.27	40	4.75
		3	36.7	4.66	43.3	4.83
0.05	0.70	1	20	4.16	20	4.16
		2	20	4.16	26.7	4.38
		3	30	4.48	30	4.48
0.025	0.40	1	6.7	3.50	6.7	3.50
		2	10	3.72	13.3	3.89
		3	13.3	3.89	13.3	3.89
0.0125	0.10	1	3.3	3.16	3.3	3.16
		2	6.7	3.50	6.7	3.50
		3	10	3.72	13.3	3.89

Fig.1. Log Dose - Probit Mortality relationship between DDT and different larval instars of _E. vigintioctopunctata_ 24 hours after spraying.

Fig.2. Log Dose - Probit Mortality relationship between DDT and different larval instars of _E. vigintioctopunctata_ 48 hours after spraying.



DDT 24 HOURS FIG 1



DDT 48 HOURS FIG 2

— 1st INSTAR LARVA. — 2nd INSTAR LARVA. — 3rd INSTAR LARVA.

Temperature during experiment: 86.0°F - 76.5°F

Humidity during experiment: 87.6%

Other details as in Experiment I

Results: Results are given in Table 2 and represented in Figs. 3 and 4.

At 24 hours., the third instar larva appears to be more susceptible than the first and second instars. Among the latter two, the first instar is considerably more susceptible than the second. At 48 hours also the third instar is more susceptible than the rest. The first and second instars appear to show equal susceptibility.

Experiment III

Dosage mortality relationship between Parathion and the different larval instars of E. vigintioctopunctata

Experimental details

Date of experiment: First instar 31--1--1967

Second instar 5--1--1967

Third instar 6--1--1967

Doses of Parathion used: 0.0003125, 0.000625, 0.00125, 0.0025, 0.005, 0.01 and 0.02 per cent.

Temperature during experiment: 87.3°F - 77.9°F.

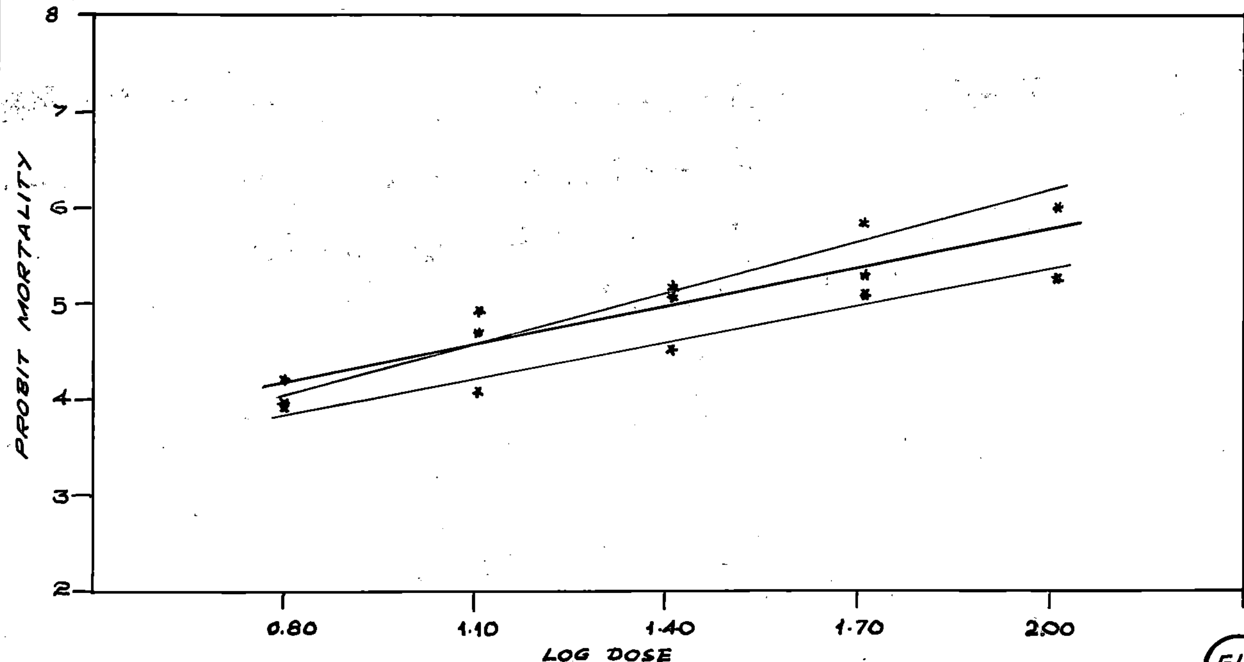
Table 2

Dosage mortality relationship between malathion and different larval instars of *E. vigintioctopunctata*.

Dose %	Log dose	Instars	Mortality at 24 hours %	Mortality at 24 hours Probit	Mortality at 48 hours %	Mortality at 48 hours Probit
0.1	2.00	1	100	..	100	..
		2	60	5.25	96.7	6.84
		3	83.3	5.97	90.0	6.28
0.05	1.70	1	60	5.25	63.3	5.34
		2	53.3	5.08	70	5.52
		3	80	5.84	83.3	5.97
0.025	1.40	1	56.7	5.17	63.3	5.34
		2	30	4.48	63.3	5.34
		3	50	5.00	60	5.25
0.0125	1.10	1	46.7	4.92	46.7	4.92
		2	16.7	4.03	23.3	4.27
		3	36.7	4.66	53.3	5.08
0.00625	0.80	1	13.3	3.89	20	4.16
		2	13.3	3.89	20	4.16
		3	20	4.16	36.7	4.66

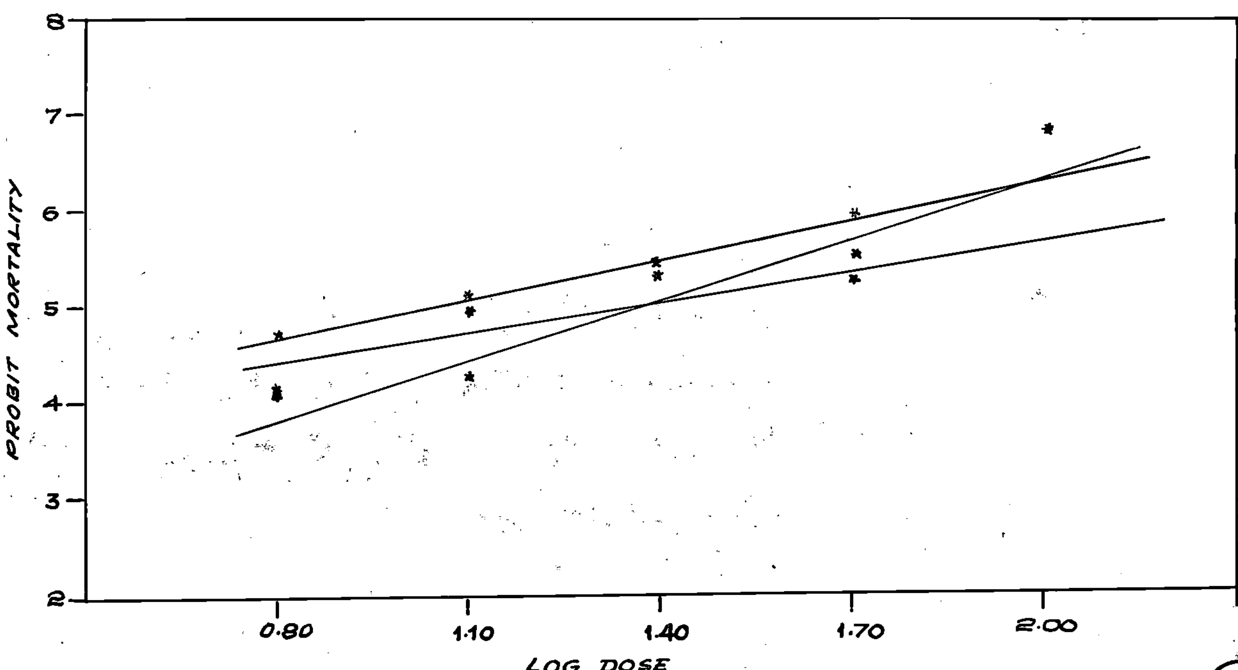
Fig.3. Log Dose - Probit Mortality relationship between malathion and different larval instars of *E. vigintioctopunctata* 24 hours after spraying.

Fig.4. Log Dose - Probit Mortality relationship between malathion and different larval instars of *E. vigintioctopunctata* 48 hours after spraying.



MALATHION 24 HOURS

FIG 3



MALATHION 48 HOURS

FIG 4

— 1st INSTAR LARVA. — 2nd INSTAR LARVA. — 3rd INSTAR LARVA.

Humidity during experiment: 85.3%

Other details as in Experiment I

Results: Results are given in Table 3 and represented in Figs. 5 and 6.

It may be seen that at 24 hours the insecticide appears to be considerably more toxic to the first instar larvae than to the second and third instars. To the second and third instars parathion is seen to be equitoxic. At 48 hours also the first instar shows the highest susceptibility to the insecticide followed by second and third instars in the descending order.

Experiment IV

Dosage mortality relationship between sevin and the different larval instars

E. vigintioctopunctata

Experimental details

Date of experiment:	First instar	Second instar	Third instar
	1--2--'67	25--1--'67	25--1--'67.

Doses of sevin used: 0.00039, 0.00078, 0.00156, 0.003, 0.00625 and 0.0125 per cent.

Temperature during experiment: 87.2°F - 77.7°F.

Humidity during experiment: 83.7%.

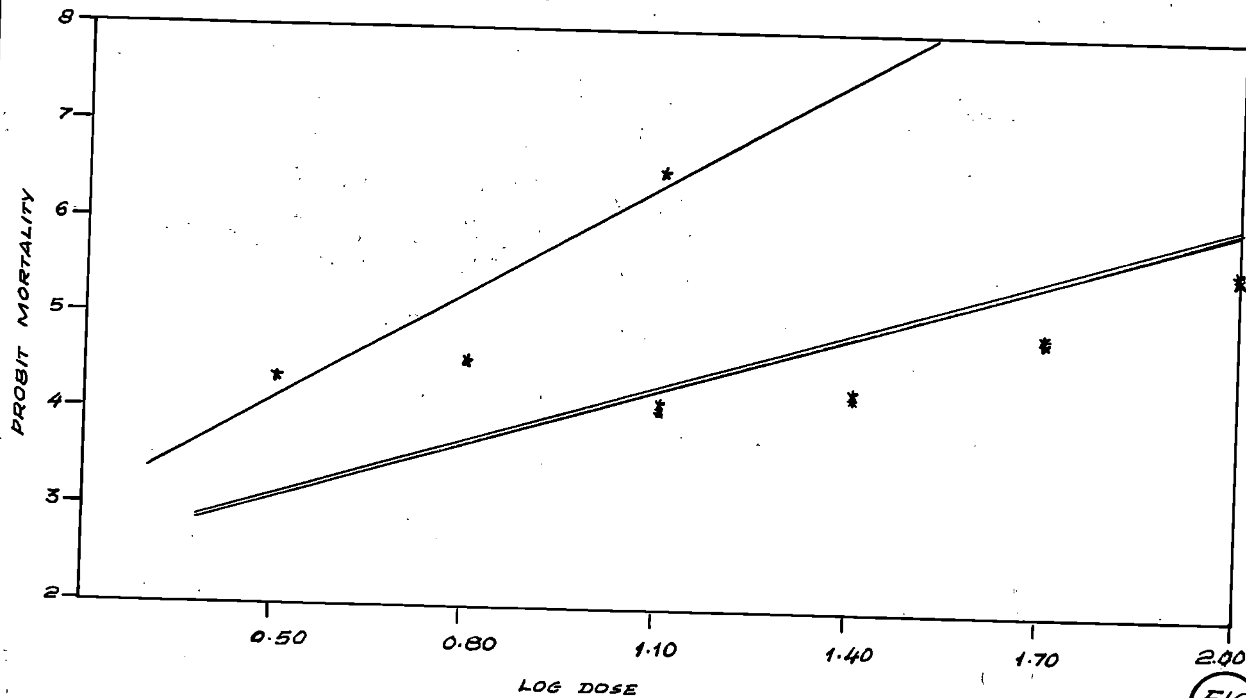
Table 3

Dosage mortality relationship between parathion and different larval instars of *E. vigintioctopunctata*

Dose %	Log dose	Instar	Mortality at 24 hours %	Probit	Mortality at 48 hours %	Probit
		2	93.3	6.50	100	..
0.02	2.30	3	93.3	6.50	100	..
		2	70	5.52	86.7	6.11
0.01	2.00	3	70	5.52	83.3	5.97
		1	100	..	100	..
0.005	1.70	2	43.3	4.83	83.3	5.97
		3	43.3	4.83	46.7	4.92
		1	100	..	100	..
0.0025	1.40	2	23.3	4.27	50	5.00
		3	23.3	4.27	43.3	4.83
		1	93.3	6.50	96.7	6.84
0.00125	1.10	2	20	4.16	36.7	4.66
		3	20	4.16	30	4.48
0.000625	0.80	1	30	4.48	53.3	5.08
0.0003125	0.50	1	26.6	4.38	50	5.00

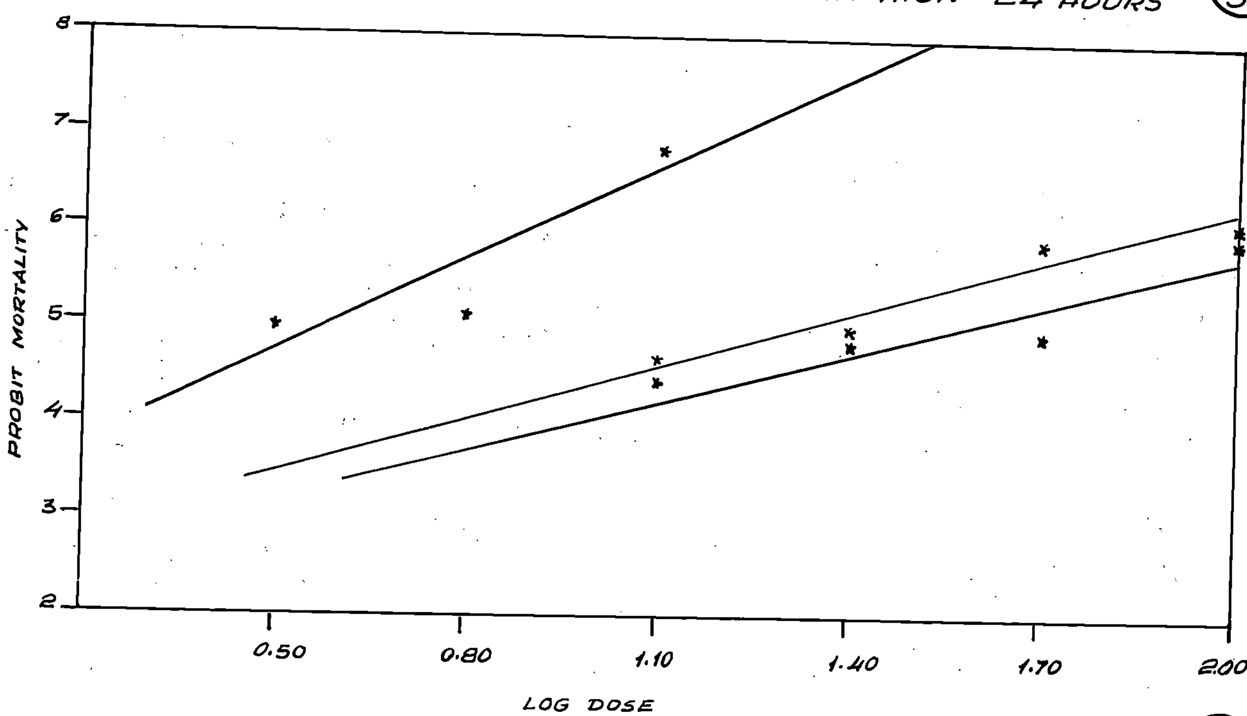
Fig.5. Log Dose - Probit Mortality relationship between parathion and different larval instars of E. vigintioctopunctata 24 hours after spraying.

Fig.6. Log Dose - Probit Mortality relationship between parathion and different larval instars of E. vigintioctopunctata 48 hours after spraying.



PARATHION 24 HOURS

FIG 5



PARATHION 48 HOURS

FIG 6

1st INSTAR LARVA. 2nd INSTAR LARVA. 3rd INSTAR LARVA.

Other details as in experiment: I

Results: Results are given in Table 4 and represented in Figs. 7 and 8.

Both at 24 hours and at 48 hours the third instar larva appears to be the most susceptible to sevin followed by the first and the second instars. The latter two instars appear to show equal susceptibility to the insecticide.

Experiment V

Dosage mortality relationship between B.H.C. and the different larval instars of *E. vigintioctopunctata*

Experimental details:	First instar	Second instar	Third instar
Date of experiment	23--2--'67	8--2--'67	8--2--'67.

Doses of B.H.C. used: 0.0125, 0.025, 0.05, 0.1 and 0.2 per cent.

Temperature during experiment: 87.7°F - 75.4°F

Humidity during experiment: 84.4%

Other details as in experiment: I

Results:

Results are given in Table 5 and represented in Fig. 9 and 10.

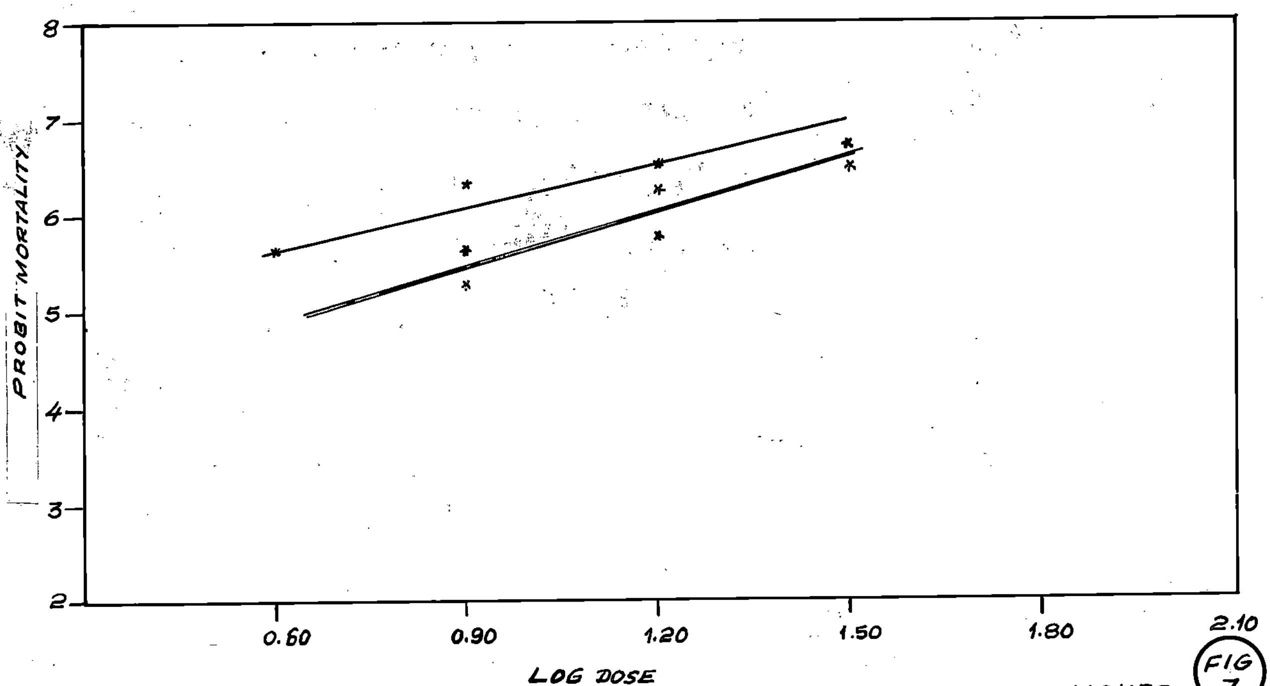
Table 4

Dosage mortality relationship between sevin and different larval instars of *E. vigintioctopunctata*.

Dose %	Log dose	Instars	Mortality at 24 hours %	Mortality at 24 hours Probit	Mortality at 48 hours %	Mortality at 48 hours Probit
0.0125	2.10	1	100	..	100	..
		2	100	..	100	..
		3	100	..	100	..
0.00625	1.80	1	100	..	100	..
		2	100	..	100	..
		3	100	..	100	..
0.003	1.50	1	96.7	6.84	100	..
		2	96.7	6.84	96.7	6.84
		3	93.3	6.50	96.7	6.84
0.00156	1.20	1	93.3	6.50	96.7	6.84
		2	76.7	5.73	83.3	5.97
		3	90	6.28	90	6.28
0.00078	0.90	1	90	6.28	93.3	6.50
		2	73.3	5.62	83.3	5.97
		3	60	5.25	66.7	5.43
0.00039	0.60	1	73.3	5.62	86.7	6.11

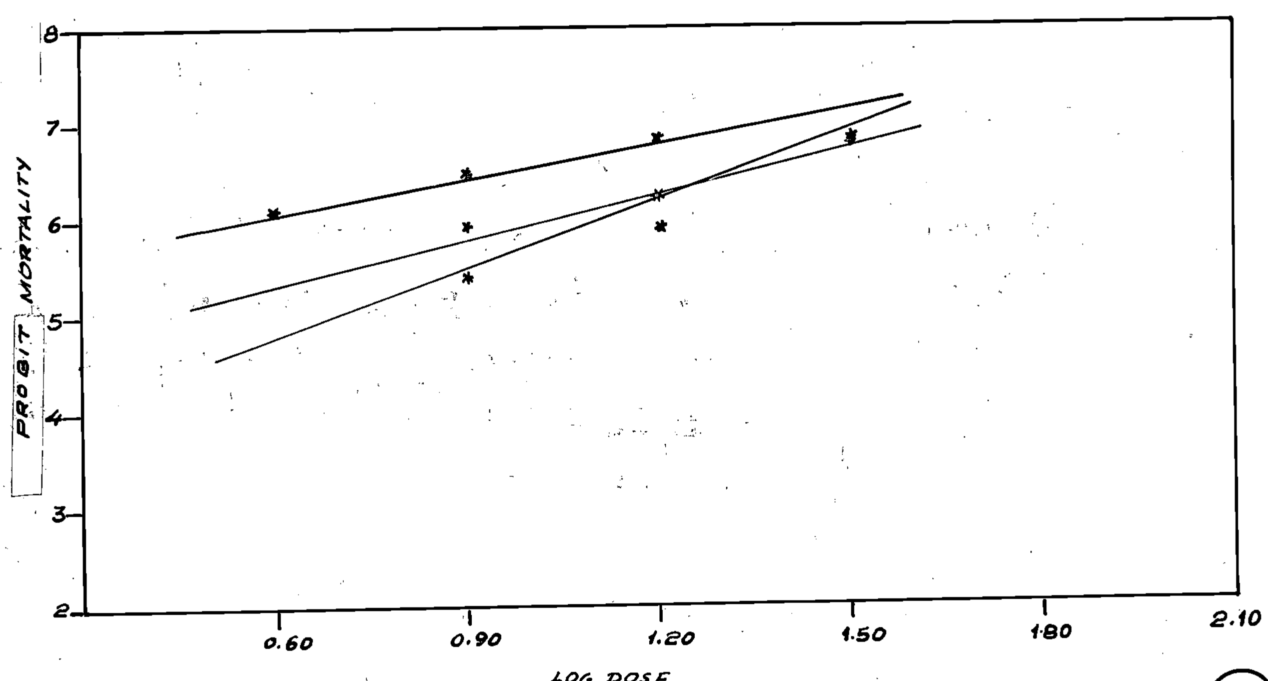
Fig.7. Log Dose - Probit Mortality relationship between sevin and different larval instar of *E. vigintioctopunctata* 24 hours after spraying.

Fig.8. Log Dose - Probit Mortality relationship between sevin and different larval instars of *E. vigintioctopunctata* 48 hours after spraying.



SEVIN 24 HOURS

FIG 7



SEVIN 48 HOURS

FIG 8

—— 1st INSTAR LARVA —— 2nd INSTAR LARVA. —— 3rd INSTAR LARVA.

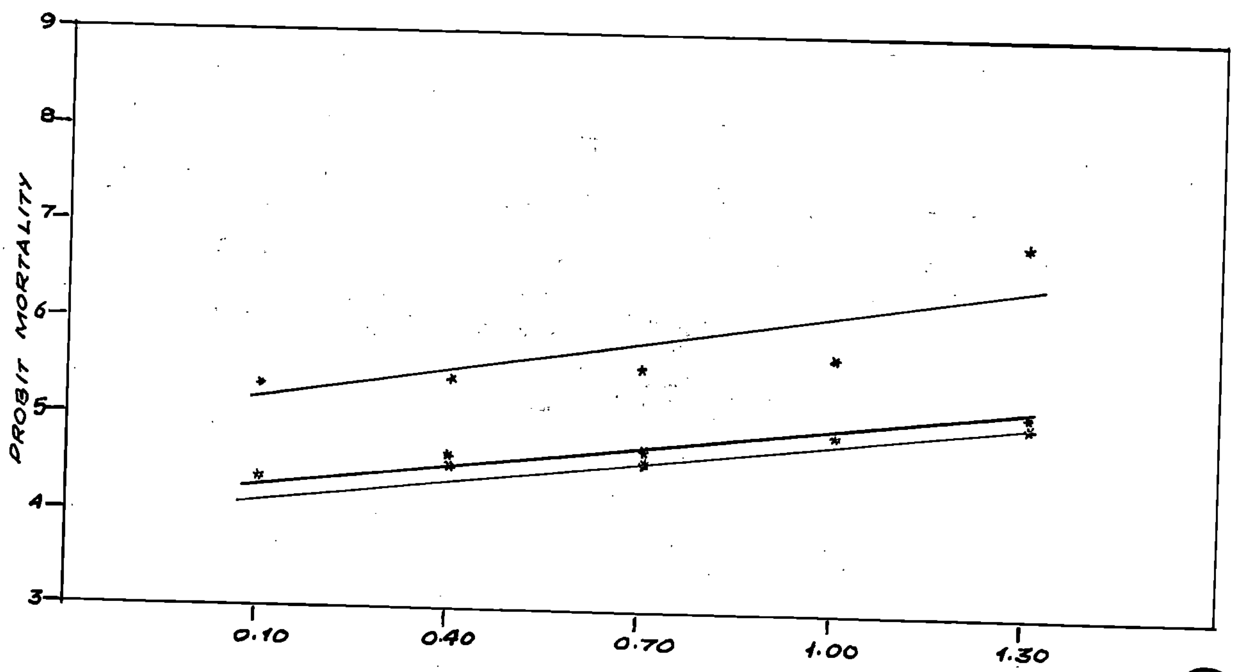
Table 5

Dosage mortality relationship between BHC and the different larval instars of *E. vigintioctopunctata*

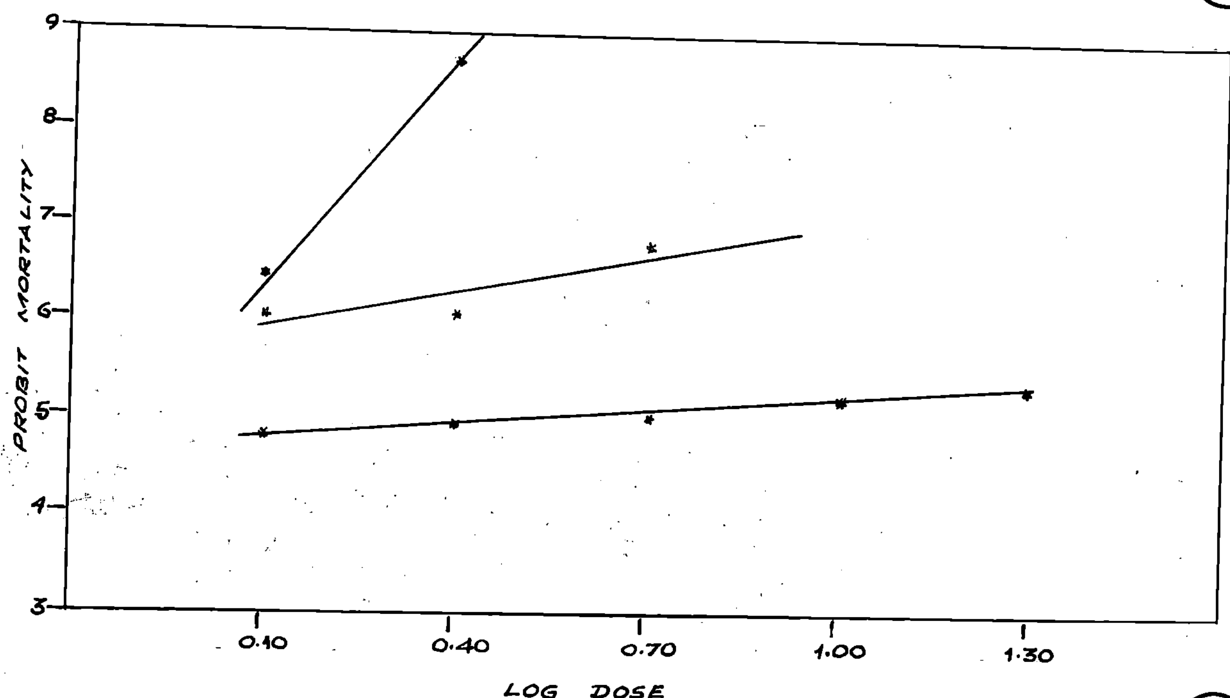
Dose %	Log dose	Instars	Mortality at 24 hours %	Mortality at 24 hours Probit	Mortality at 48 hours %	Mortality at 48 hours Probit
0.2	1.30	1	53.3	5.08	63.3	5.34
		2	96.7	6.84	100.0	..
		3	50.0	5.00	100.0	..
0.1	1.00	1	43.3	4.83	60.0	5.25
		2	73.3	5.62	100.0	..
		3	43.3	4.83	100.0	..
0.05	0.70	1	36.7	4.66	50.0	5.00
		2	70.0	5.52	100.0	..
		3	33.3	4.57	96.7	6.84
0.025	0.40	1	33.3	4.57	46.7	4.92
		2	66.7	5.43	100.0	..
		3	30.0	4.48	86.7	6.11
0.0125	0.10	1	26.7	4.38	43.3	4.83
		2	63.3	5.34	9.3	6.50
		3	26.7	4.38	86.7	6.11

Fig. 9. Log Dose - Probit Mortality relationship between BHC and different larval instars of *E. vigintioctopunctata* 24 hours after spraying.

Fig. 10. Log Dose - Probit Mortality relationship between BHC and different larval instars of *E. vigintioctopunctata* 48 hours after spraying.



B H C 24 HOURS (FIG 9)



B H C 48 HOURS (FIG 10)

—— 1st INSTAR LARVA. —— 2nd INSTAR LARVA. —— 3rd INSTAR LARVA.

The second instar larva appears to be the most susceptible to B.H.C. both at 24 hours and at 48 hours. At 24 hours the first instar larva is more susceptible than the third instar larva. However, at 48 hours, the third instar larva seems to be considerably more susceptible than the first instar larva.

Experiment VI

Relative stomach toxicity of field doses of insecticides to grubs of *E. vigintioctopunctata* when sprayed on bitter gourd leaves.

Insecticides and their doses:		
	Malathion (E.C.)	0.1%
	D.D.T. (E.C.)	0.2%
	Diazinon (E.C.)	0.2%
	Phosphamidon (E.C.)	0.04%
	Endrin (Tech.)	0.03%
	Sevin (W.P.)	0.0125%
	Parathion (Tech.)	0.05%
	B.H.C.(Tech.)	0.2%
	Thiometon (E.C.)	0.1%
	Dipterex (W.P.)	0.1%
	Formothion (E.C.)	0.025%

Test insect: Second instar grubs were used in this experiment. They were reared out in the laboratory on bitter gourd leaves.

Number of replications: 3

Number of insects in each replication: 10

Date of experiment: 1--2--1967 to 9--3--1967

Temperature during experiment: 85.3°F - 78.9°F

Relative Humidity during experiment: 87.2%

Procedure: The upper surface of bitter gourd leaves was sprayed with insecticides under the Potter's tower and the grubs exposed to the unsprayed lower surface as detailed under 'Methods'. Mortality counts were taken 24 hours after exposing the grubs to the leaf.

Results: Results are given in Table 6 and represented in Fig. 11. It may be observed that dipterex 0.1 per cent gives 100 per cent mortality among the grubs. This is followed in the descending order by parathion and endrin (which are equally effective), DDT, diazinon and phosphamidon, (which are equitoxic), BHC, sevin thiometon, malathion and formothion.

Table 6

Mortality of 2nd instar grubs of *E. vigintioctopunctata* exposed to the lower surface of bitter gourd leaves with upper surface sprayed with different insecticides.

SL.No.	Insecticide and concentration		Per cent mortality in 24 hours
1	Malathion	0.1%	50.0
2	D.D.T.	0.2%	93.3
3	Diazinon	0.02%	93.3
4	Phosphamidon	0.04%	93.3
5	Sevin	0.0125%	66.6
6	Parathion	0.05%	96.6
7	B.H.C.	0.2%	76.6
8	Thiometon	0.1%	63.3
9	Formothion	0.025%	30.0
10	Dipterex	0.1%	100.0
11	Endrin	0.03%	96.6

Fig. 11. Mortality of 2nd instar grubs of *E. vigintioctopunctata* exposed to the lower surface of bitter gourd leaves with upper surface sprayed with different insecticides.

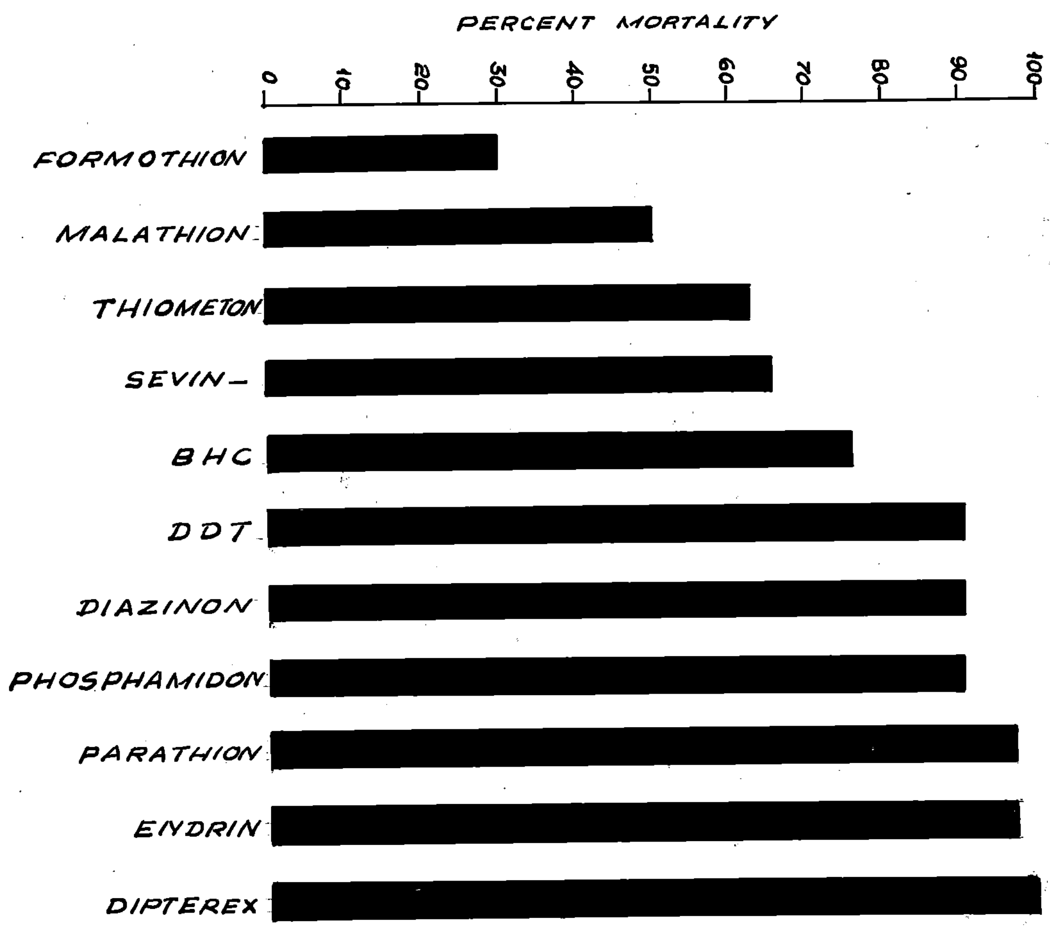

PERCENT MORTALITY
0
10
20
30
40
50
60
70
80
90
100
FORMOTHION
MALATHION
THIOMETON
SEVIN
BHC
DDT
DIAZINON
PHOSPHAMIDON
PARATHION
ENDRIN
DIPTEREX

FIG 11

DISCUSSION

DISCUSSION

Relative susceptibility of different larval instars of *E. vigintioctopunctata* to toxicity of different insecticides.

Results of the experiment conducted on this are summarised in Table 7. The comparisons of the susceptibilities have been made based on the positions of the ld-p-lines for the different insecticides and the different instars.

Table 7

Relative susceptibility of the three larval instars of *E. vigintioctopunctata* to toxicity of different insecticides

Insecticide	Order of susceptibility	
	At 24 hours	At 48 hours
DDT	III > I > II	III > II > I
Malathion	III > I > II	III > II = I
Sevin	III > II = I	III > II = I
Parathion	I > II = III	I > II > III
BHC	II > I > III	II > III > I

It will be seen that:- (1) the different instars of the grub show varying susceptibilities to the same insecticide,

(2) the order of susceptibility of the three instars is not identical for different insecticides and (3) the order of susceptibility of the three instars to the same insecticide varies at different intervals after application of the insecticides. Thus for example, to DDT, malathion and sevin the third instar stage of the grub is the most susceptible while to BHC it is the second instar and to parathion the first instar which is the most susceptible. It is interesting to note that it is the first instar grub which is the most resistant to the action of sevin and DDT. Correlation between the susceptibility to toxic action of the insecticide and the progressive instars of the insect is observed in the case of DDT and parathion (at 48 hours) this being positive in the case of parathion and negative in the case of DDT. The variations in the order of susceptibility observed at the different intervals after treatment may perhaps be due to the relative capacity of the different instars in eliminating the absorbed insecticides.

Relative toxicity of different insecticides to the grubs of *E. vigintioctopunctata*

It is seen that the different instars of the grub shows varying susceptibilities to different insecticides with no regularity or sequence. With a view to have an idea about the overall suscepttbility of the grubs taking all the instars into consideration the whole data relating to all the insecticides were subjected to analysis of variance (vide Table 8 for details of the analysis). It has been observed that there exists significant difference (significant at 5% level) between the toxicities of the different insecticides to the grubs. The five insecticides can be ranked as under based on descending toxicities:-

Sevin Parathion Malathion BHC DDT

Thus sevin is the most highly toxic insecticide to the grubs followed in the descending order by parathion, malathion, BHC and DDT. There does not exist any significant difference between the toxicity of malathion and that of BHC.

Relative stomach toxicity of insecticides to grubs of *E. vigintioctopunctata*

By spraying the insecticide on one surface (upper) of the leaf and confining the grubs on the opposite surface, direct contact of the latter with the active deposits of the insecticide is eliminated completely. The grubs feed by scraping the leaf surface, invariably leaving the epidermis of the opposite surface intact. This remains as a thin transparent membrane at the fed areas. So the insecticide can be picked up by the grubs only if it penetrates the epidermis and gets into the mesophyll on which the insect feeds. Results of this trial show that all the eleven insecticides under trial cause toxicity among the grubs indicating that they penetrate the leaf epidermis. However there exists considerable variations in the mortalities of the insect caused by the various insecticides indicating that different insecticides get translocated into the leaf tissues with varying efficiencies and that they have varying stomach toxicities. Thus dipterex (0.1%) which gives 100% mortality appears to be translocated with the maximum efficiency. Dipterex has also the additional advantage of being an effective stomach poison. Endrin (0.03%) and parathion (0.05%) also are highly effective in killing the grubs (each gives 96.6% mortality). These are closely followed by DDT, diazinon and phosphamidon. Sevin which has shown the maximum contact toxicity to the grubs shows very low stomach toxicity. This may be either due to its low penetration power or low stomach action. Malathion and formothion have very low stomach action on the grubs.

These studies will indicate that for killing the grubs which have the habit of feeding on the lower surface of the leaves it is enough that the upper surface is sprayed with the insecticide. Dipterex (trichlorphon) closely followed by DDT and endrin appear to be suitable for this.

Table 8

Analysis of Variance Table

Source	S.S.	df.	Variance	F.ratio
Total	43901.37	74		
Treatment	32149.57	24	1339.565	5.69 *
Between insecticides	24307.69	4	6076.9225	25.8553 *
Between levels of DDT	4943.69	4	1235.9225	5.2584 *
Between levels of Malathion	3633.70	4	908.425	3.8650 *
Between levels of parathion	3355.30	4	838.825	3.5689 *
Between levels of sevin	2017.06	4	504.265	2.1455
Between levels of BHC	804.49	4	201.1225	0.8557
Error	11751.80	50	235.036	

C.D. for comparison between insecticides. $= t_{50}\ (0.05)\ \sqrt{\frac{2 \times VE}{15}}$

$= \underline{\underline{11.25198}}$

* Significant at 5% level

Ranks	Treatments	Mean	Difference	
1	T_4 Sevin	232.60		
			47.82	11.25
2	T_3 Parathion	184.78		
			50.28	11.25
3	T_2 Malathion	134.50		
			0.38	11.25
4	T_5 BHC	134.12		
			34.92	11.25
5	T_1 DDT	99.20		

Ranking: $T_4\ T_3\ \overline{T_2\ T_5}\ T_1$

SUMMARY

SUMMARY

Dosage mortality relationships between the insecticides DDT, BHC, parathion, malathion and sevin and the three instars of the grubs of E. *vigintioctopunctata* were ascertained.

The orders of susceptibility of the three instars to the different insecticides based on their ld-p-lines are as follows:-

After 24 hours of contact

DDT	III	>	I	>	II
BHC	II	>	I	>	III
Parathion	I	>	II	=	III
Malathion	III	>	I	>	II
Sevin	III	>	II	=	I

After 48 hours of contact

DDT	III	>	II	>	I
BHC	II	>	III	>	I
Parathion	I	>	II	>	III
Malathion	III	>	II	=	I
Sevin	III	>	II	=	I

The order of toxicity of the insecticides to all the 3 instars of grubs taken together is: sevin > parathion >

malathion > BHC > DDT. There exists significant difference between the toxicities of the different insecticides, there is no significant difference between malathion and BHC.

Stomach toxicity of eleven insecticides each at its field dose to the second instar grub of <u>E. vigintioctopunctata</u> was ascertained by spraying the insecticide on the upper surface of bittergourd leaves and letting the grubs to feed on the opposite surface. The relative stomach toxicity of the different insecticides was in the order: - dipterex > parathion = endrin > DDT = diazinon = phosphamidon > BHC > sevin > thiometon > malathion > formothion.

REFERENCES

REFERENCES

Abou El Ghar, M.R.	1964	Effect of certain insecticides against cotton leaf worm (Prodenia litura F.). PFLANZENSCHUTZ NACHRICHTEN BAYER, 17: 45-56.
Adkisson, P.L.	1959	The relative susceptibility of the Life History stages of the Rice Weevil to certain Fumigants. J. econ. Ent., 50: 761-764.
Beneschoter, C.A.	1961	Susceptibility of life stages of Mexican fruit fly to fumigation with ethylene chlorobromide. J. econ. Ent., 53: 323-325.
David, W.A.L. and Gardiner, B.O.C.	1954	The systemic insecticidal Action of Sodium Fluoroacetate and of three Phosphorus Compounds on the Eggs and Larvae of Pieris brassicae.L. Ann. appl. Biol., 40: 403-417.
Dennis, N.M.	1952	Effect on Fumigation of adding Sulfur Dioxide to a Carbon Tetrachloride - Carbon Disulphide Mixture. J. econ. Ent., 44: 1022-1023.
Eaton, J.K. and Davies, R.G.	1951	The toxicity of certain synthetic Organic Compounds to the Fruit-tree Red-spider Mite. Ann. appl. Biol., 37: 471-489.
Ebeling, W. and Pence, R.J.	1955	Susceptibility to Acaricides of Two-Spotted Spider Mite in the Egg, Larval and Adult Stages. J. econ. Ent., 47: 789-795.

Jaubert, G.F.	1927	Destruction de la fausse teigne (_Galleria mellonella_) au moyen de la chloropicrin. _C.R. Acad. Sci. Fr._, _185_: 1608 - 1610.
Krohne, H.E. and Lindgren, D.L.	1959	Susceptibility of Life Stages of _Sitophilus oryza_ to various Fumigants. _J. econ. Ent._, _51_: 157-158.
Langenbuch, R.	1958	Investigations on the differing Susceptibility to DDT of third and fourth instar Larvae of _Leptinotarsa decemlineata_. _Z. Pflkrankh._, _62_: 564-572.
Mukerjee, T.D.	1953	The Relationship between the stage of Development and susceptibility to DDT and the Pyrethrins of _Diatarexia oleracea_ (L.) _Tenebriomolitor_ L., and _Periplaneta americana_ (L.). _Bull. Ent. Res._, _44_: 121-161.
Parkin, E.A.	1953	The Susceptibility to DDT Dust of Coleoptera infesting Stored Products. _Bull. Ent. Res._, _44_: 439-444.
Pedersen, J.R.	1961	Susceptibility of certain stages of rice weevil to a methallyl chloride fumigant formulation in wheat of various moistures. _J. econ. Ent._, _53_: 288-291.
Pepper, J.H and Hastings, E.	1944	Age Variations in exoskeletal composition of the Sugar Beet Webworm and their possible Effect on Membrane Permeability. _J. econ. Ent._, _36_: 633-634.

Pradhan, S. and Bindra, O.S. 1953 Relationship between Changes in Susceptibility and various factors connected with successive instars of the Desert Locust. Curr. Sci., 22: 380.

Pradhan, S. and Bindra, O.S. 1957 Studies on Resistance to contact Toxicity of Gamma BHC suspensions by successive Instars of *Schistocerca gregaria* Forsk. and certain associated Factors. Indian J. Ent., 18: 93-111.