MANIPULATION OF INSECT BEHAVIORAL PHYSIOLOGY BY SELECTED SYNTHETIC CHEMICALS

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IN ZOOLOGY

(PARTLY BY PAPERS AND PARTLY BY RESEARCH)

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Dedicated to

my late father

C E R T I F I C A T E

It is certified that the work incorporated in the present thesis entitled "MANIPULATION OF INSECT BEHAVIORAL PHYSIOLOGY BY SELECTED SYNTHETIC CHEMICALS." submitted by Mrs.G.D.Hebbalkar for M.Sc. degree in Zoology (Partly by papers and partly by research) embodies the findings of her original research work carried out under my supervision at ENTOMOLOGY LABORATORY, National Chemical Laboratory, Poona 8. This work has not been submitted so far for any degree or diploma.

(DR.R.N.SHARMA)
Research Guide

February 17, 1988

A C K N O W L E D G E M E N T S

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Hebbalkar

(Mrs.Geeta D.Hebbalkar)

CONTENTS

INTRODUCTION

INTRODUCTION

Insect behavior has long fascinated students and laymen alike. Veering away from the classical exposes of the vintage of Fabre and Von Frisch[1], modern perceptions of Insect behavior include physiological, biochemical and neurological processes as integral underlying mechanisms. Chemical basis of insect communication in behavioral repertoires is also widely acknowledged[2,3]. These broader comprehensions have also paved the way for the concept of pest management by behavior manipulation[4] where, again, chemicals of various origins may play different roles. That many behaviorally non-specific chemicals may affect the overall behavior has also come to be recognized, although not much work has been done on this aspect. Most prominant is the suspected avoidance behavior of mosquito adults supposedly induced by DDT[5]. If true, such phenomenon need to be examined closely and the control potential of many insecticides be assayed in the light of such investigations. Quite apart from such gross contact repellence, many subtler influences of pest control chemicals have been unmasked in recent years. Many of these may have far reaching implications in the mechanism of insect behavior as well as practical insect control[6].

Currently, two major types of chemicals dominate the insect pest control arena. Of primary importance and in major use are the conventional synthetic organic insecticides. In lesser use and possibly the safest, target specific new class of compounds emerging as insectistatics are various chemicals affecting insect growth, development, metamorphosis and reproduction among them are hormones or the insect growth regulators (IGR)[7].

In the present investigation the pest control agents belonging to two different generations mainly the second generation of synthetic organic insecticides and the third generation insecticides i.e. insect hormonal compounds. (viz. juvenile hormone analogues/JHA's) have been used to study their effects on two selected behavioral responses of chosen insects. Among insecticides, a representative from the class of carbamates and few representatives from the class of pyrethroids have been chosen. In case of JHAs, totally new compounds - geraniol based diethers[8] - synthesized at National Chemical Laboratory, Pune; have been used. The behavioral responses chosen to evaluate the behavior modifying potentials of insecticide and JHAs were, proboscis extension reflexes of house flies and mating activity levels of the red cotton bugs, respectively. Number of insecticides[9] and JHAs[10,11] when applied at sublethal dosages are reported to have effects on the tarsal responses of house flies and mating activity of the red cotton bug, respectively. However, the effects of insecticides and JHAs chosen in the present study, on the respective behavioral responses are not examined by earlier workers.

The data presented in this dissertation not only substantiate the validity of behavioral bioassays in the evaluating insecticides and JHAs at sublethal dosages but also help in exploring the possibility of building the correlation between the biological potency of the compound and the degree of behavioral response chosen. The data in this work also bring support to the earlier findings.

Alternations of neurological functioning in insecticide poisoned house fly and subtle anomalies in the development of antennal sensory

hairs of JHA affected red cotton bug are advanced as the possible underlying causes for the observed behavioral aberrations. The possibilities of exploitation of such behavioral abberations for pest control are discussed.

The thesis has been divided into four chapters the Ist chapter recapitulates information available in the literature on two separate aspects studied as well as relevant data on the chemicals employed. The IInd chapter describes material and methods used in two distinct sections A and B for the two different aspects. The IIIrd chapter consists of results obtained from the studies on the effects of insecticides and JHAs on gustatory and mating responses, respectively. The IVth and final chapter takes into account the available information as well as results obtained in this study and attempts to present a meaningful syntheses under the title of discussion.

CHAPTER 1

Discovery of insecticidal property of DDT by Paul Müller (1939) marked the beginning of the synthetic organic insecticides in the real sense. The following years witnessed the dramatic success in the pest control leading to substantial growth in global agricultural produce due to the introduction of several newly synthesized insecticides. However, documentation also exists in plenty regarding the remaining disadvantages and hazards of insecticides particularly after they were brought into focus for the lay public by Rachel Carson (1962)[12] with the publication of 'Silent Spring'. Partly as a matter of evolution and partly as a consequent of wide spread hue and cry about environmental contamination by insecticidal residues, a series of relatively less objectionable chemicals have been synthesised and are fast replacing the older once. Prominent among them are synthetic pyrethroids amongst synthetic insecticides and the juvenile hormone analogues among the so called non-insecticidal chemicals.

In the following sections the historical antecedants of various types of insecticides and their effects on insects are briefly summarised.

HISTORICAL RESUME

INSECTICIDES

Chemicals used in the insect control are generally classified into following chronological order :

1) First generation insecticides : These include insecticides used in the ancient days. The insecticides used were mostly derived from plants. Pyrethrum is one of the oldest insecticides known; it was used for louse control in Iran (Persia) in 400 B.C. In 1849 the flowers of Crysanthemum cinerariaefolium were found to have highest concentration of insecticidal principle. Nicotine from tobacco leaves and rotenone from the roots of the plants such as Derris sp., Lonchocarpus were used as a contact insecticide for aphids, fumigants against poultry mites and thrips, hemipterous insects, lepidopterous larvae respectively. Other chemicals included under first generation are inorganic insecticides such as paris green, lead arsenate, calcium arsenate, salts of hydrofluoric acid, fluosilicic acid, cryolite. These were basically stomach poisons, however they were highly phytotoxic as well.

2) Second generation insecticides : Most of the synthetic organic insecticides are included in this category. These insecticides mainly comprise four classes as follows :

a) Organophosphorous compounds (OP) : They are one of the most important class of compounds discovered by Gerhard Schrader (1930). They are broad spectrum pesticides as they are active as insecticides, acaricides fungicides defoliants, herbicides and nematicides. The various examples herbicidal and nematicidal are, schradan, Dimecron, Methyl parathion, fenthion (Baytex), temephos (Abate), malathion (cythion), phorate (Thimet), diazinon and chlorpyriphos (Dursban).

b) Carbamates : The active principle 'physostigmine' or eserine was found in the seeds of 'Physostigma venenosum (Balfour), which was chemically elucidated by Stedman (1926)[13]. The well known examples of carbamates are carbofuran (Furadan) carbaryl (Sevin) and propoxur (Baygon). Out of these carbary is relatively broad spectrum but weak against aphids and houseflies. Carbofuran is both soil and systemic, highly persistent insecticide but possesses high mammalian toxicity. Baygon is effective against pests of house hold, medical and veternary importance.

c) Chlorinated hydrocarbons : Of all the organic insecticides best known and widely discussed insecticide DDT belongs to this group. DDT was synthesized for the first time by Zeilder (1874)[14] and it's insecticidal property was discovered by Paul Müller (1939). Between 1940 and 1960 DDT gained paramount importance due to the dramatic results it produced in the areas of pests of public health importance and agriculture. Indiscriminate use DDT in past has resulted in accumulation of about 1 billion pounds of DDT in the biosphere[15,16] Other members of this group are chlordane, heptachlor, dieldrin, endrin, endosulfan (Thiodan) and lindane.

d) Synthetic pyrethroids : The natural insecticides (i.e. pyrethrum from chrysanthemum cinerariaefolium) and their synthetic analogues are called pyrethroids. Pyrethrins are heat and light sensitive and are easily oxidized, with increasing concentration and purity they tend to become increasingly unstable. This group of insecticides is characterised by their high toxicity to insects and low toxicity to mammals. However, these insecticides have low residual activity.

To guard the ecosystems from accumulation of hazardous insecticidal residues, pesticides like pyrethroids are highly suitable. Over the years chemists have synthesised stable forms of pyrethroids such as permethrin, cypermethrin and decamethrin.

3) Third generation pesticides : Williams (1967)[17] heralded insect juvenile hormones as 'third generation pesticides' which were shown to disrupt growth, development, metamorphosis, reproduction and even behaviour in number of insects[72].

4) Fourth generation insecticides : Bowers[18] discovered anti-juvenile hormone precocene I,II from the extracts of bedding plant Ageratum houstonianum and called them fourth generation insecticides residual treatment of which caused curtailment of juvenile hormone dependent processes causing regression of maturing ovaries, production of precocious adults by ommitting intermediate nymphal stages in case of Dysdercus cingulatus, induction of adult diapause in mexican beetle and inhibition in pheromone production by virgin Periplaneta americana females[19].

JUVENILE HORMONE (JH)

Wigglesworth's work on Rhodnius prolixus [20,21] demonstrated that the endocrine glands corpora allata situated behind the insect brain secrete a factor which promotes larval growth and development but prevents adult metamorphosis. This factor was given the name juvenile hormone. Juvenile hormone reappears in the adult stage and it is shown to be responsible for growth and maturation of the ovary[22]. Apart from the vitellogenesis[22] in the female, secretory activity of male accessory glands[23] maintenance of larval diapause[24] monitoring of adult reproductive diapause[25,26] pigmentation of epidermis[27] and determination of the quality of the cuticle (viz. larval, pupal, adult) synthesized by epidermis in

presence of ecdysone[28] are some of the physiological processes wherein involvement of juvenile hormone has been demonstrated.

Influence of juvenile hormone on the insect behaviour such as locomotion[29,30] flight[31,32,33] cocoon spinning[34], pheromone production[35,36], stridulation[37] and sexual behaviour[38] has been demonstrated by experimental repression of these functions by allatectomy and their restoration by reimplantation of corpora allata or synthetic juvenile hormone analogues treatment.

Sexual behavior of female and JH

Conflicting reports are available in the literature regarding the control of corpora-allata (i.e. JH) on the receptive behavior of the female.

Engelmann (1960)[39] was first to produce the evidence of juvenile hormone's positive influence on determining the receptivity in the Leucophaea maderae females.

Unique and well documented, total control of corpora-allata (CA) over receptive behavior of the female is shown in Gomphocerus. Here, the allatectomized female which has entered into permanent state of "primary defence" turns receptive after reimplantation of active CA or application of juvenile hormone analogue (JHA).[40,41,42,43,44]

On the other hand, CA do not appear to be involved in the maintenance of receptive behavior of Diploptera punctata[45], cricket Gryllus bimaculatus[46] lepidoptera Galleria mellonella[47], Bombyx mori[48,49], Antheraea pernyi[51] and roaches[50] since,allatectomy in these insects was ineffective.

In Diptera, the first report on control of females receptivity by CA was demonstrated in Drosophila[52,53] where implantation of active

CA-CC complex in the pharate female resulted in precocious expression of receptive behavior 24 hrs after eclosion, rather than normal expression after 48 hrs. Similar dependence of female receptivity on CA was reported in Aedes aegypti[54], Musca domestica[55] wherein receptivity was restored even after application of JHA to allatectomized females. In another report, application of JHA to Aedes aegypti females within 30 min. after emergence caused precocious onset of sexual receptivity[56,57]. Similar induction of precocious sexual behavior was observed in Drosophila grimshawi after JHA treatment[58].

Sexual behavior of male and JH

Onset of male sexual behavior was totally prevented in allatectomized Schistocerca and Nomadacris adults[59,60,61] which however reappeared after implantation of several active CA[62,63].

On the other hand in Locusta migratoria migratorioides, CA have partial control over male sexual activity[61] since total abolishment of male's sexual activity was noticed only after the removal of neurosecretory cells (i.e. c-type) in the pars intercerebralis[64].

Similar experiments involving re-implantation of active CA in allatectomized male adults, demonstrated JH involvement in the control of male sexual behavior of crickets[65] carabid beetles[66], dung flies[67], and rabbit fleas[68].

In Hemipteran bugs Oncopeltus fasciatus[69] and Dysdercus koenigii[70] control of male sexual activity by JH was demonstrated by restoring the mating activity level of the anti-juvenile hormone (precocene) treated males to normal levels after simultaneous treatment of JH.

Effects of JHA treatment

Physiological and Morphological effects : In the representatives of Thysanura, Orthoptera, Hemiptera, Homoptera Coleoptera, Lepidoptera, Diptera, Blattaria and Anoplura application of the juvenoids to the eggs earlier to the blastokinesis stage cause severe disruption in embryogenesis leading to non-emergence[71,72].

Exogenous application of JHAs to the sensitive (where JH titer is low) larval, pupal stages often results in the formation of adultoids, larval pupal, or pupal adult intermediate forms or even supernumerary larval instars. JHA treatment to the last larval instars of Hemipteran bugs causes metamorphic inhibition of adult wings. This response, being dose dependent widely used in bio-evaluation of hormonomimitic activity of the test compound[73].

Juvenoid treatment to the males of Hemipteran bugs is reported to induce sterilizing effects which can be transmitted to the females through multiple matings[74,75,76]. In other insects JHA treatment is reported to cause histological deformities of ovaries such as defective chorion, inhibition of yolk synthesis, resorption of egg follicles and even deformation of whole ovarioles[77,78,79,80].

Application of juvenoids to black brown colored swarming stages of Locusta migratoria and Schistocerca gregaria (Forsk) caused disappearance of this colour and reappearance of light pigmentation which is characteristic of solitary phase[81] change in pigmentation after juvenoid treatment is also observed in lepidopteran larvae[82,83].

Juvenoid treatment to the eggs prevented embryonic diapause in Aulocara elliotti[84], Bombyx mori[85] but similar treatment to the last-

instar of certain coleopteran larvae induced larval diapause[24]. Juvenoid treatment to the diapausing pupae[73] and the adults under reproductive diapause[71] invariably terminated the diapause.

Histolysis of flight muscles[86,87] stimulation of flight muscles[88] induction of pheromone production[89] and disruption of process of caste formation in social insects[90] are some of the miscellaneous effects produced by juvenoid treatment.

Effects on mating behaviour : Exogenous application of juvenoid to the insect has diversly affected the mating behaviour. The effects produced by juvenoid treatment may be classified as (a) immediate effects and (b) delayed effects.

a) Immediate effects : Topical treatment of JHA to the diapausing males of Oncopeltus fasciatus[69] and Dysdercus koenigii[70] increased their mating activity levels and brought closer to that of non diapausing colony. On the other hand, juvenoid treatment to Trypodendron lineatum caused supression of mating behavior[91].

b) Delayed effects : Under this category, the larval/nymphal stages of insect were exposed to the juvenoid treatment and adults emerging from these larvae were examined for possible displacement in their mating behaviour patterns. In Lepidoptera, Oberlander et.al. (1975)[92] were first to report that exposure of larval stages of Plodia interpunctell to sublethal dosages of JHA caused supression in the mating behavior of emerged population. Only report on Hemipteran bugs came from Hebbalkar, Sharma (1980)[10] wherein, the adults emerging from the JHA treated (at sublethal dosages) fifth instar population showed substantial

reduction in their mating activity. Similar studies on cockroaches showed that JHA treatment caused suppression of adult behavior (viz. mating behavior) and retention of nymphal behavior in Periplanta americana[93,94] and Blatella germanica[95].

CHAPTER 2

MATERIALS AND METHODS

Section (A)

Experiments with the housefly

Rearing : The local strain of house fly, Musca domestica Nebulo (Diptera Muscidae) was reared at 28±2°C temperature, 60±5% R.H. under LD 12:12 photoperiod. Adults held in glass jars were offered with milk. Eggs deposited by them on the milk soaked cotton pads were collected and allowed to hatch. Late first larval instars were subsequently transfered from the pads to the jars containing rearing medium prepared by adding/ sprinkling small amount of water in presterilized, half broken pieces of animal diet pellets. A layer of dry cotton was placed on the top of the larval medium to facilitate pupation. Pupae from the cotton layer were collected and held in clean glass jars, for adult emergence.

Chemicals : Insecticides used for the study were as follows. (Fig. A)

I) 3-phenoxybenzyl ester of 2,2-dimethyl-3-(2,2 dichlorovinyl) cyclopropane carboxylic acid i.e. Permethrin*

II) 3-phenoxybenzyl ester of cis-2,2-dimethyl-3-(2-chloro-2-methyl vinyl) cyclopropane carboxylic acid i.e. Indothrin**.

III) α-cyano-3-phenoxybenzyl ester of 2,2-dimethyl-3 (2,2 dichlorovinyl) cyclopropane carboxylic acid i.e. Cypermethrin**

IV) α-cyano-3-phenoxybenzyl ester of cis-2,2-dimethyl-3-(2,2-dichloro-vinyl) cyclopropane carboxylic acid i.e. Alphamethrin**

v) 1-naphthyl N-methyl carbamate i.e. Carbaryl*

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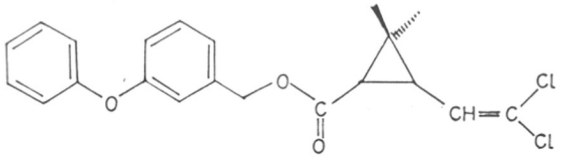
* Courtesy : Gift sample from vector control, WHO, Geneva

** Courtesy : Synthesized in the Division of Organic Chemistry (I), National Chemical Laboratory, Pune

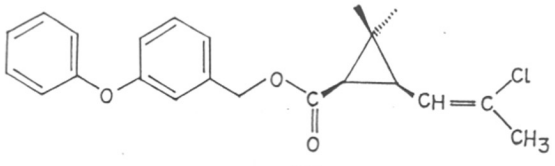
FIG. A

PERMETHRIN



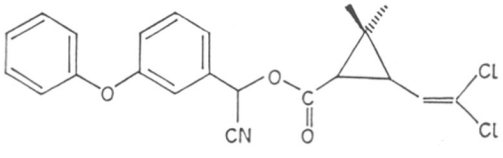
I

INDOTHRIN



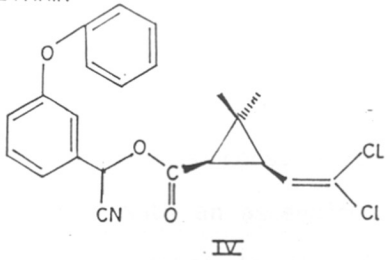
II

CYPERMETHRIN



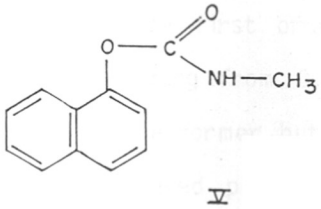
III

ALPHAMETHRIN



IV

CARBARYL



V

Sucrose from which solutions of various concentrations were prepared to elicit tarsal responses from flies was procurred from BDH, AR quality. Molecular wt. 342.30

Determination of sublethal doses : To examine the effects of the insecticide poisoning on the tarsal responses of the housefly, it was necessary to determine the dose level at which the flies experienced marginal mortality and at the same time induce tangible displacement of the tarsal rsponse. Such a sublethal dose was found to be the LD20 level. However, it may be noted that this LD20 value was obtained from flies which were 0-16 hrs old and kept on distilled water since (i.e. not provided food) emergence. Mortality was recorded 24 hrs after the insecticide treatment. Percentage mortality was converted to probits and plotted against the Log values of various concentrations. LD 20 value was estimated from the curve fitted by Busvine, Nash (1957)[96] method. LD20 values of the five chosen insecticides were determined in the similar manner. Topical treatments to the flies were carried out with the help of 'Aglamicrosyringe', carrier solvent's (acetone) volume was 1 μl.

Tarsal response bioassay : 0-16 hrs old houseflies kept on water since emergence were used. These flies were fixed by their wing tips on the distal wax portion of glass rods. Tarsal responses were obtained by presenting their tarsi with an ascending series of sucrose solutions in watchglasses, care was taken to check water satiety as well as viability of the test flies by first offering (i) water and (ii) range of sucrose solution conc. varing from 0.0025 to 1 Molar (M) to their tarsi. Flies responding to the former but not to the latter were discarded. A tarsal response was considered positive if there was full uninhibited extension of the proboscis immediately after dipping of tarsii in an

offered solution. The different concentrations of sucrose solution used were 0.0025 M, 0.005M, 0.01 M, 0.025 M, 0.05 M, 0.1 M, 0.25 M, 0.5 M and 1 M. Different batches of flies were tested on different days, so as to obtain authentic data on tarsal responses of the untreated flies to the different concentrations of sucrose solutions mentioned above. Number of tarsal responses thus observed were converted to % tarsal response as follows.

$$\% \text{ Tarsal response} = \frac{\text{No. of flies responding to test sucrose conc.} \times 100}{\text{Total no. of flies examined}}$$

For testing tarsal responses of the insecticide poisoned flies 0-16 hrs old water satiated flies were treated with the different insecticides in 1 μl of acetone, on the dorsal metathorax. "Arnold Burkard" micro-applicator was used for topical delivery of different sublethal doses of the test insecticides. The various doses were as follows.

I) Permethrin 0.0038 μg/fly LD20

II) Indothrin 0.0027 μg/fly LD20

III) Cypermethrin 0.0014 μg/fly LD20

IV) Alphamethrin 0.00039 μg/fly LD20

V) Carbaryl 0.1384 μg/fly LD20

The treated flies were kept in separate glass container and provided with a water soaked cotton swab. In general, treated flies revived after 4 hrs. These revived flies were permitted an additional 3 hrs. for water ingestion and satiety before being mounted on the distal waxed portion of glassrods as described earlier for untreated flies. These flies were then assayed for their tarsal responses to the same range of sucrose solution of different molarities as were offered to untreated flies. Responses obtained to the different concentrations

were recorded and averaged after atleast 8 replications using a minimum of 20 flies per replicate. For one concentration of sucrose solution minimum of 100 flies were assayed for tarsal response behavior.

Percentage tarsal responses were converted to probits and plotted against Log concentration of sucrose solution. ED50 value was estimated from the curve fitted by Bousvine, Nash (1957)[96] method. ED50 values of the insecticide poisoned flies wre compared with ED50 value of the untreated (control) flies. ED50 is the value of the sucrose solution concentration required to elicit 50% tarsal response from the test flies.

Section (B)

Experiments with the red cotton bug

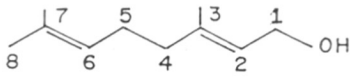
Rearing : Colony of the red cotton bug Dysdercus koenigii F. (Hemiptera: Pyrrhocoridae) was reared on water and soaked cotton seeds at temperature 27±1°C and 60-65% R.H. under LD 12:12 photoperiod. Freshly molted (0-12 hrs) fifth instars required for juvenoid treatment were collected from the jars containing late fourth instars kept only on water.

Chemicals : The juvenoids* used were geraniol based diethers synthesized from geraniol where, C-8 position of geranyl ether was oxidized and further etherified to give 1,8-diethers[8,97] . The chemical structures (Fig. B) and formulae of these juvenoids (JHAs) were as follows.

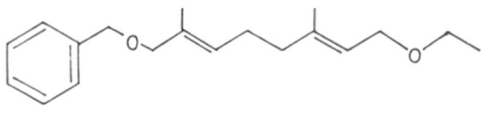
1) 1-Ethoxy, 8-benzyloxy-3,7-dimethyl-2, 6-octadiene i.e. A_1
2) 1-(p-chloro)phenoxy-8-methoxy-3,7-dimethyl-2,6-octadiene i.e. A_2
3) 1-(p-chloro_phenoxy-8-ethoxy-3,7-dimethyl-2,6-octadiene i.e. A_3
4) 1-Ethoxy-8-(p-chloro)phenoxy-3,7dimthyl-2,6-octadiene i.e. A_4
5) 1-Ethoxy-8-phenoxy-3,7-dimethyl-2,6-octadiene i.e. A_5

* Courtesy, Organic I Division, National Chemical Laboratory, Pune 8

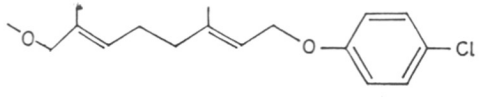
FIG. B



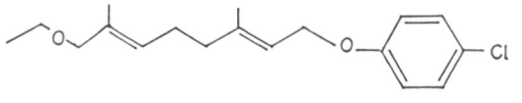
GERANIOL



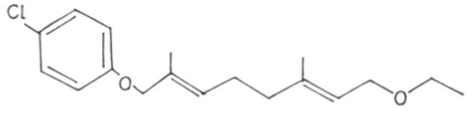
A_1



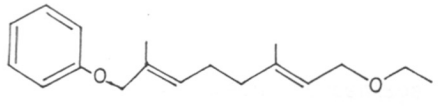
A_2



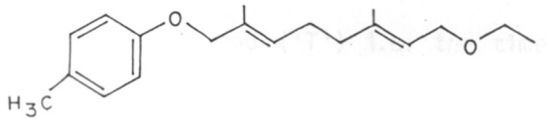
A_3



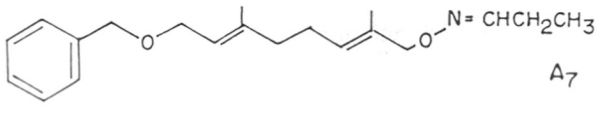
A_4



A_5



A_6



A_7

6) 1-Ethoxy-8-(p-methyl)phenoxy-3,7-dimethyl-2,6-octadiene i.e. A_6

7) Propion aldehyde oxime i.e. A_7

JHA treatments : Different doses of these juvenoids were applied to the mesonota of the fifth instar nymphs, with the help of 'Hamilton' syringe. The volume of the carrier solvent acetone, used for topical treatment was fixed to 1 µl. The sublethal dose (i.e. causing 0% morphological inhibition in the emergent adults) range required for experimental purposes, of an individual juvenoid was decided after initial study which established the range of the dosages required to produce 0% to 100% morphological inhibitions (MI). At least 150-200 fifth instar nymphs were used, per dose, per replicate. The treated batches were held at the temperature humidity and photoperiod metioned earlier. These batches were examined every day for adult emergence and morphological inhibition, apparently unaffected (i.e. 0% MI) adults (from sublethal doses) were collected, labelled and sexed. The sexes were kept separately in different containers on water and soaked cotton seeds. These insects were used to study the effect of the JHAs on mating behavior. Mating behavior of test insects was compared with that of the adults emerged from the acetone treated fifth instar population.

Mating behavior bioassay : Mating behavior was evaluated by recording the variations in (a) mean percentage matings (i.e. % pairs formed = % m.f.) observed over a fixed period of time (30 mins) and (b) mean pre-copulatory period ('T') i.e. the time taken for the formation of a stable pair. Both (a) and (b) observations were recorded simultaneously using batches containing males and females of identical age. The obsevations of mating behavior bioassay were taken by introduing one female per glass petridish (of 5 cm diameter), already containing

a single male. A minimum of 30 such replicates formed one experimental batch. Several such batches were screened for daily mating behavior bioassay. The precopulatory period or the time taken for establishment of a stable pair (one which remains intact for at least 1 min or more) was measured with the fhelp of stop watch and number of such pairs formed out of total assayed in 30 min. were recorded both for control and experimental batches. Mean % matings were calculated as follows.

$$\text{Mean \% matings} = \frac{\text{No. of pairs mated} \times 100}{\text{Total No. of pairs examined}}$$

OR

% m.f.

Observations of precopulatory period (in minutes) recorded in several pairs were pooled and the mean was derived (T). Observations on mating behavior of test as well as control adult populations were carried out from the day of emergence till 12th day. Mating behavior bioassay were conducted daily between 0800-13.00 hrs. under constant light intensity, at room temperature conditions and a single such bioassay ran for 30 min. only. At the end of this bioassay the mating pairs were separated and sexes were held in separate containers till the next test 24 hrs. later. The mean percentage repression of mating activity (observed between 1-12 days) was calculated as follows.

$$\text{Mean \% Repression of mating activity} = 100 - \frac{\text{Mean \% m.f. in TEST} \times 100}{\text{Mean \% mf. in CONTROL}}$$

Since various JHAs used had different biological potencies at times similar values of % mating repression were obtained with two different compounds at different levels of dosages. To achieve meaningful comparison of repressive action of JHAs on mating activity a parameter 'ratio' was devised. The ratio was calculated as follows.

$$\text{Ratio} = \frac{\text{\% Mating activity Repression caused by JHA}}{\text{The dose of the JHA applied}}$$

Statistical methods : Standard error for mean was derived by usual formula. Significance of difference between the mean values of test and control was statistically analysed by subjecting the data to single tail analyses of independent variables, followed by students' t-test[98].

CHAPTER 3

RESULTS

Section (A)

Effect of insecticide poisoning on the tarsal response of housefly

Determination of sublethal doses : Housefly extended it's proboscis in response to the perception of various grades of sucrose concentrations by it's tarsi. This behavioral response was termed as tarsal response. To study the effect of insecticide poisoning on the tarsal response, it was necessary to estimate the sublethal dose which would induce changes in the pattern of tarsal response without causing significant mortality in the test flies. Preliminary studies showed LD_{20} as an ideal sublethal dose for these behavioral studies. By studying the regression of % mortality (as probits) on the dose of insecticide applied (as log concentration) a linear regression equation was derived from which LD_{20} value of the insecticide was calculated. The linear regression equations and LD_{20} values obtained for various insecticides were as under :

1) Permethrin : (Fig..1)

Equation : $Y = -0.0492 + 2.673\ X$

$LD_{20} = 0.0038 \pm 0.0001012\ \mu g/fly$

2) Indothrin : (Fig. 2)

Equation : $Y = 0.00162792 + 2.9009\ X$

$LD_{20} = 0.0027 \pm 0.0001004\ \mu g/fly$

3) Cypermethrin : (Fig. 3)

Equation : $Y = 0.001 + 3.677\ X$

$LD_{20} = 0.0014 \pm 0.000100105\ \mu g/fly$

4) Alphamethrin : (Fig. 4)

Equation : $Y = 0.0009 + 2.609\ X$

$LD_{20} = 0.00039 \pm 0.0000102\ \mu g/fly$

FIG. 1 PERMETHRIN DOSE/MORTALITY DATA FOR M.domestica.REGRESSION CURVE

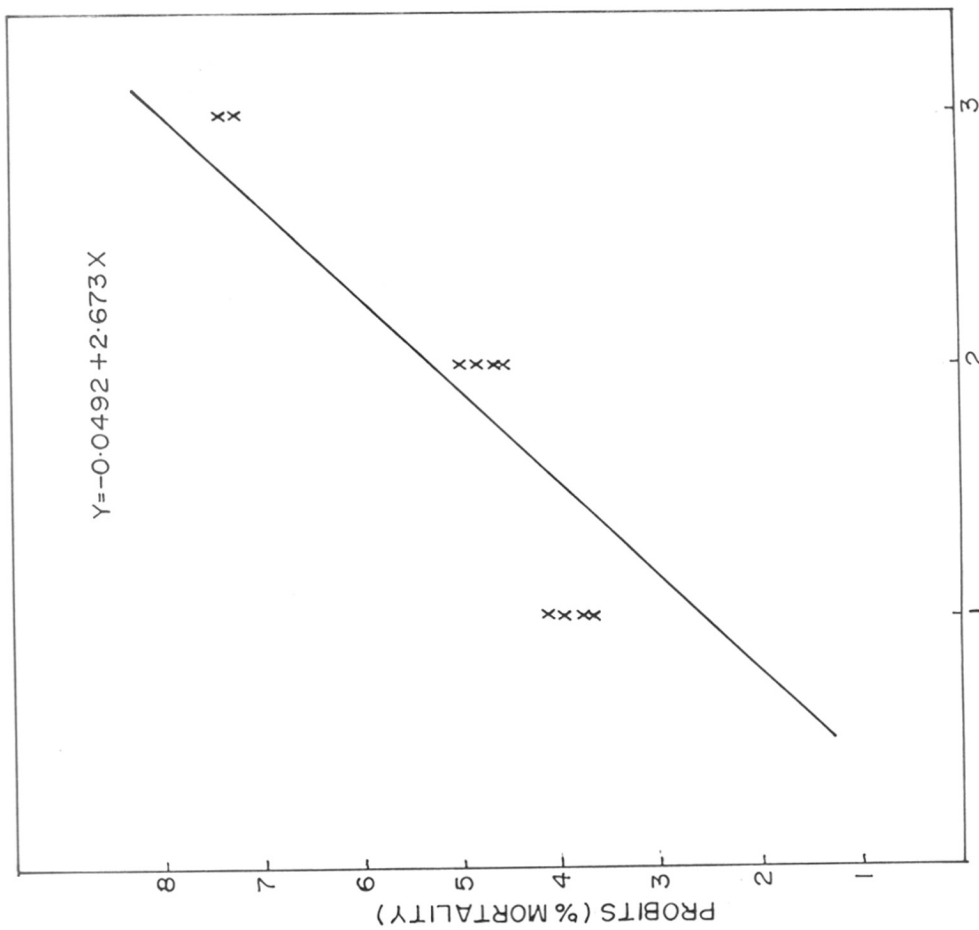

Y=-0·0492+2·673X
PROBITS (% MORTALITY)
LOG DOSE (µg PERMETHRIN/FLY X10000)
1
2
3
4
5
6
7
8

FIG.1

FIG. 2 INDOTHRIN DOSE/MORTALITY DATA FOR M.domestica. REGRESSION CURVE

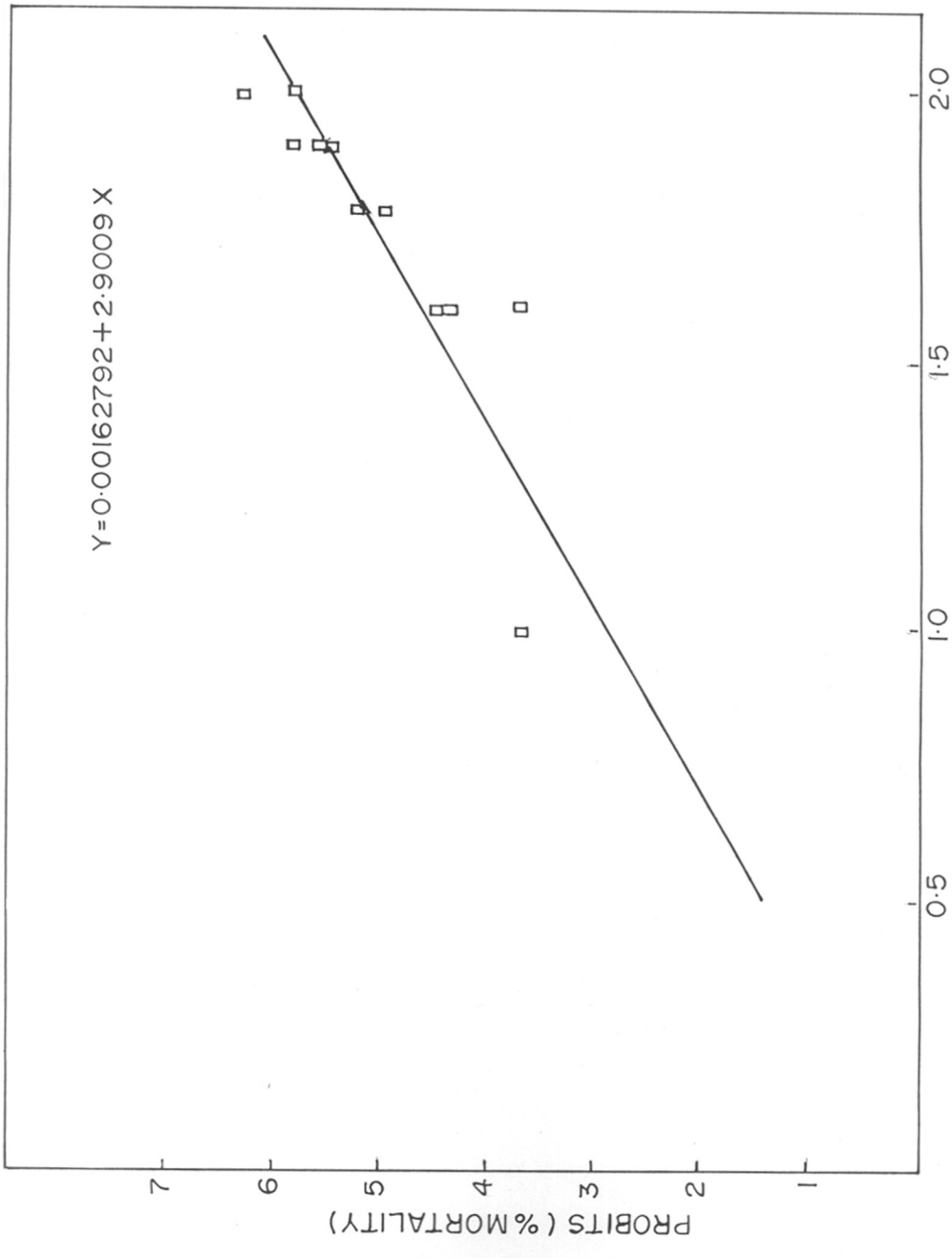

Y=0·00162792+2·9009 X
PROBITS (% MORTALITY)
7
6
5
4
3
2
1
0·5
1·0
1·5
2·0
LOG DOSE (μg INDOTHRIN / FLY X 10000)

FIG. 2

FIG. 3 CYPERMETHRIN DOSE/MORTALITY DATA FOR M.domestica. REGRESSION CURVE

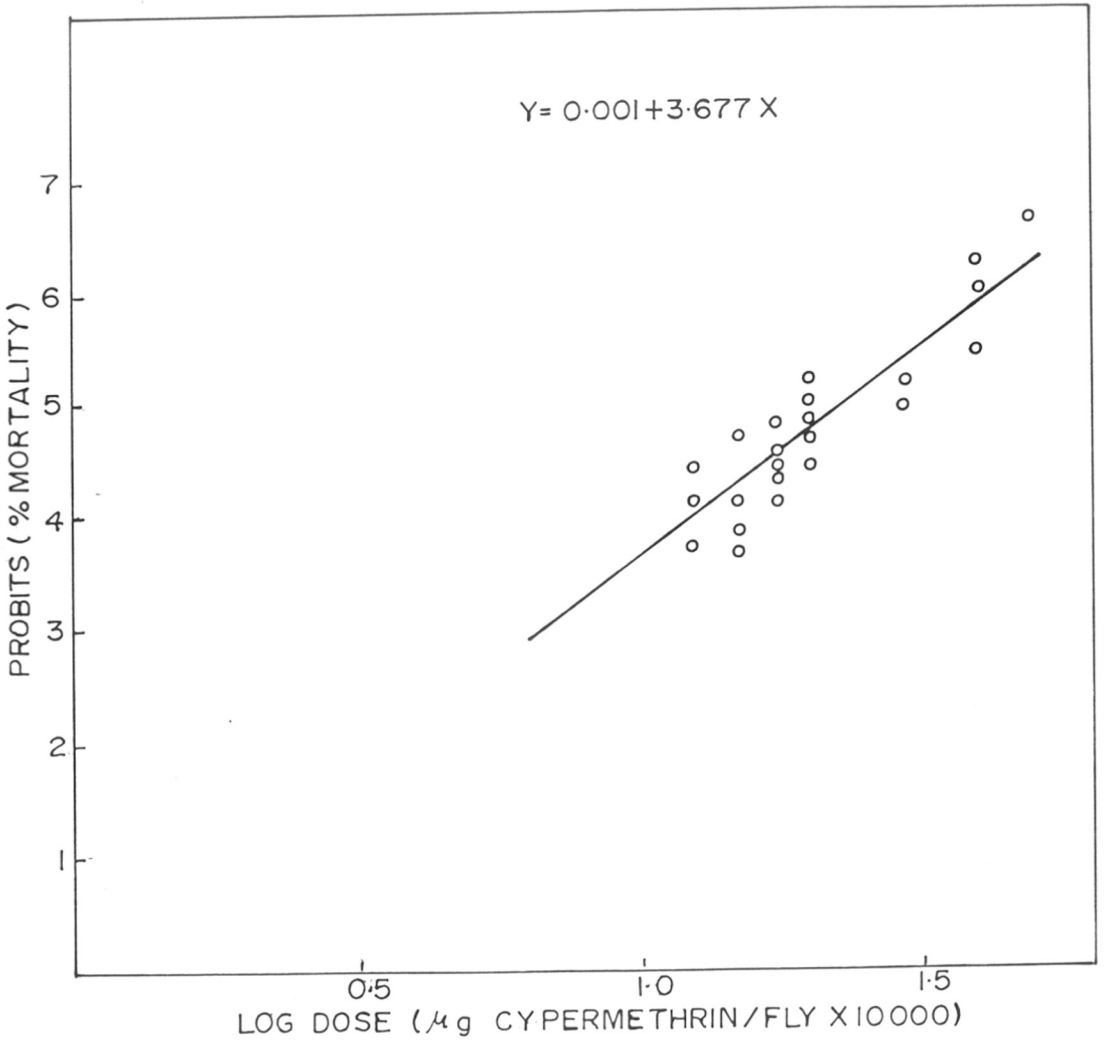

Y= 0·001+3·677 X
PROBITS (% MORTALITY)
7
6
5
4
3
2
1
0·5
1·0
1·5
LOG DOSE (μg CYPERMETHRIN/FLY X10000)

FIG.3

FIG. 4 ALPHAMETHRIN DOSE/MORTALITY DATA FOR M.domestica.REGRESSION CURVE

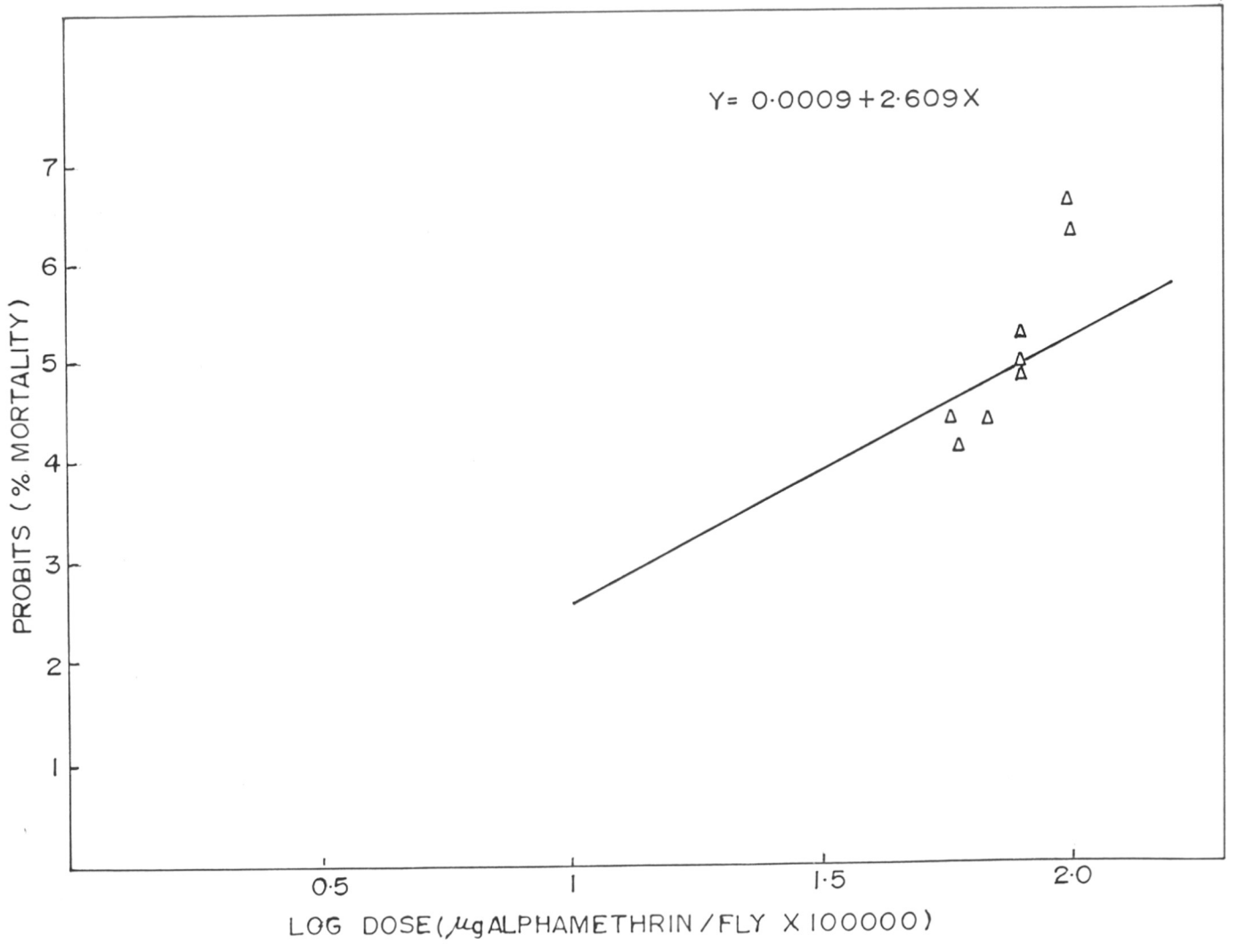

Y= 0·0009 + 2·609X
PROBITS (% MORTALITY)
7
6
5
4
3
2
1
0·5
1
1·5
2·0
LOG DOSE (μg ALPHAMETHRIN / FLY X 100000)

FIG. 4

FIG. 5 CARBARYL DOSE/MORTALITY DATA FOR M.domestica. REGRESSION CURVE

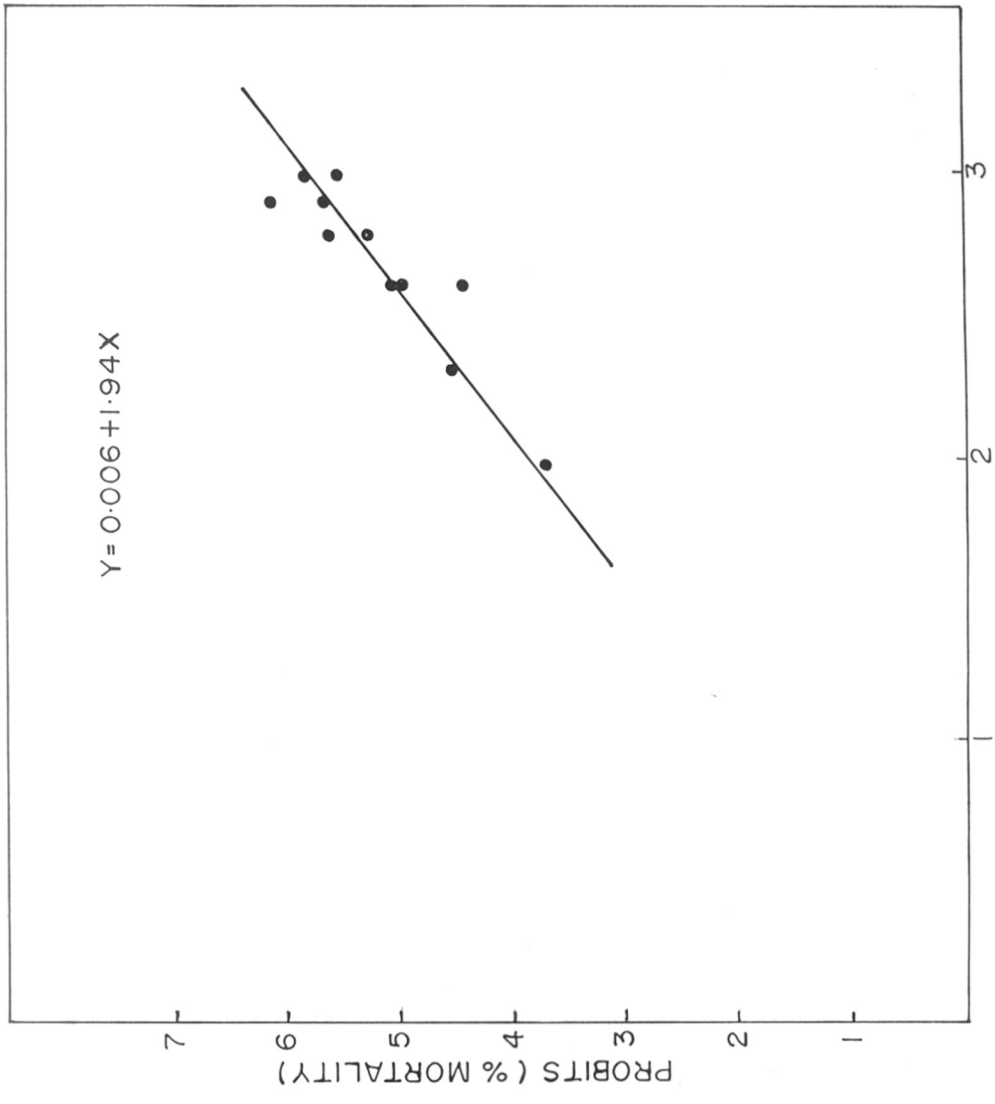

Y= 0·006+1·94X
PROBITS (% MORTALITY)
LOG DOSE (μg CARBARYL/FLY X 1000)
1
2
3
4
5
6
7

FIG.5

5) Carbaryl : (Fig. 5)

Equation : $Y = 0.006 + 1.94\ X$

$LD_{20} = 0.1384 \pm 0.001033\ \mu g/fly$

But for carbaryl LD_{20} treatment of above insecticides to the test flies caused 100% knockdown. However, in these treatments, within a span of 4 hrs flies recovered completely. After the revival, of test flies additional 3 hrs were allowed for aclimatization and water satiety. This avoided the possibility of error in the determination of median threshold response (i.e. tarsal response) levels.

Tarsal responses in untreated flies : The data on % tarsal response of the control/untreated flies towards the various concentrations (Molar = M) of sucrose solution were subjected to satistical analyses wherein, the regression of % tarsal response (as probits) on the sucrose concentrations (as log concentration) was studied (Fig. 6). The graph (Fig. 6) showed that there was a positive linear correlation between the % tarsal responses elicited and the concentration of the sucrose offered.

Comparison of tarsal responses in untreated and insecticide treated flies:

Data on, % tarsal response of the insecticide treated flies to the similar (to control) range of sucrose concentrations were also subjected to similar statistical treatment. it was found that inspite of the insecticide treatment (sublethal) the basic pattern of tarsal response (i.e. observed in the flies without treatment) viz. increase in the % tarsal response with the increase in the sucrose concentration (fig. 6) remained essentially unchanged (Fig. 6 Versus fig. 7,8,9,10 and 11). This positive correlation was further confirmed by the observation that the slope values obtained

FIG. 6 CORRELATION BETWEEN THE CONCENTRATIONS OF SUCROSE SOLUTION AND % TARSAL RESPONSE DISPLAYED BY UNTREATED M.domestica.

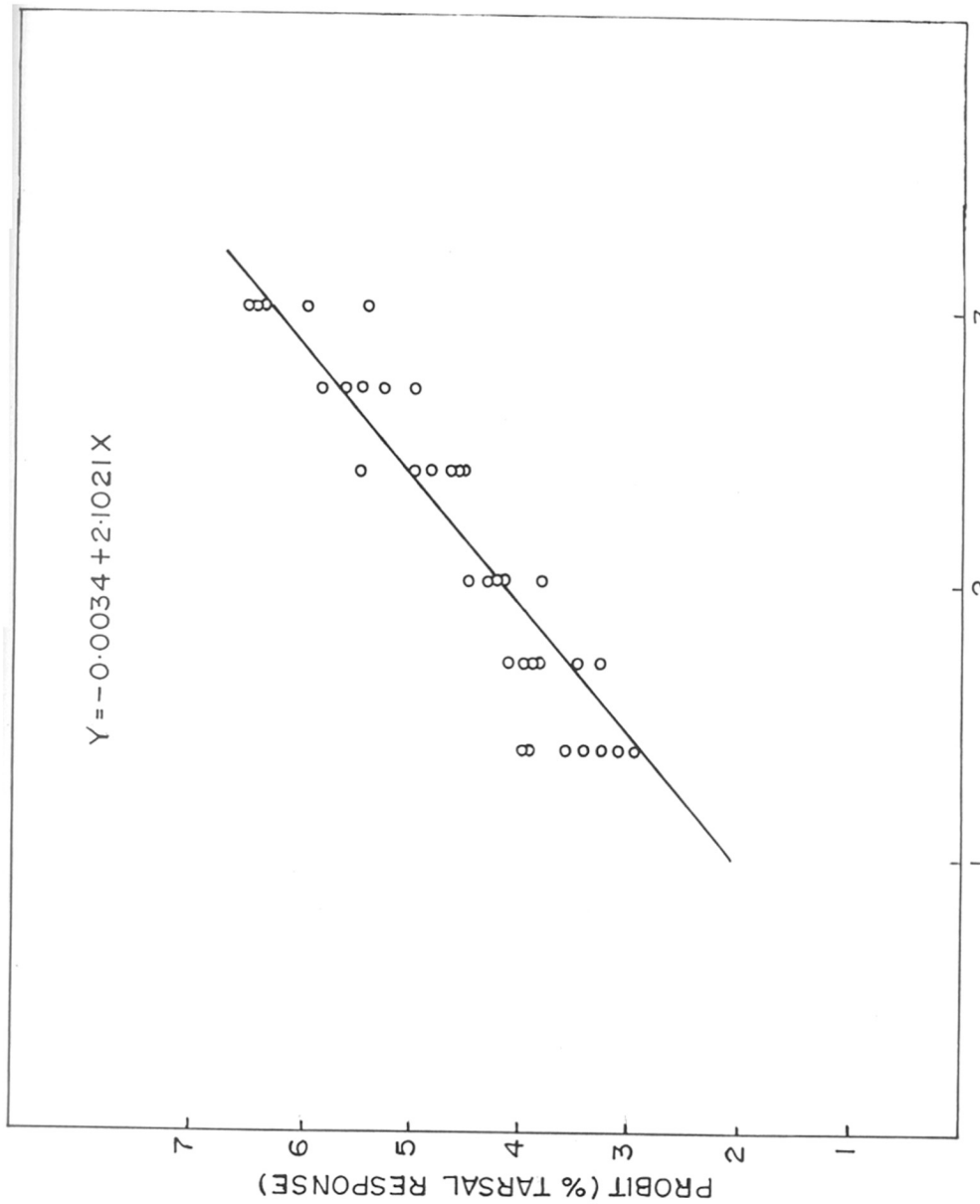

Y = -0·0034 + 2·1021 X
PROBIT (% TARSAL RESPONSE)
LOG CONCENTRATION (MOLAR OF SUCROSE X 10000)
1
2
3
4
5
6
7

FIG. 6

FIG. 7 CORRELATION BETWEEN THE CONCENTRATIONS OF SUCROSE SOLUTION AND % TARSAL RESPONSE DISPLAYED BY PERMETHRIN TREATED *M. domestica*

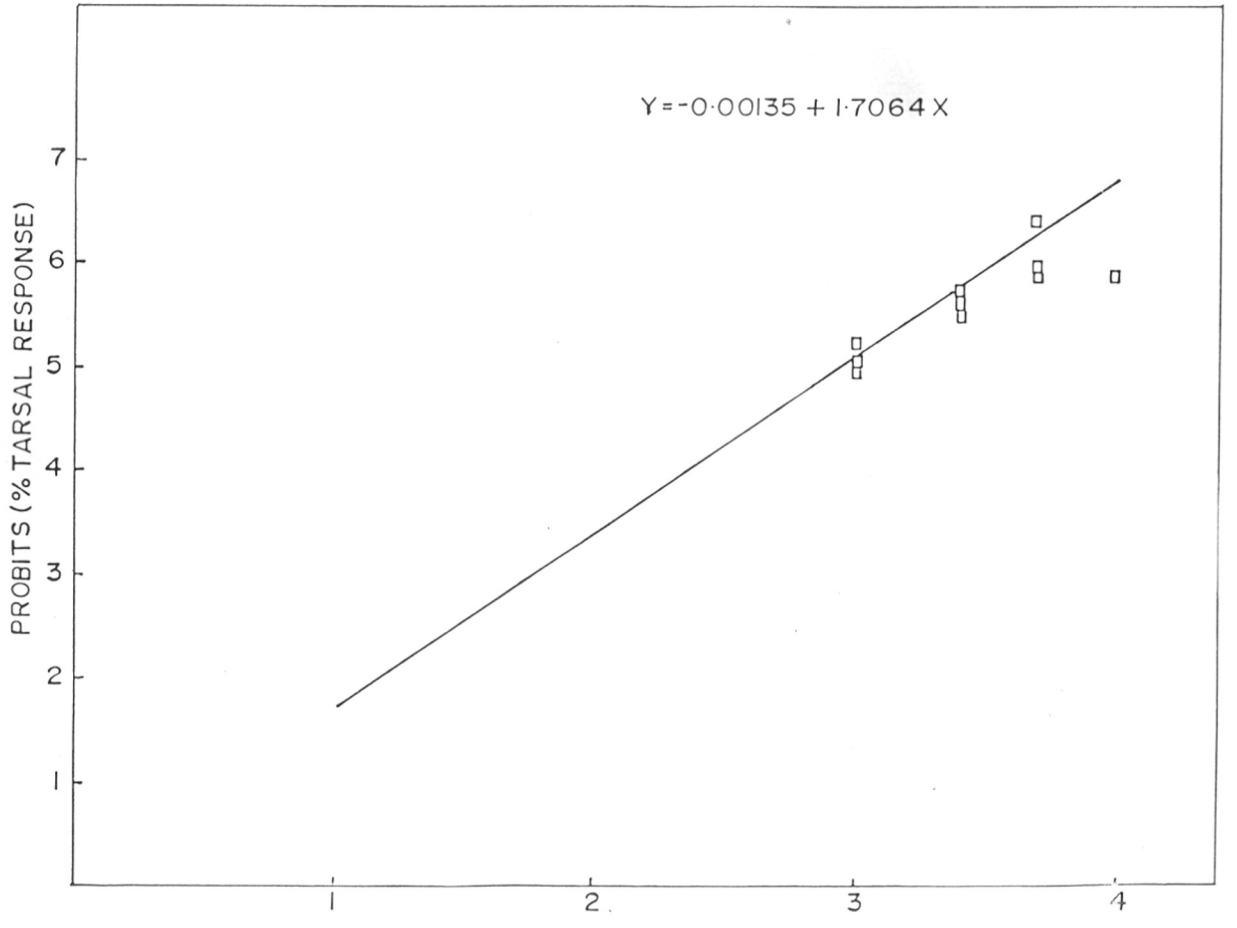

Y=-0·00135 + 1·7064 X
PROBITS (% TARSAL RESPONSE)
7
6
5
4
3
2
1
1
2
3
4
LOG CONCENTRATION (MOLAR SUCROSE X 10000)

FIG.7

FIG. 8 CORRELATION BETWEEN THE CONCENTRATIONS OF SUCROSE SOLUTION AND % TARSAL RESPONSE DISPLAYED BY INDOTHRIN TREATED *M. domestica*.

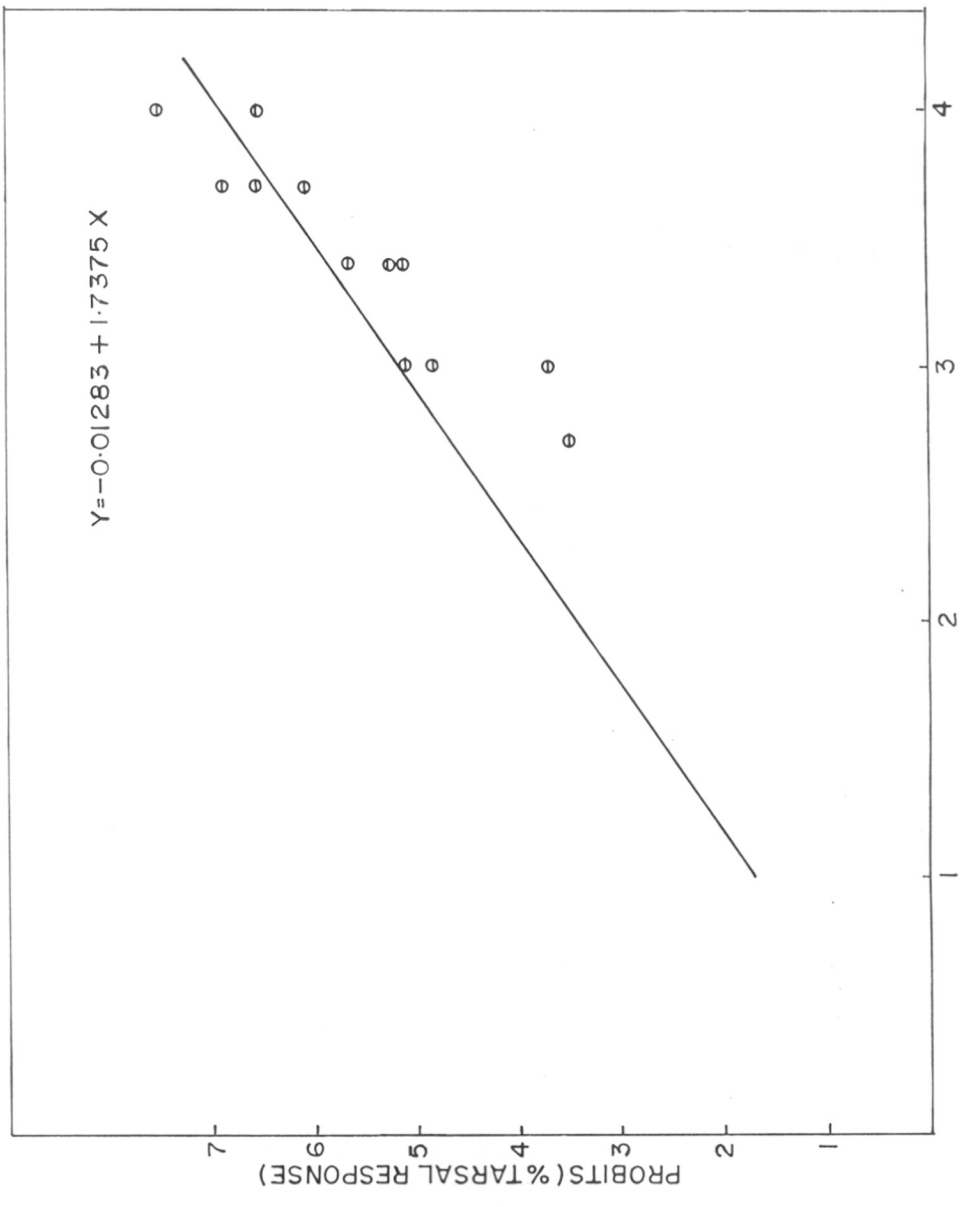

Y=-0·01283 + 1·7375 X
PROBITS (% TARSAL RESPONSE)
LOG CONCENTRATION (MOLAR SUCROSE X10000)
1
2
3
4
2
3
4
5
6
7

FIG.8

FIG. 9 CORRELATION BETWEEN THE CONCENTRATIONS OF SUCROSE SOLUTION AND % TARSAL RESPONSE DISPLAYED BY CYPERMETHRIN TREATED M.dometstica

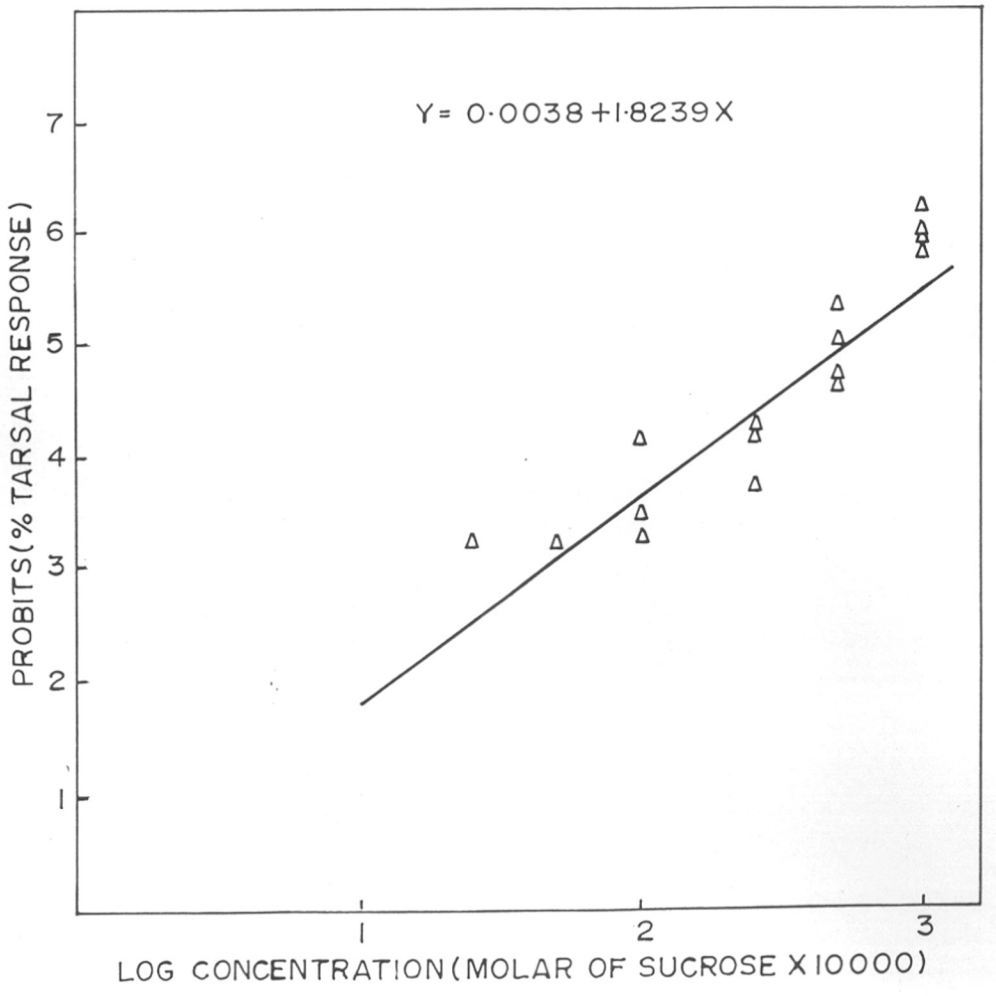

Y= 0·0038+1·8239X
PROBITS(% TARSAL RESPONSE)
7
6
5
4
3
2
1
1
2
3
LOG CONCENTRATION(MOLAR OF SUCROSE X10000)

FIG.9

FIG.10 CORRELATION BETWEEN THE CONCENTRATIONS OF SUCROSE SOLUTION AND % TARSAL RESPONSE DISPLAYED BY ALPHAMETHRIN TREATED M.domestica.

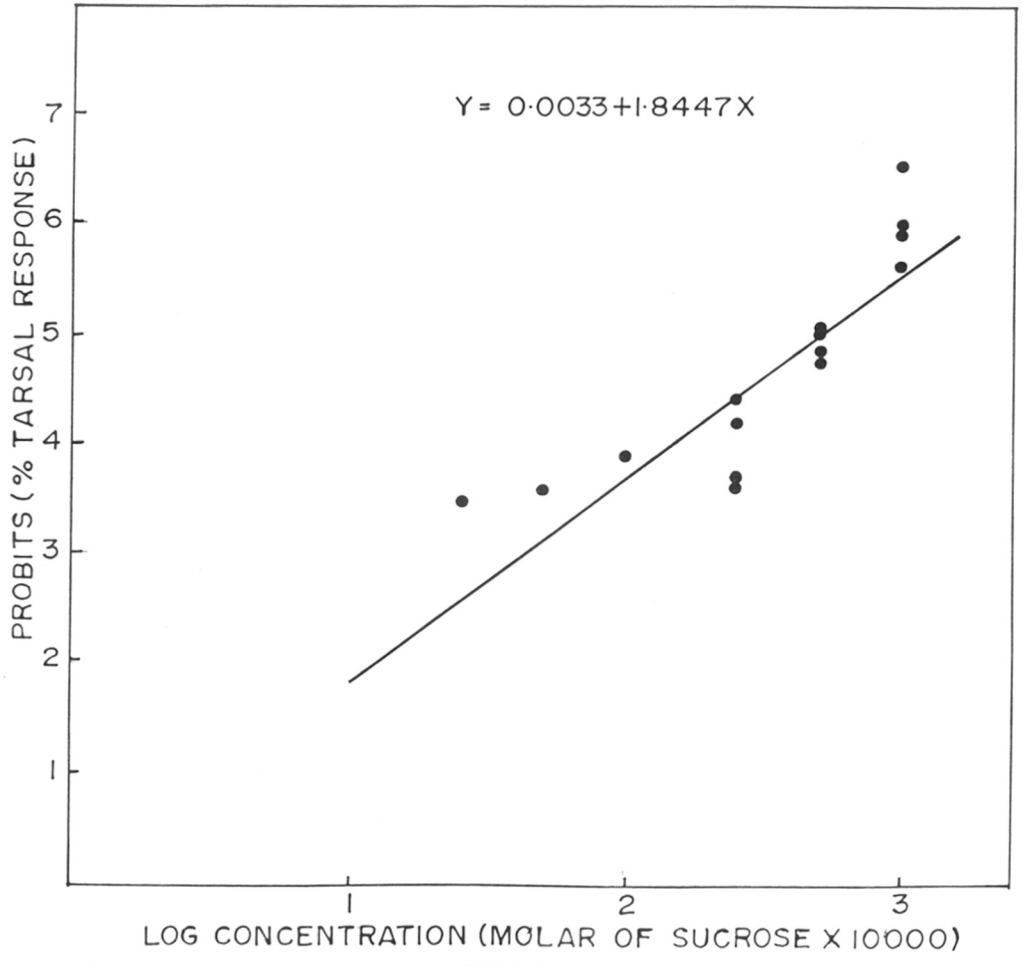

Y= 0·0033+1·8447X
PROBITS (% TARSAL RESPONSE)
7
6
5
4
3
2
1
1
2
3
LOG CONCENTRATION (MOLAR OF SUCROSE X 10000)

FIG.10

FIG.11 CORRELATION BETWEEN THE CONCENTRATIONS OF SUCROSE SOLUTION AND % TARSAL RESPONSE DISPLAYED BY CARBARYL TREATED M.domestica.

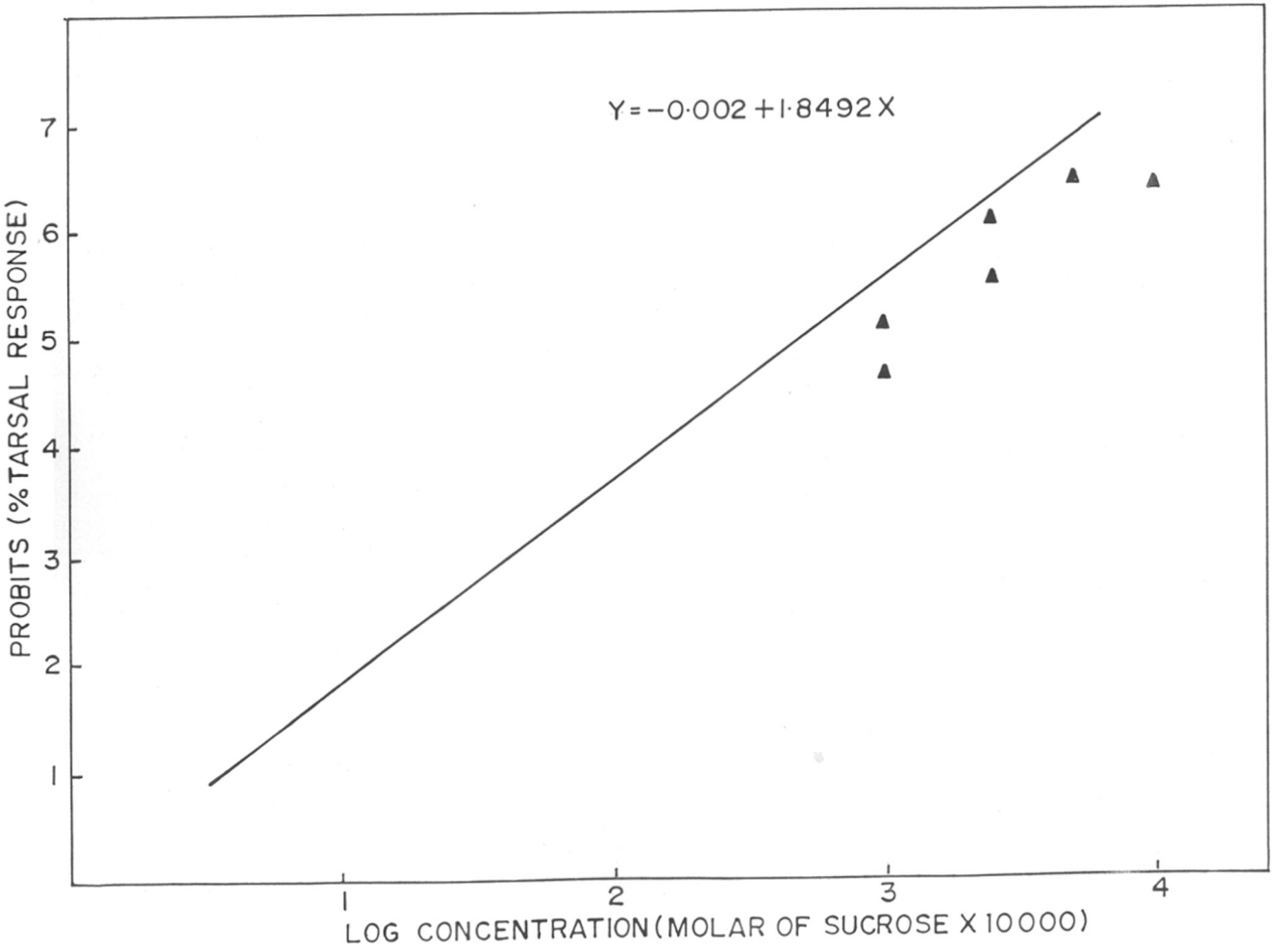

Y=-0·002+1·8492X
PROBITS (%TARSAL RESPONSE)
7
6
5
4
3
2
1
1
2
3
4
LOG CONCENTRATION (MOLAR OF SUCROSE X 10000)

FIG.11

FIG.12 COMPARISON OF ED_{50} FOR SUCROSE SOLUTION IN INSECTICIDE TREATED AND UNTREATED M.domestica .

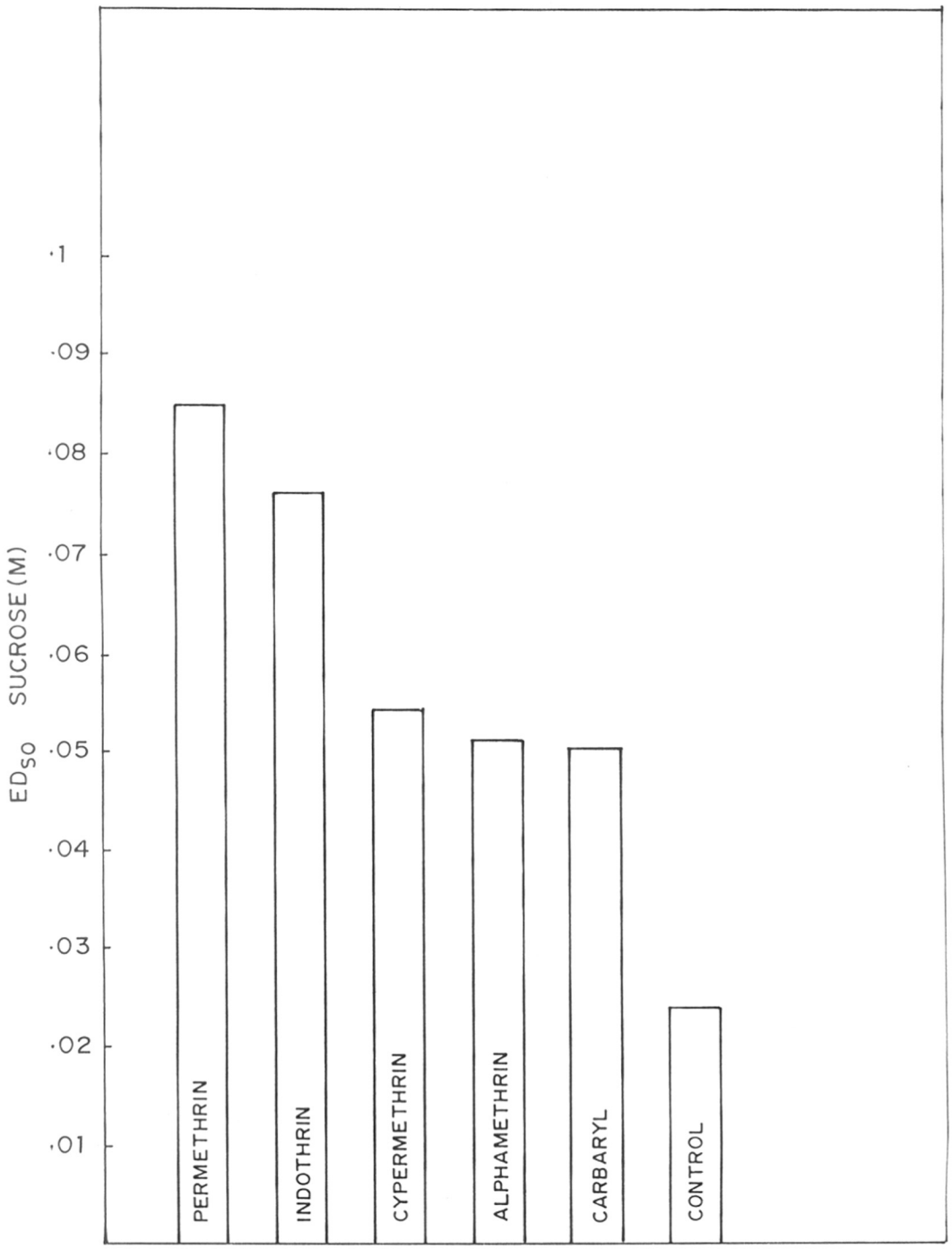

ED50 SUCROSE (M)
·1
·09
·08
·07
·06
·05
·04
·03
·02
·01
PERMETHRIN
INDOTHRIN
CYPERMETHRIN
ALPHAMETHRIN
CARBARYL
CONTROL

FIG.12

both in control and insecticide treated batches were positive.(Table 1; slope). Even though, the basic pattern of tarsal response was similar both in test and control batches, the slope values differed from each other (Table 1, slope). Therefore, median threshold response value or ED_{50} (i.e. the concentration of sucrose solution which elicits tarsal response from 50% of the housefly population) of the control batch was compared to that of the insecticide poisoned batches. The ED_{50} values (Table 1; ED_{50}) of control and Permethrin, Indothrin, Cypermethrin, Alphamethrin, Carbaryl treated batches were derived from the linear regression equations shown in the observation table [Table 1; Equation (ED_{50})] comparative examination of the ED_{50} values obtained in insecticide treated housefly batches with the ED_{50} of control (Table 1, ED_{50}) revealed that insecticide treatment (sublethal) to the houseflies had invariably increased their ED_{50} values. In other words, sublethal treatment of selected carbamate (carbaryl) and pyrethroids (i.e. Permethrin, Indothrin, Cypermethrin, Alphamethrin) to the houseflies had induced decrease in their ability to detect lower sucrose concentrations or general decrease in the chemorceptor sensitivity of the housefly.

Considering, extremely low standard errors of the ED_{50} values obtained for various insecticide treated and control batches (Table 1, ED_{50}), it could be seen from the histogram (Fig. 12) that among the chosen insecticides Permethrin caused maximum increase in ED_{50} of control while, Indothrin, Cypermethrin, Alphamethrin and Carbaryl followed in descending order of their ability to cause increase in ED_{50} value.

TABLE 1 : Comparison of the ED_{50} levels of sucrose induced tarsal responses observed in untreated and insecticide treated, starved, water satiated Musca domestica populations, in relation to the LD_{50} or toxicity of the insecticides

Insecticide used	LD_{50} (µg/fly)	ED_{50} (M sucrose conc.)	b (Slope for ED_{50})	Equation (for ED_{50})	Sublethal dose of the insecticide applied(µg/fly)
A) Pyrethroids					
1) Permethrin	0.007744 ± 0.0001005	0.08529 ± 0.000989	1.7064	Y = -0.00135 + 1.7064 X	0.0038
2) Indothrin	0.00528 ± 0.00010014	0.07675 ± 0.000814	1.7375	Y = -0.01283 + 1.7375 X	0.0027
3) Cypermethrin	0.00229 ± 0.000100054	0.05486 ± 0.0001005	1.8239	Y = 0.0038 + 1.8239 X	0.0014
4) Alphamethrin	0.000824 ± 0.00001002	0.05112 ± 0.0001	1.8447	Y = 0.0033 + 1.8447 X	0.00039
B) Carbamate					
1) Carbaryl	0.37517 ± 0.0010039	0.05069 ± 0.000929	1.8492	Y = -0.002 + 1.8492 X	0.1384
C) Control	-	0.0239 ± 0.0001	2.1021	Y = -0.0034 + 2.1021 X	-

Comparative examination of the relative toxicities (i.e. Table 1; LD_{50}; Pyrethroids) of the selected pyrethroids and their corresponding ED_{50} values (Table 1; ED_{50}; Pyrethroids) revealed that Permethrin which was relatively least toxic (i.e. LD_{50} : 0.007744 ± 0.0001005 µg/fly) had caused highest level of tarsal response suppression (and therefore, had highest ED_{50} value i.e. 0.08529 ± 0.000898 M.Sucrose) while, Alphamethrin relatively most toxic (i.e. LD_{50} : 0.000824 ± 0.00001002 µg/fly) caused lowest level of tarsal response suppression. (hence, lowest ED_{50} value i.e. 0.05112 ± 0.0001 M sucrose). The remaining pyrethroids Indothrin and Cypermethrin which showed intermediate level of toxicity (Table 1; LD_{50}) showed intermediate levels of tarsal response suppression (hence intermediate level of ED_{50} values, Table 1; ED_{50})

On the other hand, Carbaryl which was relatively least toxic insecticide also caused least suppression of tarsal response (Table 1; Carbamates : LD_{50} Versus ED_{50}).

Section (B)

Comparison of mating responses observed in normal and JHA affected adult population of the red cotton bug

Preliminary tests involving measurement of % morphological inhibition of wing development (% MI) caused by the topical treatment of the selected juvenoids to the fifth instar nymphs, revealed that to produce maximum % MI (i.e. 100% or formation of supernumerary larva) the dose required by juvenoids A5, A7, A4 was 5 µg (Table 2) which was three times lesser than that of A1, A3, A6 and A2. (Table 2). On the basis of afore-

TABLE 2 Comparison of mating responses observed in normal and juvenoid (JHA) affected adults of Dysdercus koenigii obtained from the fifth instar nymphs exposed to sub-lethal doses of various synthetic JHAs

Juvenoid used	Dose (µg/v instar)	% Morphological inhibition	Total mean mating response (/30min/day : 1-12 days)		% Mating repression (% M.R.)	Ratio (% M.R./dose)
			Mean precopulatory period T (min)	Mean % matings (% m.f.)		
Control I	Nil	0.0	3.0 ± 0.5	63.9 ± 6.5	0.0	-
II	Acetone	0.0	3.2 ± 0.5 a	61.77 ± 7.03 d	0.0	-
A_5	5.0	100	-	-	-	-
	1.0	0.0	3.71 ± 0.66	61.85 ± 9.69 e	0.0	
	2.0	0.0	3.87 ± 0.83	35.53 ± 4.37 f	42.48	21.24
	2.5	0.0	5.81 ± 1.25 q	34.58 ± 6.05 g	44.02	17.61
A_7	5.0	100	-	-	-	-
	2.0	0.0	7.15 ± 1.35 r	37.04 ± 6.76 h	40.04	20.02
A_4	5.0	100	-	-	-	-
	2.0	0.0	3.45 ± 0.63	47.92 ± 9.54 i		
	2.75	0.0	9.31 ± 1.29 s	36.81 ± 6.05 j	40.41	14.7
A_1	15.0	100	-	-	-	-
	4.0	0.0	7.61 ± 1.02 t	34.83 ± 4.35 k	43.62	10.91
	5.5	0.0	6.5 ± 1.1 u	35.56 ± 8.27 l	42.44	7.71
	6.0	0.0	8.59 ± 0.68 v	38.57 ± 5.76 m	37.56	6.26
A_3	15.0	100	-	-	-	-
	6.0	0.0	4.09 ± 0.49 w	45.13 ± 4.66 n	26.93	4.49
A_6	15.0	100	-	-	-	-
	7.0	0.0	5.47 ± 0.91 x	42.37 ± 7.76 o	31.41	4.49
A_2	15.0	100	-	-	-	-
	6.0	0.0	6.2 ± 2.01 y	48.17 ± 2.93 p	22.02	3.67

e not significantly different from 'd' ($P > 0.05$), where, $t = 0.01$, $df = 22$

f significantly lower than 'd', where, $t = 2.36$, ($P < 0.025$) at df = 22

g significantly lower than 'd' where, $t = 2.87$ ($P < 0.005$) at df = 22

h significantly lower than 'd' where, $t = 2.48$, ($P < 0.025$) at df = 22

i not significantly different than 'd' where, $t = 1.14$, ($P > 0.05$) at df = 22

j significantly lower than 'd' where, $t = 2.63$, ($P < 0.01$) at df = 22

k significantly lower than 'd' where, $t = 3.19$ ($P < 0.005$) at df = 22

l significantly lower than 'd' where, $t = 2.36$, ($P < 0.025$) at df = 22

m significantly lower than 'd' where, $t = 2.5$, ($P < 0.025$) at df = 22

n significantly lower than 'd' where, $t = 1.96$ ($P < 0.05$) at df = 22

o significantly lower than 'd' where, $t = 1.81$, ($P < 0.05$) at df = 22

p significantly lower than 'd' where, $t = 1.78$ ($P < 0.02$) at df = 22

q significantly higher than 'a' where, $t = 1.9151$, ($P < 0.05$) at df = 22

r significantly higher than 'a' where, $t = 2.6495$, ($P < 0.01$) at df = 22

s significantly higher than 'a' where, $t = 3.7760$, ($P < 0.005$) at df = 22

t significantly higher than 'a' where, $t = 3.5413$, ($P < 0.005$) at df = 22

u significantly higher than 'a' where, $t = 2.6228$, ($P < 0.01$) at df = 22

v significantly higher than 'a' where, $t = 5.0458$, ($P < 0.0005$) at df = 22

w not significantly different from 'a' where $t = 1.2701$, ($P > 0.05$) df = 22

x significantly higher than 'a' where, $t = 1.9579$, ($P < 0.05$) at df = 22

y significantly higher than 'a' where, $t = 2.4799$, ($P < 0.025$) at df = 22

mentined observations it could be infered that the former group of juvenoids has relatively more JH activity than the latter. Consequently, comparison of sublethal dosages (ones which produced no morphological inhibition [0% MI] but caused significant repression in the mating behavior of the emerged adults) obtained for selected juvenoids also showed that the sublethal dose range of 2 - 2.75 µg/v, obtained for A5, A7 and A4 was comparatively lower than that of (i.e., 6-7 µg/v) A1, A3, A6 and A7 (Table 2, dose).

Comparison of the mean % matings (between 1-12 days) observed in control I and II (Table 2, % m.f.) showed that acetone treatment to fifth instars did not affect the mating activity of emerged adults. Therefore, mean % matings in control II (Table 2, % m.f. : d) were compared to that of the test populations, in the subsequent experiments. Comparison of mean % matings observed in A5 & A4 to that of the control population clearly demonstrated the process of induction of mating repression. Thus, both A5 and A4 at respective sublethal dosages 1 and 2 µg/v, caused no repression in the mean mating activity (Table 2 : % m.f. : e Versus d, i Versus d) however, by increasing the sublethal dosages to 2, 2.5 and 2.75 µg/v in respective JHAs, statistically significant reduction in the mating activity was achieved. (Table 2, % m.f. : f Versus d, g Versus d and j Versus d) which was further denoted as % mating repression (Table 2, % M.R. : A5, A4).

The dose dependent induction of mating repression as observed incase of A5 and A4 (Table 2; % M.R. for Doses, 1,2,2.5 and 2, 2.75 µg/v) however, was not of common occurrence because, incase of A1 inspite of wide range of ascending dosages (i.e. 4 to 6 µg/v) chosen the value

of the % mating repression went on decline (Table 2; % M.R. for A1 at Doses 4, 5.5 and 6 µg/v) nevertheless,they did induce substantial amount of reduction in the mean mating activity when compared with control (Table 2; % m.f. : k Versus d, l Versus d and m Versus d). Unlike A5 and A4 the level of % mating repression obtained by remaining JHAs was not directly proportional to the dose applied. Therefore, only the sublethal doses which induced maximum mating repression were chosen as "representative doses" for these compounds (A7, A3, A6 and A2). Here too, mean % mating activities observed in the adult populations treated by these compounds were found to be significantly lower than that of the control population (Table-2 % m.f.: h Versus d; n Versus d, O Versus d, and p Versus d)

Perusal of the data on % mating repression values obtained for the various juvenoids showed (Table 2, % M.R.) that A5 and A1 induced identical % M.R. (42.4%). However, the doses required to induce this % M.R. were entirely different. (2.0 µg for A5 and 5.5 µg for A1 - Table 2). It was felt that such disparities could be resolved by calculating the ratio of % M.R. to the dose (Table 2; Ratio). This 'Ratio' was then used as an index to compare the relative potencies of the selected juvenoids. Examination of the ratio values (Table 2; Ratio) obtained for the "representative doses" of respective juvenoids clearly showed that juvenoid A5 induced maximum mating repression, (Ratio : 21:24) closely followed by A7 (Ratio : 20.20) while, A2 caused the least amount of mating repression denoted (ratio : 3.67) among the series. The juvenoids A4, A1, A3 and A6 (in descending order of ratio. values) showed intermediate level of mating repression activity (table 2; Ratio : A4 to A6). This order of mating redpression activity could also be deemed to reflect the order of biolgical potency of these juvenoids.

In addition to the mean % matings, another parameter viz. mean precopulatory period (in minutes) was also studied simultaneously and variations in this parameter were examined both in the JHA affected and control populations. Barring the sole exception of A3 all other JHAs (which had also caused repression of mating activity) were found to cause statistically significant increase in the mean precopulatory period of the JHA affected populations (Table 2 ; T (min) : q Versus a, r Versus a, s Versus a, t Versus a, u Versus a, v Versus a, x Versus a and y Versus a) when compared with the mean precopulatory period of control population.

CHAPTER 4

DISCUSSION

Section (A)

Effect of insecticide poisoning on tarsal response of housefly

On the basis of octanol water partition coefficient the four classes of insecticides can be arranged in descending order of their polarity[99]. The carbamates being most polar top the list followed by organophosphates. Most organochlorine insecticides occupy the third position and the least polar are the most non-polar pyrethroids which occupy the fourth position. It follows that carbamates and organophosphates possess systemic and translaminar properties by virtue of their high polarity and resultant water solubility. The relatively less polar organochlorines do not posses systemic properties but have high residual activity. Pyrethroids, being the least polar are practically insoluble in water and hence do not posses systemic property but unfortunately, they are photolabile and hence non-persistent. In the present work the carbamates and pyrethroids with such contradictory properties have been evaluated for effects on a common behavioral (gustatory) response in the housefly.

Most of the synthetic organic insecticides are largely neurotoxic in nature. Subtle changes in the excitatory state of nervous system of insects treated with sublethal doses of such insecticides may be reasonably expected. Dethier (1952)[100] observed that proboscis extension reflex of dipterous insects could be taken as a reliable index of the excitatory state of the central nervous system. Using a simple experimental design wherein various concentrations of sucrose solution were used

as sources of chemical stimuli. The labellar responses (as measured by the proboscis extension reflexes) of untreated versus insecticide treated (at sublethal dose level) houseflies were compared[101]. It was found that DTT treatment to susceptible flies resulted in lowering of their behavioral threshold[102]. This however, was not true incase of DDT resistant flies. Leski and Cutkomp (1962)[103] on the basis of electrophysiological experiments carried out on the labellar hairs of flies viz. Musca domestica L and Phormia regina (Meig), demonstrated increase in the frequency of spikes in organophosphate treated flies and argued that labellar response is an index of excitatory state of both central as well as the peripheral nervous system of the insect. This argument was supported by the works of Soliman and Cutkomp (1963)[104] wherein both behavioral bioassay and electrophysiological techniques were employed to evaluate the effects of DDT and parathion on chemoreceptor sensitivity of housefly. They found that parathion had no effect on chemoreceptor sensitivity until the hyperactive stage whereafter, there was complete cessation of the response till death ensued. DDT on the other hand, initially induced increase in sensitivity of the labellar chemoreceptors followed by failure of response till death. They attributed this unique response towards DDT to it's probable mechanism of action. Barton Browne and Kerr (1967)[105] carried out electrophysiological analyses of the labellar taste receptors of resistant and sensitive strains of houseflies and found that the former showed higher behavioral thresholds. They concluded that resistance was fully expressed at the level of chemoreceptor hairs and was due mainly to the ability to recover from DDT poisoning. Using labellar response technique Sharma (1973)[9] examined effects of dieldrin on susceptible and resistant strains of housefly and found that both

in suscedptible and resistant dieldrin treated flies ED_{50} values of sucrose were lowered below the level exhibited by untreated/control flies. Overall patterns of dieldrin induced alterations in chemosensitivity were similar in both the strains except for the difference in the doses of insecticide required to produce these changes.

Apart from one preliminary report from the author's laboratory[106] there are no reports on the effect of pyrethroids and carbamates on the tarsal responses of housefly. The results of the present work indicate that pyrethroids and carbamates, regardless of their different modes of insecticidal action[107] such as former affecting sodium channels in the nerve membrane and the latter inhibitindg enzymatic action of acetylcholinesterase, have produced common inhibitory effects on the tarsal responses of the housefly. However, this phenomenon of decrease in the chemoreceptor sensitivity by sublethal treatments with selected pyrethroids and carbamates is exactly opposite to the hyper sensitivity induced by DDT[104] and dieldrin[9].

While comparing the amount of increase in ED_{50} value over that of control with their relative insecticidal activity (LD_{50}) it is observed that Permethrin which is least toxic (LD_{50} 0.007744 ± 0.0001005 µg/fly) causes maximum increase in the ED_{50} to sucrose. On the other hand, Alphamethrin which appears among them as most toxic (LD_{50} 0.000824 ± 0.000010002 µg/fly) produces least increase in ED_{50} value. It would therefore appear that suppression of tarsal responses cannot be correlated with insecticidal activity of the pyrethroids. This finds support from the observation that there does not seem to be significant correlation between the ability of the pyrethroids to knock insects down and their

ability to kill them[108]. Basically insect knock down is attributed to the effect of pyrethroids on the sensory neurons[109]. In this study also, the proboscis extension response intrinsically depends upon the impulses received from the chemosensory neurons located in the tarsi. Therefore, it is difficult to explain the lethal action (LD_{50}) of pyrethroids solely on the basis of their effects on sensory neurons or the reflexes dependent on them (viz. tarsal responses)[108]. However, this investigation has furnished additional information on hitherto unknown effects of sublethal dosing with two classes of insecticides (viz. Pyrethroids and carbamates) on the tarsal responses of the housefly. The validity and importance of simple behvioral bioassays such as the proboscis extension reflex used herein stand reiterated as powerful tool for probing ancillary but nevertheless physiologically significant effects of chemicals such as the insecticides.

Section (B)

Effect of nymphal exposure to sublethal doses of juvenoids on mating behavior of the emergent adults of the red cotton bug.

Apart from confirming the earlier findings[10] on mating repression in JHA affected D.koenigii adults, the present investigation has cleared some of the doubts expressed in that work. The results in this work have demonstrated conclusively that the reported mating activity repression induced by Hydroprene, Kinoprene Methoprene sublethal treatments is not an exclusive phenomenon, confined to the outstanding potential of these juvenoids but it occurs even in other chemically unrelated (i.e. geraniol-diethers) juvenoids as well. On the basis of earlier

report[10] the most potent juvenoid, Methoprene produced about 48.3% mating repression at the dose of 0.0025 µg/v. On the other hand, the most active juvenoid, A5 examined in the present investigation produced near about similar (i.e. 44.02% M.R.) mating repression at the dose 2.5 µg/v. Thus, it is clear that JHA A5, which is about 1000 times less active than Methoprene is also capable of inducing mating activity repression in the red cotton bug. Therefore, it can be infered that induction of mating repression is a characteristic phenomenon related to the juvenilizing property of the compound and does not depend upon it's bioloical potency. The present investigation also brings support to the earlier view[10] that the parameter "mating activity repression" can be used as a supplementary behavioral bioassay tool to evaluate the biological activity of small quantities of juvenoids.

According to the earlier findings[10] the mean value of the pre-copulatory period observed in the JHA affected population was not significantly different than the untreated/control population. On the other hand, the results in the present study showed that apart from one juvenoid (i.e. A3) the values of the mean precopulatory period in the remaining six juvenoid affected populations were significantly higher than that of control. These observations also suggest that apart from mean % matings (% m.f.) the parameter mean precopulatory period (T) can also be employed for comprehensive evaluation of repression of mating responses by sublethal treatment of juvenoid.

Phenomenon of repression of adult mating activity due to the larval exposure to the sublethal doses of juvenoids was first reported in lepidoptera[92], followed by dictyoptera[93,94] and hemiptera[10,11]. However, this phenomenon can be explained on a common basis of hormonal

physiology. High titer of JH represses the expression of adult genes[110]. However, in the terminal instar the CA cease or drastically reduce the production of JH leading to the subsequent adult metamorphosis[111]. Exogenous application of JHA causes considerable increase in JH titer in the terminal instar/nymph resulting in inhibition of the realization of adult characters and retention of nymphal characters. In P.americana nymphs, application of exogenous JHA prevents the development of adult antennal sensilla[112] consequently, leading to the failure of perception of odorants by male[93]. As repression in mating activity of JHA affected D.koenigii population is basically due to the reduced sexual activity of the affected male[113]. It may be reasonably inferred that inability of the male to display normal level of mating activity may be due to retention of nymphal sensilla on it's antennae or occurrence of both the adult and hymphal antennal receptors which would not be able to perceive the female sex pheromone[113] entirely or perfectly. Possibility of JHA induced anatomical deformities in the copulatory organs impairing the process of copulation and causing mating repression is ruled out because such deformity is observed at higher dosages (causing 30-50%MI) while, dosages used in these experi ments are sublethal (causing 0%MI) Inhibition of adult antennal sensory hairs due to the exogenous application of sublethal doses of juvenoids to the fifth instar of D.koenigii is most plausible reason for the observed mating repression because in another hemipteran bug, Rhodnius Wigglesworth (1964)[114] has reported that the dose of JHA required to produce morphological deformities in the development of wings is substantially higher than the dosages required to produce other morphological deformities. On the other hand, in Periplaneta americana the dose of the juvenoid required to produce

antennae with nymphal complement of antennal sensilla was higher than the dose required to produce deformities in the wings[115].

The possibility of suppressing the mating activity of the D.koenigii adults by exposing their earlier nymphal stages (viz. V instar) to relatively low amounts of JHAs appears to be attractive proposal from the point of integrated pest management. However, the effect of this mating repression on the fecundity and pest propagation etc. should be investigated. In any case, pest control strategy involving use of juvenoids should include examination of sublethal effects on the exploitable behavior patterns and subtle changes in the development of morphology of sensory organs.

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