# PHYSIO - TOXICOLOGICAL STUDIES ON THE EFFECTS OF DIFLUBENZURON ON SOME LEPIDOPTEROUS PESTS

A

THESIS

Presented to the

**SUKHADIA UNIVERSITY**

For The Degree

Doctor of Philosophy

By

**MADAN MOHAN SHARMA**

**1986**

SUKHADIA UNIVERSITY

UDAIPUR

CERTIFICATE I

DATED : April, 1986

THIS IS TO CERTIFY THAT THIS THESIS ENTITLED " PHYSIO-TOXICOLOGICAL STUDIES ON THE EFFECTS OF DIFLUBENZURON ON SOME LEPIDOPTEROUS PESTS " SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE SUBJECT OF ENTOMOLOGY OF THE SUKHADIA UNIVERSITY, UDAIPUR IS A BONAFIDE RESEARCH WORK CARRIED OUT BY MR. MADAN MOHAN SHARMA UNDER MY SUPERVISION AND THAT NO PART OF THIS THESIS HAS BEEN SUBMITTED FOR ANY OTHER DEGREE. THE ASSISTANCE AND HELP RECEIVED DURING THE COURSE OF INVESTIGATION HAVE BEEN FULLY ACKNOWLEDGED.

14/5

HEAD
DEPARTMENT OF ENTOMOLOGY

14/5

ADVISOR

16.5.86

DEAN
RAJASTHAN COLLEGE OF AGRICULTURE
UDAIPUR

SUKHADIA UNIVERSITY
UDAIPUR

CERTIFICATE II

DATED: 23.2.87

THIS IS TO CERTIFY THAT THE THESIS ENTITLED "PHYSIO-TOXICOLOGICAL STUDIES ON THE EFFECTS OF DIFLUBENZURON ON SOME LEPIDOPTEROUS PESTS" SUBMITTED BY MR. MADAN MOHAN SHARMA TO THE SUKHADIA UNIVERSITY, UDAIPUR IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE SUBJECT OF ENTOMOLOGY HAS BEEN APPROVED BY THE COMMITTEE AFTER AN ORAL EXAMINATION OF THE SAME ON 23.2.87

ADVISOR

HEAD
DEPARTMENT OF ENTOMOLOGY

APPROVED

DEAN
POST GRADUATE STUDIES
FACULTY OF AGRICULTURE
SUKHADIA UNIVERSITY
UDAIPUR

COMMITTEE MEMBER

1.

2. V.J. Sukhadia

3. Parmathma

ACKNOWLEDGEMENT

I take this opportunity to express my deep sense of gratitude to Rev. Dr.R.P. Srivastava, Professor and Head, Department of Entomology, Rajasthan College of Agriculture, Udaipur for his able guidance, helpful suggestions, constructive criticism, indefatigable interest and unfailing help during the course of this investigation and reconstruction of the manuscript.

I am extremely grateful to Dr.M.S. Manohar, Dean, Rajasthan College of Agriculture, Udaipur for providing the necessary facilities during the course of investigation.

I am highly grateful to Dr.R.C.Srivastava, Assistant Professor, A.R.S. Durgapura (Jaipur) who deserves special mention for his everwilling generous help and creative suggestions during the experimentation and preparation of the manuscript. I am also, grateful to Dr.H.C.L.Gupta, Toxicologist, and Dr.P.N. Mathur, Biochemist, R.C.A. Udaipur for their timely help during the course of investigation.

I also feel highly graced to express my regards to Dr.V.G. Gokhale, Ornithologist, A.R.S. Kota, Dr.R.C. Saxena, Maize Entomologist and Dr.D.L. Lavti , Assistant Professor, Soil Science, R.C.A. Udaipur for their affectionate inspiration and constant encouragement.

I am thankful to Dr.V.J. Shrikhande, Professor and Head, Shri M.S. Bhatnagar, Assistant Professor, Statistics, Udaipur; Mr. R. Swaminathan, Assistant Entomologist (Date Palm), Bikaner and Mr.V.K. Agarwal, Research Associate, A.R.S., Durgapura for their valuable advice and help in statistical evaluation of the results and to Dr.N.M. Mathur, Assistant Entomologist (Maize), Udaipur for his critical suggestions made during preparation of manuscript. Thanks are also due to all Departmental colleagues for their co-operation and help throughout the course of this work and to M/s. Mysore Insecticides Co. Pvt. Ltd., Madras for supplying the chemical diflubenzuron as gratis.

At this occasion, I must not forget to acknowledge the words of inspiration and constant encouragement by Mrs. Dr. R.P. Srivastava, my relatives and friends; and their desire to see this task reach its destination.

My heart felt thanks are also due to my wife who managed all homely responsibilities with passion and emotively added to my perseverance enough to attain this academic achievement.

Above all, I render my reverence to the Lord Almighty whose blessings could bring me upto the goal and to whom I very humbly dedicate my academic achievements.

(M.M. Sharma)

Udaipur
Dated : 17th April, 1986

C O N T E N T S

Page

LIST OF TABLES

-o-

LIST OF FIGURES



--o--

LIST OF PLATES

Fig. 3 Transverse section of the integument of S. litura larva 72 hrs after DFB treatment

Fig. 4 Transverse section of the integument of S. litura larva 96 hrs after DFB treatment

-o-

# INTRODUCTION

Since the dawn of human civilization man has been endeavouring to harvest a pest-free crop. The birth, use and ultimate commercialization of pesticides on an ever increasing scale, more so of chemical insecticides, got built-up as powerful aids in achieving this ever cherished goal - rather a dream of human society. When one insecticide, after a little initial success, failed, yet another - a stronger and more potent one, was discovered to make the crop free from pests. Whether we could achieve 'a pest free crop' so far or not, is a big question that still awaits a convincing answer, yet one thing is certain that the agriculturists today keep a heavy reliance on the use of chemical insecticides (Lewo 1982). This has imperceptably infused more and more poisons in the biosphere causing unforeseen physical, mental and genetical dangers not only to living but also to coming generations of human beings. Consequently to avoid and check the building up of these dangers into future catastrophes new methods of pest control are being developed. Lately the use of IGRs, which include besides JH and their mimics, 'the chitin inhibitors' also, have shown a promise as safer, better and economical agents of controlling the insect pests.

The discovery of 'chitin inhibitors' had its roots in the study of those agents which inhibited growth and metamorphosis of insects. Some of the hypocholesterolemic agents and azasteroids had been known to inhibit the growth and metamorphosis of insects (Svoboda and Robin, 1967; Thompson and Robins, 1972) by interfering with biosynthesis of steroids (Svboda et al., 1975). Recently, phenylureas have been known to inhibit the deposition of insect chitin and prevent the growth by imposing difficulties in moulting (Post and Vincent, 1973). Available literature shows that most widely studied member of phenyl-urea-group is 1-4(-chlorophenyl)-3-(2,6-difluorobenzoyl)urea, commonly known as Dimilin. It interferes with chitin deposition and as such has a powerful action on eggs also, making it difficult for the young larvae to hatch out.

It has yet another edge over the conventional insecticides : it has low toxicity to mammals (Anonymous, 1974) and fishes, no toxicity to birds and does not accumulate in food chain to an appreciable extent. It has no apparent teratogenic or mutagenic effects too (Bijloo, 1975). It is fairly persistant but somewhat slow in action (Bijloo, 1975 and Busschbach 1975).

In order to assess the effectiveness of Dimilin and its ultimate economic utility as a potent means to control the pests, without adding any further poison to our biosphere,

the present study had been taken up on two serious pests with divergent morphological and behavioural attributes - viz., S. litura and E. virgincula. The former is without hairs whereas, the latter bears a thick layer of hairs on its body. The former causes serious damage to many economically important crops including cotton, tobacco, groundnut, castor, tomato, cabbage and other cole crops while the latter causes extensive damage to the leaves of cotton, pigeon pea, cowpea, castor and fruit trees. The selection of these noxious pests as test insects also provide an opportunity to compare the effects of diflubenzuron on the insects having differences in their outer protective coverings - an aspect which has a direct bearing on the control of hairy caterpillars which are normally difficult to be controlled with traditional insecticides.

Since diflubenzuron is the outcome of very recent investigations, a little is known about the details of its actual modus operandi on different insects and as such available literature is also very sketchy.

In view of the above facts the present investigation had been planned with the following objectives :-

1. To assess the toxicological effectiveness on two morphologically and ethologically divergent pests of economic importance by evaluating dermal and oral toxicities.

2. To investigate symptomatic and behavioural changes alongwith morphological deformities through the production of abnormal stages and to assess the extent of damage in the test insects.

3. To determine the immediate and cumulative mortalities alongwith inhibition of regular expected metamorphic changes at different developmental stages viz., eggs, larvae, pupae and adults.

4. To estimate the effectiveness and persistence of DFB on the test insects through field evaluation.

5. To assess the modus operandi of DFB in bringing about inhibition of chitin deposition through histopathological and biochemical investigations (to be carried out on S. litura only).

6. To evaluate the possibility of DFB as an alternative to conventional insecticides for the control of test insects/or the use of DFB as a major effective link in the management of the test insects.

* *

# REVIEW OF LITERATURE

The literature pertaining to diflubenzuron (DFB)* and other representatives of phenylurea group has been reviewed stressing only those aspects which have a direct bearing with the present investigation.

Toxicity of DFB :

This caption deals with various aspects of the toxicity of DFB viz., contact toxicity, stomach poisoning, systemic action, repellency and mode of action. However, no separate headings have been given to either of these aspects due to scanty information available on them. Evidently, the text has been presented in a chronological sequence of the dates of the publication of the references.

The earliest traceable information on DFB dates back to the eight decade of this century. It was Vaan Daalen et al., in 1972, who for the first time observed symptoms of poisoning by this compound as a moulting inhibitor in Schistocerca gregaria, Dysdercus supersticiosus and Leptinotarsa decemlineata. Subsequent to this finding a large group of phenylureas was synthetised and tested for insecticidal activity by Wellinga et al. (1973). In the same year (1973) Mulder and Gijswijt evaluated PH 60-38 and

---

* Code names : PH 60-40, TH 6040, DU 112307, ENT - 29054, OMS 1804, PDD 6040 I. Registered trade mark: DIMILIN

PH 60-40 and reported that larvae of Leptinotarsa decemlineata, Pieris brassicae, Baratra brassicae and Musca domestica when fed on treated food were susceptible to PH 60-40; however, young ones of Aphis fabae were not affected. They further maintained that topically treated and sprayed larvae of P. brassicae with aquous suspension of PH 60-40 completed development without showing adverse effects. Their results inferred that PH 60-40 was only a good stomach poison having no contact toxicity or systemic effects in plants. They also noted that larvae of Pieris brassicae treated with the chemical PH 60-40 remained seemingly unaffected till the setting up of the process of apolysis but failed to wriggle out of their exuviae; they exuded their body fluids, became black and died. The insecticidal activity of this chemical, according to these authors, was mainly due to failure of moulting or inability to pupate, resulting in death. They attributed the cause of this failure to defect in the process of cuticle deposition. They also found lesions in the endocuticular tissue of affected larvae.

Post and Vincent (1973), although could not give the exact nature of reactions of diflubenzuron in Pieris brassicae, yet their observations proved an enhancement to our knowledge; they found that it affected the chitin synthesis and appeared to have inhibited the deposition of chitin thereby causing death during moulting. They used this compound as a controlling agent against this noxious pest. Willinga et al. (1973) stated

that TH 6040 apparently induced the degradation of newly synthesized chitin in insects. Post et al. (1974) had also expressed a similar opinion.

According to a technical bulletin on Dimilin published in 1974 by Thompson-Hayward it was maintained that DFB had low mammalian toxicity. It was also reported that it did not accumulate in the food chain to an appreciable extent.

Ishaaya and Casida (1974) made a remarkable headway by indicating that TH 6040 affected the hormonal balance in Musca domestica; the chemical interferred with its endocrine system causing retention of larval characters in pupating insects. Based on the findings of Ishaaya and Casida (1974) diflubenzuron was accepted by the subsequent workers as a member of IGR(Insect Growth Regulator) group. Interestingly enough, Neal (1974) considered DFB not as a growth regulator but a growth disrupter with stomach toxicity only. However, scanning of the available literature revealed that DFB was regarded as IGR having functional properties much akin to JH activities in insects. Some of the workers picking up the clues, established by their contemporary scientists, used DFB in the control of a number of insects. The latter process continues unabated till today as exhibited by the subsequent publications (Tamaki and Turner, 1974, on zebra caterpillar; Neal, 1974 on alfalfa beetle; Rizk and Radwan, 1975, on cotton pests; Granett and Dunbar, on gypsy mouth, Porthetria dispar ; Ratnakaran et al. , 1976, on forest tent caterpillar ; Natesan and Balasubramanian, 1979 ,

on Spodoptera litura; Alfred et al., 1982, on boll weevil, Anthonomas grandis and Rishi and Shah, 1984, on Indian gypsy moth.

Audemard et al. (1975) has described in his research paper about mode of action of diflubenzuron that it effects when it is ingested by the larvae.

Busschbach (1975) also reported Dimilin being a stomach poison with no contact and systemic action, could not be used against sucking insects or mites. His demonstrations projected a redeeming aspect of this chemical that it had no effect on predators whatsoever, However, he maintained that it had slow action but high persistence in field; it could not prevent damage from all the insects put together.

Bijloo in the same year (1975) described the physical, chemical and biological properties of DFB and reported that the particle size of this chemical influenced its effectiveness, decomposition and acute and chronic toxicity. He observed that once this compound was ingested by the larvae of Lepidoptera, Coleoptera and Diptera, they were unable to complete their next moult properly and died of cuticle rupture or starvation. His studies had indicated that the main changes in the cuticle of the insects were caused in the endocuticle which sometimes failed to develop at all. He also had maintained that this chemical had a low mammalian toxicity with no apparent teratogenic or mutagenic effects in them. However, he did found low toxicity to fishes, but no toxicity in birds.

Moore and Taft (1975) investigated that Anthonomus grandis adults when put in contact with a dry film of TH 6040 or dipped in acetone solution of this compound, produced eggs with reduced hatching or no hatching.

In the same year (1975) Taft in association with Hopkins reported that TH 6040 acted as sterilant against Anthonomus grandis female when applied as sprayable invert sugar bait in the field.

In the year 1976 Wright and Harris noted the ovicidal activity of TH 6040 when they exposed the eggs of stable fly to a paper impregnated with this chemical.

Abo-Elghar et al. (1976) revealed that most prominent ovicidal effect of DFB was achieved when the egg masses were deposited on DFB residues on treated plant leaves.

Ascher and Nemny (1976b) demonstrated that S. littoralis was 10 times as susceptible to topical or contact treatments as dietary treatments of DFB giving an evidence of its high cuticular absorption. Contrary to this, a lack of toxicity by DFB topical treatments was reported on coleopteran grubs by Beavers et al. (1976).

Ratnakaran et al. (1976), on the basis of their investigations in laboratory on the effects of Dimilin on Malacosoma disstria larvae, had presented an additional finding that this chemical lack any repellent effect as the larvae readily fed on the treated pallets of artificial diet.

While studying the effects of TH 6040 on the metamorphosis of Dacus oleae Fytizas (1976) observed the contact toxicity of TH 6040 when the final instar larvae were dipped in a concentration of 500-2500 ppm of the chemical. He observed a high pupal mortality in addition to the abnormalities in pupal formation. He also recorded inhibition of the transformation of larval cuticle to the puparium. McLaughlin (1976) had identified the stomach toxicity of TH 6040 alongwith sterilizing effect when it was fed to the females of Anthonomus grandis. Sundermurthy (1976) had expressed his opinion that diflubenzuron acted like a JH analogue as it induced such morphogenetic deformities which resembles those caused by the exogenous application of juvenile hormone analogues.

Salama et al. (1976) observed that Dimilin had a specific mode of action which interferred with the rate of chitin deposition in the endocuticle; it became less rigid and could not withstand the internal pressure during ecdysis, or give sufficient support to the muscles involved in ecdysis, thus resulting in an inability to cast the exuviae; finally death ensued. They also observed that lethal doses of the chemical caused death of the larvae during ecdysis whereas lower doses retarded development and induced some deformities causing symptoms similar to JH analogues.

Technical information on Dimilin (7th edition) published by Philips-Duphar in 1977 had also attributed inhibition of chitin as a mode of action of this chemical. Moreover, it acted at higher $LD_{50}$ values and produced delayed mortality. However,

it was non-toxic to beneficial insects and was compatible with other insecticides.

Sundarmoorthy (1977) found that feeding the last instar caterpillars of S. litura with leaves treated with various doses of DFB resulted inhibition of moulting upto 96 per cent and also caused various degrees of morphological deformities in the larvae and pupae. They reported that DFB also reduced the food ingested, weight gained and the adult emergence. The adults emerged from the treated larvae at low doses were malformed and non-functional.

Ker in 1977 observed that when a juvenile insect of Locusta migratoria was fed diflubenzuron it failed to ecdyse. He found that the ecdysis failed to come up in the cuticle of the pharate stage. Yu and Terriere (1977) expressed their opinion that diflubenzuron inhibited ecdysone metabolism in dipteran species.

Elings and Dieprink (1977) reported that diflubenzuron was found to act on the larval stages of certain insect species by interfering with moulting when fed with food and as such they suggested it acted mainly as a contact poison without systemic action in plants, and was therefore unlikely to control sucking pests. However, it did not affect the parasites and predators except by disappearance of their food.

Clarke et al. (1977) while studying the effect of Dimilin on the production of peritrophic membrane in the locust, suggested a different mode of action of this chemical. They

observed that Dimilin partially blocked chitin synthesis during the production of the peritrophic membrance in the insect. Reduction in chitin led to a reduction in protein in the same proportion. They further suggested that protein incorporation was affected by the stability of the protein in the matrix so that unbound protein tended to inhibit the addition of further protein.

Abo El-Ghar et al. (1978), while screening 21 non-systemic gramular pesticides to assess direct contact action, exposed Spodoptera liittoralis for 4 days to treated soil with DFB and recorded the contact toxicity index of the chemical; they found the index as 0.63 in comparison to Dursban which was taken as 100.00. The lethal rate ($LR_{25}$) calculated by them as 3.0 kg ai/feddan (4200 sq.m.).

El-Sayed (1978) also screened three chitin inhibitors including Dimilin and found that Dimilin was more effective than the other two chemicals, i.e. PH 66-13 and PH 66-14, against S. littoralis larvae. He further observed that the activity of Dimilin appeared after feeding for 2 or 3 days on treated leaves depicting its slow action.

Radwan et al. (1978) investigated contact toxicity of 9 IGR's including DFB when administered the chemical topically on the 4th instar larvae of Spodoptera littoralis. They observed PH 6040 was more toxic by topical application when short term activity ($LD_{50}$ at 96 hours post treatment) was concerned. The topical application of DFB to newly formed pupae inhibited

severely the number of deposited eggs and per cent of hatchability of the eggs laid by adults emerged from the treated pupae. The oral toxicity of this chemical was also observed by them.

McCoy (1978) applied Dimilin on the citrus rust mite eggs topically but did not succeed to get any ovicidal effect. However, after 6-7 days of treatment he observed 2nd stage nymphal mortality. He found that dead mites were entrapped in their exuviae thereby indicating inhibition of the moulting process. He also did not find any effect on fecundity or hatchability of eggs in Dimilin treated individuals.

Grosscurt (1978) observed that the permeability of elytra in adults of Leptinotarsa decemlineata was blocked by administering diflubenzuron. The blocking of permeability showed identical kinetics as the inhibition of chitin formation by this compound.

Deul et al. (1978) observed that diflubenzuron reduced the rate of production of chitin in insects during cuticle deposition by competitive inhibition of final enzyme, chitin synthetase.

Gijswijt et al. (1979) suggested that Polyoxin D, which was known as a competitive inhibitor of chitin synthetase in fungi, acted in a similar way in insects as Dimilin. They demonstrated that Polyoxin D, DU 19.111 and diflubenzuron, inhibited chitin synthesis. However, the former showed such activity only when it was injected in the body of the insect.

Sundarmurthy and Santhanakrishnan (1979), while studying the morphogenetic effects of DFB on Nephantis serinopa, observed that this chemical interferred with the growth and development of the pest.

Subramaniyam et al. (1980) studied the adverse effects of DFB on Achaea janata and observed different stages of larval-pupal intermediates and incomplete tanning of the cuticle. They suggested that such stages also denoted the hormonal imbalance caused by DFB, besides its normal mode of action i.e. chitin inhibition.

According to Ascher and Nemny (1980) ovicidal action of diflubenzuron was reported to be due to the increase in respiratory metabolism of the developing eggs. They observed mortality of 1 day old eggs of Spodoptera littoralis when dipped in aquous DFB formulation.

Hoying and Riedl (1980) observed that technical DFB was more toxic to the eggs of codling moth Laspeyresia pomonella. They also maintained that increasing toxicity of technical DFB was due to the presence of water and acetone which was believed to enhance the penetration of DFB across the chorion.

Reed and Bass (1980) determined dosage-mortality curves for different instars of soybean looper fed on DFB treated diet and reported that 1st and 3rd instars were more susceptible than 5th instars. Consequently 5th instar was more susceptible in comparison to 6th instar. They also concluded that consumption of higher dosages of DFB, when consumed only once, were less effective than the dosages fed ad libitum at lower concentrations.

Saad et al. (1981) studied joint action of DFB with other insecticides against S. littoralis strains. They observed that DFB, when used alone was least toxic but a potentiation effect occured when it was mixed with deltamethrin (1:1 ratio). It also showed antagonistic effect when cypermethrin + DFB was used against resistant strains. Potentiation to addition effect was observed in the case of chlorpyriphos + DFB against Alexandria strain. On the contrary, the combination of fenvelrate/phosfolan and DFB gave an antagonistic effect.

Anderson and Elliott (1982) found that codling moth eggs were very sensitive to diflubenzuron when treated topically after oviposition. They also observed a linear relationship between hatching percentage and the age at which the eggs were treated with diflubenzuron. They further reported that egg-hatch was also inversely related to the length of time the chorion was in contact with the diflubenzuron solution.

Thangavelu (1982) reported that diflubenzuron partially inhibited oviposition and completely inhibited fertility in ash weevil females obtained from the larvae reared on the treated food. However, he did not observe any intereference with courtship and mating of such weevils.

Elliott and Iyer (1982) studied the toxicity of DFB to nymphs of the migratory grasshopper. This pest was found to be very sensitive to this compound when reared continuously on treated wheat seedling.

Villavaso (1982) reported sterilization of the adults of Anthonomus grandis after they were fed on a diet containing DFB at 100ppm, for 5 days.

According to a technical bulletin on Dimilin (8th edition; 1983) published by Philips-Duphar B.V., diflubenzuron probably interferred with the formation of chitin in the cuticle by way of blocking the chitin synthatase. The action of this chemical was found to be larvicidal and ovicidal in nature. In larvae Dimilin (diflubenzuron) acted mainly as a stomach poison. However, contact toxicity on larvae of some species, particularly Spodoptera littoralis was also observed.

Granett et al. (1983) showed that there was considerable variability in the topical treatment toxicity in comparison to the toxicity after dietary treatments. They treated beet armyworm, Spodoptera exigua with three benzoylphenyl ureas and found Penfluron as most toxic by cuticular treatment followed by BAY SIR 8514 and diflubenzuron.

Satyanarayan and Kumuda Sukumar (1984) observed contact toxicity of penfluron, a chitin inhibitor on the red cotton bug by topical application and observed gradual degeneration of oocytes thus affecting the fecundity.

Saradamma *et al.* (1984) recorded contact toxicity of DFB and triflumeron by topical application on larvae of spotted beetle *Henose epilachna vigintioctopuntata* preventing emergence of normal adults.

Das *et al.* (1984) also recorded some contact toxicity of DFB. They found that doses ranging from 0.2 to 0.025 brought about maximum mortality of 30.7 per cent in potato weevil.

In their review Retnakaran *et al.* (1985) discussed in detail regarding toxicity of benzoylphenyl urea compounds and described various laboratory bioassay techniques commonly used; among which topical treatments were not preferred for comparison between species with these compounds and surface treating on foliage or other food by dipping or spraying and feeding to insect *ad libitum.* However, they had an openion that without the dosage value precise comparisons between species in regard to the toxicity of these compounds were impossible. They also described numerous laboratory tests done to assess the toxicity of these compounds to various species and inferred that few generalizations can be drawn from the work to date, although there are some intriguing trends which will hopefully help justify continued efforts.

Laboratory evaluation and symptomatic studies :

Vaan Daalen et al., who discovered the insecticidal activity of DFB in 1972 for the first time, reported the symptoms of poisoning of this compound : the exuviae of the insect remained attached to it and the body fluid exuded from it causing death.

Mulder and Gijswijt (1973) confirmed Vaan Daalen's observations in P. brassicae; they found that the skin of the larvae of this insect could not split during moulting. Likewise at the marginal lethal concentrations, they found that the anterior parts of pupae remained tucked in the larval skin although abdominal exuviae was shed. Similar results were obtained by them while testing the effects of Dimilin on B. brassicae. They concluded that in other test insects also similar abnormalities were obtained at larval or pupal stage with different dosages. Even with lowest dose they found that though pupation was normal, the adults emerged from them were abnormal.

Wellinga et al. (1973) evaluated TH 6040 in laboratory and reported that it apparently induces the

degradation of newly synthesized chitin in insects. Post and Vincent (1973) also had a similar opinion. They further mentioned that this chemical prevented growth in P. brassicae. Ishaaya and Casida (1974) also agreed to Wellinga et al. (1973) and further observed that TH 6040 caused retention of larval characters in pupating Musca domestica.

Ables et al. (1975) found that in Musca domestica Dimilin concentrations of 1.0, 2.5 and 1.25 ppm active ingradient produced over 90 per cent mortality in intermediate and late larval stages.

Granett and Dunbar (1975) observed several stages of moulting abnormalities in gypsy moth larvae fed on TH 6040. They graded those abnormalities as under- molt complete and unable to feed, molt partially complete but either head capsule or abdominal skin remains, molt started or indicated by frayed or ruptured cuticle partially, insect death in pre-molt stage etc. They observed 0.013 ppm of TH 6040 in the artificial diet proved 50 per cent effective concentration for the 3rd stadial larvae of gypsy moth.

Bijloo (1975) also noted rupture of cuticle, incomplete moulting and starvation in the larvae of lepidoptera, coleoptera and diptera when fed on diflubenzuron treated food.

Miller et al. (1975) fed TH 6040 in rations to laying hens at decreasing amount from 50 to 1.6 ppm and

observed that the level that gave complete inhibition of development of M. domestica L. larvae., fell between 6.2 to 12.5 ppm. They also found the residues of TH 6040 in the eggs of the treated animals.

While studying the effect of TH 6040 on the metamorphosis of Dacus oleae, Fytizas (1976) observed abnormalities in pupal formation when final instar larvae were dipped on concentrations of 500-2500 ppm. He also recorded high pupal mortality.

McLaughlin (1976) evaluated stabilizing effect of TH 6040 in laboratory by feeding it for 24 hours to the females of Anthonomus grandis. He reported that the concentrations required to inhibit 50 per cent hatch of the eggs laid at about 72 and 216 hours after ingestion were 431 and 2797 ppm respectively. He noted the intervals required for inhibition to decrease 50 per cent hatching were 32, 62 and 218 hours for initial doses of 201, 576 and 4034 ppm respectively. The dose required to inhibit 50 per cent hatch of the eggs laid at about 84 h recorded by him was 0.11 /ug/o+.

Tsutomu et al. (1976) observed prolapse of rectum in the larvae of tobacco cut worm Spodoptera litura when an IGR ZR-515 was applied in the middle of 6th instar larval stage. They further observed that these larvae did not survive because the prolapsed rectum ruptured, leading to haemorrhage.

On lowering the dose they obtained larval-pupal intermediates and on increasing the dose extra larvae were obtained. They also reported similar symptoms indicated by other two IGRs ZR-512 and ZR-619.

In DFB treated larvae of S. littoralis Ascher and Nemny (1976b) noted the symptoms of failure to wriggle out of exuviae, body fluid exuded, larvae became black and ultimately died.

Salama et al. (1976) also reported that treatment of Dimilin resulted an inability to cast exuviae in Lymantria dispar, L. Monacha and Ectropis bistortata larvae. They also observed that lower doses retarded development and induced some deformities causing symptoms similar to those developed with the treatment of JH analogues.

Ker (1977) also observed that when a juvenile insect of Locusta migratoria was fed diflubenzuron it was unable to continue ecdysis or to move and death followed.

Sundarmurthy (1977) found that feeding the last instar caterpillars of S. litura with leaves treated with DFB resulted various degrees of morphological deformities. The per cent inhibition of moulting and severity of deformities were dose dependent. A dose of 0.10 per cent completely inhibited the larvae to transform into pupae. The other doses did not prevent completely the moulting but resulted in mosaic

pattern of deformities. Kinds of abnormalities observed were pupae with degenerated proboscis, pupae with malformation and larval skin retained on the body, pupae with larval head, larval-pupal intermediates with different stages of larval skin attachment, larvae transformed into a tubular skin, larvae shortened and the non functional adults with crippled wings and prominent maxillary palpi. Such non-functional adults were obtained in 0.04 per cent concentration, which lived only for two days with a total absence of any sexual behaviour. He also observed the symptoms like reduced food ingestion and weight reduction.

Calkins et al. (1977) in their laboratory tests found that ingestion of DFB by female plum curculio did not affect the mating and number of eggs laid but it did reduce the emergence of mature larvae from the fruits infested by treated females. In their laboratory soil tests they obtained 80 per cent mortality or greater during pupation and eclosion in all the boxes with DFB mixed soil @ from 108.7 to 1.09 ppm in comparison to 14 per cent mortality in control. The $LD_{50}$, they recorded, was 0.14 ppm. They also reported no adult emergence upto 40 days from the treated soil with DFB @ 358 ppm in which larvae were released every week indicating prolong life of the chemical in laboratory tests.

Flint and Smith (1977) evaluated TH 6040 against Pectinophora gossypiella and found that 1 ppm of the chemical

in larval diet reduced 64 per cent adult emergence from control level.

Abo El-Ghar et al. (1978) screened Dimilin and two other IGR's - ACR 2036 and ACR 2044 alongwith eighteen different insecticides in the form of gramules by their pot experiments in the laboratory. When the $LR_{25}$ values were compared, they found that Dimilin was more effective ($LR_{25}$ = 3[illegible].000 kg/feddan) than ACR 2044 and less than ACR 2036; this group showed slight difference with chlorinated hydrocarbons. On the other hand the difference between both of these groups and the organophosphorous compounds was remarkable.



Radwan et al. (1978) also evaluated PH 6040 along with 8 other IGR's and reported that PH 6040 was the most effective and the oral application showed better response ($LD_{50}$ 0.007 - 0.01 /ug/larva) over the topical application ($LD_{50}$ 0.04 - 0.06 /ug/larvae). When they treated pharate pupa with 0.2 /ug IGR/pupa; pupal period was found increased with a reduction in average weight of pupa (100, 63 and 70 mg/ pupa ) and emergence of malformed adults (32.6, 24.2 and 22.2 per cent) respectively following the application of RO-10-3108, RO-08-9801 and PH 6040. Similarly they reported these compounds as most effective in respect to fecundity inhibition and sterility activity in S. littoralis.

El-Sayed (1978) tested Dimilin, PH 66-13 and PH 66-14 in the laboratory on eggs and larvae of Spodoptera littoralis. He

found Dimilin to be effective against the larvae at low concentrations and obtained 96 hr $LC_{50}$ ranging between 0.0005 and 0.0013 per cent for various larval instars. He reported that Dimilin was about 5 and 41 times more effective than PH 66-14 and PH 66-13 respectively. Although he obtained heterogenous effect of Dimilin on the eggs, he could conclude its weak ovicidal activity compared to its effectiveness on the larvae.

Sundarmurthy and Santhakrishnan (1979) found that the maximum concentration of DFB tested (4.0 g litre$^{-1}$) could result only 75 per cent mortality in case of Nephantis serinopa.

Subramanyam et al. (1980) observed characteristic morphological disturbances in A. janata at larval pupal moult; such insects were intermediary to larvae and pupae and invariably retained larval head and thoracic legs. They observed that indication of pupation were restricted only to the abdominal region in many cases. They observed that pupal skin was seen raising above the larval head and in some larvae tanning of the cuticle was apparently disturbed, especially on the ventral part. They also recorded clearly prolapsed rectum in 6 per cent of the larvae fed on 1 ppm DFB. Rabindra and Balasubramanian (1981) observed that feeding of A. janata larvae on Dimilin treated food inhibited the normal growth and metamorphosis of the insect resulting in various morphological deformities in the pupae. The insect died with

incomplete cuticle. They further observed a very high mortality rate of 96 per cent with lowest concentration tested i.e. 0.05 g litre$^{-1}$ and 100 per cent with all the higher concentrations.

Reed and Bass (1980) studied larval and post-larval effects of DFB on the soybean looper Pseudoplusia includens and reported different types of post-larval abnormalities in DFB fed loopers viz., haemolymph leakage at anus, hind-gut everted at anus, larval development ceased but appearance normal, prepupal-pupal intermediate, same with haemolymph leakage, pupal appearance normal but adult did not emerge, pupa deformed, pupa with haemolymph leakage, adult pupa like, head or abdomen stuck inside pupal case, deformed wings, adult unable to escape silk cocoon, wing stuck to pupal case and proboscis stuck to pupal case etc. They also observed lengthened developmental time for larval and post larval period in treated insects.

Argauer and Cantelo (1980) conducted laboratory tests to determine the stability of some insecticides in compost and found that the 3 uriede compounds DFB, BAY SIR 8514 and Lilly 7063 were much more stable than the phosphorus insecticides. They also assayed residues of the compounds by established bioassay with Sciarid fly and with a newly developed chemical method based on high performance liquid chromatography and recorded highest stability of DFB and Lilly 7063 even upto 100 days and about 97 per cent residual effect even after 60 days.

Rabindra and Balasubramanian (1981) reported disruption of the growth and metamorphosis in the larvae of Achoea janata Linn. by feeding DFB treated food resulting in death. They detected various morphological deformities in pupae viz larviform pupae with anterior portion completely larval, larval pupal mosaics, forwardly bent pupae with larval head and two pairs of legs, elongate pupae with short wing pads on one side only; sclerotised cuticle absent ventrally in the mid portion, twisted larval pupal intermediates, almost normal pupae with larval head and legs and the curved pupae. They noticed that such deformed insects survived for 2-3 days but did not develop into adults. They also observed patches of dark brown and well sclerotized pupal integument below the larval skin in some prepupae. They had an openion that it was most likely due to inhibition of moulting of the larvae and irregular deposition of the cuticle. They recorded 96 per cent mortality even at the lowest concentration tested (0.05 g litre$^{-1}$) and 100 per cent mortality with higher concentrations.

Villavaso (1982) reported that the adults of Anthonomus grandis were sexually sterilized after they fed for 5 days on a diet containing DFB at 100 ppm. After the treatment, the mating ability of treated males were significantly lower than that of untreated males. Laboratory evaluations showed that both treated males and females were 100 per cent sterile at 100 ppm.

Elliott and Iyer (1982) studied the toxicity of DFB to nymphs of the migratory grasshopper. They reported that concentration of 10 ppm or above of the compound completely inhibited moulting when fed contimuously through treated wheat seedling . The $LC_{90}$ values found after 8, 12 and 20 days were 10, 2.4 and 0.8 ppm respectively.

Pushpa Gund and Sharma (1984) studied the effect of Dimilin on the potato tuber moth and observed several symptoms resulting in various degrees of morphological abnormalities.

Saradama et al. (1984) observed morphogenetic effects of DFB and Triflumeron on spotted beetle Henose epilachna vigintioctopuntata when fed on bitter gourd leaves treated with 0.1 to 0.0001 per cent emulsions of chitin synthesis inhibitors.

Das et al.,(1984)evaluated DFB against four important pests and observed that no rhinoceros beetle grub could develop into adult, when fed on cowdung mixed with even the lowest dose of 0.05 per cent chemical. They also observed it very effective against rice leaf roller and recorded cent per cent mortality at above concentration but found ineffective against brown plant hopper Nilaparvata lugens in lab trials and in pot culture experiments. Satyanaryana and Kumuda Sukumar (1984) reported that topical application of 0.1 per cent solution of Penfluron caused gradual degeneration of oocytes - thus affecting the fecundity.

Histological investigations :

Although strictly histological investigations involving the tissues and their components could not be done so far, yet gross changes ensued in the internal structure had been incorporated under this caption.

While testing the new chemicals PH 6038 and PH 6040, which interfere with the cuticle deposition, Mulder and Gijswijt (1973) examined the histological changes of the cuticle of the treated larvae of P. brassicae. They found severe obliterations in the connections between the epidermis and the cuticle. They also noted that when the treatment was started long enough before ecdysis (24 hours) the newly formed cuticle consisted only of epicuticular and exocuticular tissues, which were not properly attached to the epidermis. The newly formed cuticle was very delicate and not stable; consequently it could not resist the muscular traction and the increased turger during moulting. They reported scattered globules of coagulated material in the space between the exocuticle and the new cuticle. They further observed that when the treatment was given shortly after ecdysis, the larvae ceased to strengthen their endocuticle whereas when the treatment was given some time after the ecdysis the cuticle became thick. However, they noted that the epicuticle and exocuticle of the treated larvae were normal, thereby concluding that histological changes took place only in the endocuticle.

In the same year (1973) Post and Vincent reported the blockade of deposition of chitin in the endocuticle of Pieris brassicae fed on PH 6040 treated food. Similar blockade was found in the treated housefly larvae by Ishaaya and Casida (1974).

Hunter and Vincent (1974) also stated that P. brassicae larval cuticle apparently did not grow after DFB treatment. However, according to them DFB did not affect the protein in insect cuticle. As regards the tanning, they concluded from their experiments that tanning was not influenced by DFB.

According to Bijloo (1975), the histological studies indicated that the main changes caused by DFB were in the endocuticle which failed to develop in some cases in the treated larvae.

Salama et al. (1976), when fed the artificial diet containing lethal doses of DFB to the larvae of Lymantria dispar, L. monacha and Ectropis bistortata, did not detect any histological change in their internal tissues especially the midgut, muscles, fat bodies and malpighian tubules, but the integument was found affected. They observed that the endocuticle was not properly attached to the eqidermis and showed globules of apparently co-agulated material, mainly protein. From the histological observations Ker (1977) came to the conclusion that tanning was not influenced in locust species by DFB treatment.

Grosscurt (1978) also reported that in DFB treated L. decemlineata the structures of the elytra were drastically changed. He observed the distortions in each of the several types of mesocuticle in the elytra of treated adult beetles. However, he observed that in DFB treated insects, inspite of arrestation of chitin synthesis, the thickness of the mesocuticle still increased; and concluded that the protein synthesis was not affected by DFB. He noted that the permeability of the elytra of treated beetles did not coincide with the increase in thickness of the treated mesocuticle. He concluded that effects on penetrability were due to interference of DFB with chitin-protein bonding in the elytra. However, he was of the openion that direct effect of DFB on tanning could not completely be ruled out.

Ker (1978) reported that suppression of cuticle formation was not uniform throughout the insect and only from some regions of the insect, it was possible to obtain affected cuticle.

Gijswijt et al. (1979) on histological examination of P. brassicae larvae apparently looking normal but once injected with DFB or Polyoxin D or DU 19.111 chemicals, noticed severe cuticular lesions. They observed that all the chemicals caused obliteration of the connections between epidermal cells and the cuticular layers. They observed scattered globules of apparently co-agulated material stained

red by the Biebrich Scarlet component of Mallory's stain in the space in between. They observed that the measurement of the cuticular growth at intervals after ecdysis and before apolysis in treated and untreated larvae showed inhibition of growth as a result of DFB or Polyoxin D treatment. This indicated that the effect could be obtained at any time during larval growth and that Polyoxin D, the antibiotic also showed similar inhibitory effect.

Reed and Bass (1980) when dissected some of the soybean looper prepupae which developed "hindgut everted at anus" after treatment of DFB, reported that the posterior region of the gut lining had broken near the beginning of the hindgut. They postulated that haemolymph pressure probably pushed the broken membrane towards the anus and formed an evertion at the anus. They had the opinion that this breakage of the membrane was due to the weakening of the gut cuticle at the junction of mid and hind gut, probably by decreasing the amount of chitin incorporated into the endocuticle of the hind gut due to ingestion of DFB treated food.

Rabindra and Balasubramanian (1981) examined the histological sections of the deformed A. janata pupae developed from DFB fed larvae and having incomplete deposition of cuticle, especially on their ventral side. Their study revealed that such sections depicted irregular deposition of the cuticle and

clearly showed the absence of exocuticle in such areas. They further stated that under the influence of DFB, stable layer of cuticle was not deposited. However, the suppression of cuticle formation was not uniform throughout the insect and only from some regions of the insect it was possible to obtain affected cuticle. They concluded that this type of irregular disruption of cuticle deposition could be one of the possible reasons for the formation of mosaic forms in DFB treated A. janata.

The histological observations described in a technical bulletin (8th edition - 1983) published by Duphar B.V. revealed that in DFB treated larvae the cuticle was disturbed. It was illustrated that the endocuticle was distorted after treatment. Gund and Sharma (1984) tested Dimilin against potato tuber moth larvae and could show that chitin deposition in treated larvae was quantitatively reduced.

Satyanarayan and Kumuda Sukumar (1984) studied histopathological effects of a chitin inhibitor A 13-63223, penfluron, in female red cotton bug Dysdercus cingulatus. They also observed that higher concentrations caused gradual degeneration of oocytes.

Choklingam et al. (1984) assessed the impact of a thiourea compound SR 103514 on the survival, food consumption and utilization in Amsacta albistriga, Danaus chrysippus and Ergolis merione larvae. They obtained $LD_{50}$ values of 25 $\mu$g,

15 /ug and 21 /ug per larva respectively. The food consumption was higher in former species whereas, it was lesser in E. merione larvae. However, they found that the rate of conversion of food into body tissue was reduced in all the larvae fed on thiourea treated leaves in the following order; 49.66%, 47.93% and 16.52% respectively in the three species. They suggested that polyphagous lepidopterous insects can efficiently withstand the stress of chitin synthesis inhibition by chanelling more of food energy to overcome the toxic effect of this chemical than using it for the conversion into the body tissue when compared to oligophagous and monophagous pests.

Kramer et al. (1985), while reviewing researches pertaining to chitin metabolism in insects, have listed some of the published reports of the effects of pesticides including members of benzoylphenylurea and other IGRs on chitin/cuticle metabolism. They mentioned that histological studies note a variety of structural changes after the administration of benzoylphenylureas and an absence of normal deposition of endocuticle.

Biochemical investigations :

Wyatt (1961) on the basis of his investigations on the protein make up of the larvae of Bombyx mori, made a generalized observation that the number of protein bands differed from species to species and within the species from one stage to another. Hackman (1964) while reviewing the progress of researches on the chemistry of insect cuticle, also mentioned that not only the electrophoretic behaviour and amino acid composition of different fractions of cuticular proteins differ but the proteins of different insect species also differ. Pant and Agarwal (1965) reported quantitative radical changes in the haemolymph of Philosamia ricini pupa during metamorphosis. These findings led to a new trend of researches providing further techniques or improving the existing techniques in the separation of haemolymph and body proteins of test insects.

For the better separation of proteins from the haemolymph and the cuticle of an insect Ching Muh and Pitton (1968) and Srivastava (1970) found that acrylamide gel electrophoretic techniques gave better results.

Srivastava (1970) studied electrophoretic behaviour of the water soluble cuticular proteins of Galleria mellonella. A comparison of the bands obtained at different developmental stages indicated that in the adult cuticle only those protein

bands appeared which were already present in the cuticle of larva or of young pupa.

Greene and Dahlman (1973) also observed quantitative changes in haemolymph proteins from each physiological phase of last three instars of tobacco hornworm Menduca sexta. Twelve anodial migrating protein bands were found by them. They observed that total protein increased from third to late fifth instar larva but decreased slightly in the pharate pupal stage. They further reported that some individual bands showed cyclic pattern with each instar similar to the overall cyclic pattern of total protein.

Post and Vincent (1973) suggested that DFB caused a blockade of deposition of chitin in the endocuticle in P. brassicae. A similar blockade was observed in housefly by Ishaaya and Casida in 1974.

Post et al. (1974) described how DFB affects the chitin and reported that Du 19.111 (a diflubenzuron related compound) prevented chitin synthesis in P. brassicae. However, they found no indication of an effect of DU 19.111 on cuticular protein synthesis while studying the incorporation of radio-labeled tyrosine and proline. They also recorded amino acid incorporation in endocuticular tissue of P. brassicae after DFB treatment.

Hunter and Vincent (1974) found no effect of DFB on the protein or on the amino acid composition in locusts. As regards tanning of the cuticle also they concluded that it was not influenced by DFB.

Willis (1974) stated that possible interaction of DFB molecules either with neuroendocrine system or biosynthesis of steroids cannot be ruled out as the larval-pupal mosaic are associated with uneven distribution of hormone in the system.

Baumler and Salama (1976) observed that haemolymph protein pattern looked normal although proteins became quantitatively less after the treatment of Dimilin. In cuticular extracts, they recorded that diflubenzuron induced some changes in the pattern of distribution of some hydrolysed proteins, whereas, lipids are effected a little. They did not observe any affect on the activity of enzymes. The amino acids, amino sugars and peptides also did not show any effect with the treatment of Dimilin.

Salama et al. (1976) recorded about 33 per cent reduction of chitin deposition in the endocuticle in L. dispar larvae fed on artificial diet containing lethal dosages of DFB. They observed that lipid content of the treated larvae increased suggesting a reduced rate of synthesis of glucose in the haemolymph to form chitin but the protein content remained unaffected. They concluded that DFB acts by interfering with the rate of deposition of chitin in the endocuticle so that endocuticle

becomes less rigid. Clarke and Temple (1977) reported that partial blocking of chitin synthesis during the production of peritrophic membrane in locust led to a reduction in protein in the same proportions.

Sundarmurthy (1977) on the basis of his observations postulated the possibility of interaction of DFB molecules with neuro-endocrine systems or biosynthesis of steroids. He observed a reduction in the intake of food by treated caterpillars of A. janata, which increased with the increase in dose, while the per cent weight gained by the larvae were found to be inversely related to the dose.

Ker (1977) found no effect of DFB on tanning in locust species. They also observed that the thickness of the cuticle continued to increase even after the treatment which indicated that protein synthesis is not affected by DFB. He did not found any effect on protein content or amino acid composition of the cuticle of DFB treated locust species.

Miltin et al. (1977) also, in their experiments did not observe any inhibition of protein synthesis in DFB treated boll weevils. However, they reported a decrease in lipoprotein synthesis in males.

According to Grosscurt (1978) in DFB treated elytra of L. decemlineata chitin synthesis was arrested. He obtained 81 per cent decrease in chitin synthesis in the elytra occurred within one day. He did not observe cent per cent inhibition of chitin formation within one day for which they had an opinion that inhibition was not instantaneous. He reported that DFB treatment caused increase in the dry weight of deproteinized elytra. He concluded that in larvae of L. decemlineata there was no evidence of any other direct effect of DFB on the cuticle.

Gijswijt et al. (1978) demonstrated the effect of DFB and Polyoxin D on cuticle deposition. In an experiment on incorporation of radioactivity from D [6-$^{14}$C] glucose into various tissue fractions of DFB/Polyoxin D injected larvae of P. brassicae, they revealed that only the chitin fraction of the cuticle was seriously reduced by these chemicals while the other fractions did not show any such reduction. The inhibition of chitin synthesis was further supported by them by observing the lack of increase in the dry weight of the deproteinized cuticle after the treatment. In their next experiment of incorporation of radioactivity by incubation method in tissue fractions of the treated P. brassicae larvae, they noted that all these chemicals resulted in 80 to 100 per cent chitin inhibition while the metabolism of glucose was unaltered in other larval components.

Duel et al. (1978) examined the chitinase activity in P. brassicae after treatment of DFB and found no effects with either DU 19111 or DFB reported that TH 6040 reduced the rate of production of chitin during chitin deposition by competitive inhibition of the final enzyme, the chitin synthetase. They observed decreased chitin in the cuticle of treated locust adults.

Saxena and Kumar (1981) quantitatively estimated glucose, glycogen, fructose, glucosamine and N-acetylglucosamine in the integument of diflubenzuron and penfluron fed Chrotogonus trachypterus nymphs. They found an increase in the glucose and glycogen levels and a decrease in fructose level, while other components were found unaffected. They suggested that at least partial blockage in the chitin synthesis was there. In the same year, they also analysed chitin, protein and lipid contents of the integument of C. trachypterøus nymphs treated with the same compounds. They recorded great reduction in chitin content, an increase in lipid content and no marked effect on the cuticular protein content.

Philips et al. (1982) also analysed the total protein, chitin and soluble protein contents in the adult locust and found that after an initial period of increase, cuticular protein and chitin contents levelled off with the onset of sexual maturation. Specific cuticular protein electrophoretic bands decreased in staining intensity with development and were presumed to become bound within the cuticle.

The bulletin on Dimilin (8th edition; 1983) published by Duphar B.V. had described that DFB interferred with the formation of within in the cuticle. Protein synthesis was unaffected but its deposition in the cuticle can be disturbed. Further studies had shown that the inhibition of chitin synthesis could be almost instantaneous. It depended on the dose and the method of application. The rapid inhibition can be caused at any moment as long as chitin formation took place. The bulletin further described the effect of a single dose; it was reversible. They felt the necessity of a contimuous intake of DFB to produce a weak cuticle at the time of the next moult. It also described that probably DFB blocked chitin synthetase mainly and there were several other effects of DFB on insect enzymes. However, such effects were observed after treatment, hence had been suggested as being of a secondary nature.

Satyanarayana and Kumuda Sukumar (1984) in a short publication (abstract) mentioned the biochemical variations in controlled and sterilized eggs after the application of a chitin synthesis inhibitor (AI 3-63223).

Retnakaran et al. (1985) made an extensive review on different biochemical effects of the benzoylphenyl urea analogs on different insect species. They mentioned that all the studies seemed to implicate the enzyme chitin synthetase as the actual biochemical moiety which interact with the toxicant. However, they stated that some other biochemical anamolies were also

produced by these compounds in insects such as differential effects on chitinase, β ecdysone, trehalase, amylase invertase, phenoloxydase etc., however, studies relating to protein synthesis in DFB treated insects were also described.

Kramer et al. (1985) while reviewing about the research work on chitin metabolism in insects enlisted some of the published reports of the effects of pesticides including IGRs on chitin/cuticle metabolism. They stated that exact mode of action of DFB and other antichitin compounds is unknown although it has been shown in the researches that both chitin synthesis and degradation are affected in insects. They further concluded that because inhibition of chitin synthesis occurs first, this phenomenon rather than chitin degradation appears to be predominant mechanism leading to death. They have also described effects on enzymes, carbohydrate and other metabolities.

Field persistence of diflubenzuron and its efficacy against pests :

The development of IGR's during the last few years had increased the options available for the control of important pests. Diflubenzuron which is a moulting inhibitor (Vaan Daalen et al., 1972) and regarded as a member of I.G.R. faming, had been found to be an effective agent in managing a large number of pests. The observation of Vaan Daalen (1972) projecting the insecticidal activity of phenylurea compounds led several workers to exploit this knowledge.

Picking up the thread Post and Vincent (1973) tried control of cabbage butterfly Pieris brassicae L. through diflubenzuron (DFB). In the same year (1973) Mulder and Swinnen also attained success in the control of Brevicorne brassicae with DFB at a lower rate of 0.0039 lb AI/10 gal water. Tamaki and Turner (1974) controlled zebra caterpillar, Ceramica picta of sugarbeet with two phenylurea compounds.

Turnipseed et al. (1974) found in field tests that DFB was effective against the velvetbean caterpillar, Anticarsia gemmatalis and the soybean looper Pseudoplusia includens. Neal (1974) also controlled alfalfa weevil with a single application of TH 6040 in small plots. Rizk and Radwan (1974) reported that in bollworm infestation, mainly of Pectinophora gossypiella, a moderate reduction in per cent infested bolls was induced by DFB at a dose of 0.48 kg/feddan as soil treatment. They further

proved in 1975 that PH 6040 soil treatment contained the cotton pink bollworm, P. gossypiella and concluded that this compound had potentiality for the control of this insect. They also observed promising effect of this compound against S. littoralis when it was sprayed in field and treated leaves were fed to the larvae in the laboratory and obtained 100 per cent kill of the cotton leafworm in 5 days after the treatment.

Field and lab studies, conducted by Ables et al. (1975), revealed that concentrations of 10, 2.5 and 1.25 ppm a.i. of Dimilin produced over 90 per cent mortality of Musca domestica in intermediate stage to late stage larvae.

Granett and Dunbar (1975) in his laboratory and field trials found that on apple trees infested with gypsy moth a treatment of TH 6040 at the rates from 0.125 to 0.0039 lb a.i./ 10 gal, with a mist blower, protected the foliage effectively. They also observed that the parasitoid, Apanteles melanoscelus, was dealt with within the gypsy moth by TH 6040 treatment.

Taft and Hopkins (1975) applied in field sprayable invert sugar bait containing TH 6040 (as a coarse spray with maximum droplet size in 3 to 4 mm dia. range) to sterilize female Anthonomus grandis. They observed that adult emergence was reduced to 98 per cent in all the infested squares. They maintained the effect by applying the material 14 times in 5 weeks.

Buschbach (1975) reported that Dimilin was fairly persistant but somewhat slow in action. He obtained satisfactory results against lepidopteran and coleopteran insects. El Tantawi et al. (1976) also reported that the activity of DFB in the field was slow and some defoliation occurred subsequent to treatment.

Ratnakaran et al. (1976) could achieve eradication of the tent caterpillars, Malacosoma disstria and prevented the defoliation of the trees with an application of 1% suspension of diflubenzuron.

Abo-Elghar et al. (1977) conducted small scale field trial and assessed the efficacy of soil treatment of Dimilin and other insecticides against cotton leafworm Spodoptera littoralis, pink bollworm Pectinophora gossypiella and spiny bollworm Earias insulana. Based on overall performance, they reported Dimilin as highly potential soil insecticide when used @ 2% and 1% in granular form. They achieved 80.4 and 72.0 per cent reduction in boll weevil infestation with two successive treatments each of 2% and 1% respectively. Their experiments revealed that first application of 2% Dimilin G at 10 kg/feddan increased the potentiality of the following application of Volaton 5% G at 20 kg rate or Dursban 5% G at 15 kg rate against the cotton leafworm and the bollworms.

Contrary to these findings Flint et al. (1978) indicates that the pests like P. gossypiella which burrows in the bolls were not susceptible to DFB treatment as larvae and as such the material was not effective in the field.

Calkins et al. (1977) reported that DFB was ineffective for control of the plum curculio when it was applied in soil. They also sprayed DFB on the plum trees and inferred that it did not give the total protection.

Elings and Dieperink (1977) had suggested that DFB showed potential for the control of Diptera and Lepidoptera; especially lepidopterous larvae infesting forest and shade trees. However, they found that this chemical being non-systemic in action, could not control the sucking insects.

Ables et al. (1977) reported that DFB when sprayed in field had minimal effects against beneficial arthropods. Keever et al. (1977) also arrived at the same inference through their studies.

Saad et al. (1977) stated that Dimilin acted as antimoulting compound with relatively low initial but long residual activity against cotton leafworms; El-Gayer et al. (1978) confirmed the latter findings.

McCoy (1978) applied Dimilin in field @ 0.04 and 0.15 g a.i./litre in water with and without medium oil (0.25%) and obtained effective control of citrus rust mite upto 13 weeks. He observed residual effect of this compound upto 21 weeks.

Abo Elghar et al. (1978) screened 21 non-systemic granular pesticides including Dimilin against S. littoralis through direct contact action and soil treatment in potted clay soils. Based on $LR_{25}$ values they found that chlorinated hydrocarbons and IGR's had a little difference in their action. They observed that among the three IGR's tested, Dimilin exhibited only 25 and 30 per cent mortality in S. littoralis after 4 days of exposure at the rates of 4.0 and 8.0 kg a.i./feddan, respectively.

✓Natesan and Balasubramanian (1979) tested the efficacy of diflubenzuron and chlorpyriphos individually as well as in combination against tobacco caterpillar, Spodoptera litura. They found that chlorpyriphos 0.02 per cent caused reduction in larval population to the extent of 78.26 per cent in 72 hours whereas DFB at the same concentration, resulted 39.19 per cent reduction in the stated period which increased to 78.81 per cent after 144 hours. This delayed impact of DFB was attributed to the slow action of the material. Donaubauer (1979) also observed such slow action of DFB; he recorded highest larval mortality of Lymantria dispar around 6th to 7th days. Khalil and Watson (1979) too on the basis of their results concluded that Dimilin acted as antimoulting agent with relatively slow activity but long residual effects against the cotton leafworm. The sciarid

fly Lycoriella mali, a major pest of commercial mushrooms was successfully controlled upto as long as 60 days by incorporating DFB and DAY SIR 8514 into the compost used to grow the mushrooms (Argaur and Contelo, 1980).

Westigard (1979) studied the effect of DFB on non target pests and beneficial species. He found that increased rates of DFB improved the control of codling moth but it disrupted the balance of natural enemies of the pest.

Santharam and Balasubramanian (1980) also, while studying the relative efficacy of chitin inhibitors, chemical insecticides and NPV against S. litura, recorded less than 30 per cent mortality in DFB and NPV treatments after 48 hours but after a week 63% to 90% mortality could be obtained. The activities of DFB and its combination with endosulfan and chlordmeform as ovicides under field condition were evaluated by Abo-Elghar et al. (1980). They observed that DFB alone and with endosulfan exhibited an inhibitory effect in suppressing the number of deposited egg-masses. They recorded 68.70 per cent non-hatched egg-masses in DFB treatment. On the contrary El Badawy et al. (1980) concluded that DFB was a poor-ovicide but may increase the potency of Methomyl if added at optimum ratio.

Hoying and Riedl (1980) also reported that since codling moth Lasperyesia pomonella burrows in fruit escapes surface treatment of DFB in field spray at normal concentrations.

Retnakaran (1981) in his experiments noted that DFB in the field has been notably ineffective against Eastern spruce budworm. He stated that the low level of efficacy in field treatments seems to be due to low innate susceptibility of this species. Madrid and Stewart (1981) using DFB for the control of Lymantria dispar at the rate of 0.03 kg ai/ha, obtained 50 per cent larval mortality after one week and cent per cent after 10 days.

Watson et al. (1981) combined a number of insecticides with Dimilin for the control of S. littoralis. Their results indicated that out of 79 combinations tested only nine binary mixtures could be used to suppress the amounts of conventional insecticides released in the environment.

Saad et al. (1981) also studied joint action of DFB against S. littoralis strains. They observed that DFB when used alone, was least toxic but a potentiation effect occurred on mixing it with deltamethrin (1:1 ratio). They observed potentiation effects in the case of DFB and Chlorpyriphos against Alexandria strain. On the contrary, the combination of DFB and Phosfolan gave an antagonistic effect.

Alfred et al. (1982) studied the efficacy of DFB on boll weevil Anthonomus grandis grandis. They observed a significant reduction in the number of adult weevils when DFB was applied @ 70 g ai/ha.

According to technical bulletin on Dimilin (8th edition : 1983), it was established that this chemical can be recommended for the pests of agricultural and vegetable crops, fruit orchards, forest plants and other insects like flies, mosquitoes etc. As maintained in the bulletin a generalized effective dose of 0.01 to 0.02 per cent of DFB can be recommended for the control of crop and orchard pests and 10 g a.i. per cubic meter to be mixed in compost for the soil insects.

Rana (1983) applied single recommended dosage of nine different insecticides including diflubenzuron as foliar application against bhindi pests to find out their efficacy. He reported that diflubenzuron was not much effective against aphids, jassids and stem borer. He suggested that diflubenzuron was, however, suitable for use in late stage of the vegetable crop due to its shorter residual toxicity as observed by him.

Das et al. (1984) evaluated DFB against some important crop pests and reported that this chemical was not promising for the control of sweet potato weevil.

Rishi and Shah (1984) studied efficacy of DFB against Indian Gypsy-moth and recorded 90 per cent collapse of the pest population in field when the apple trees were given a foliar application of 0.7 lb a.i. DFB/100 gallon of water. Natarajan et al. (1984) studied the efficacy of DFB and BAY SIR 8514 against cotton pests and observed least effects on aphids and

jassids and no effect on ash weevil adults. They obtained more pronounced effect of SIR 8514 at 300 g ai/ha than DFB 400 g ai/ha against spotted boll worm and pink boll worm.

In the same year (1984) they also studied the efficacy of BAY SIR 8514 on groundnut pests. The chemical was tested at varied doses and in combination with other insecticides against S. litura and Aproarema modicella and compared with DFB and other insecticides. They found that initial knock down effect of SIR 8514 was less than that other chemicals used. However, at 7 days after application it was found to be equally effective. They obtained 46.4 to 58.1 per cent control of S. litura and 50 to 58.3 per cent of A. modicella by SIR 8514. However, the control of latter species was enhanced upto 75 per cent when this chemical was combined at the rate of 100 g/ha. with other insecticides.

Rabindra and Balasubramanian (1984) conducted two field cage experiments to assess the efficacy of DFB and other insecticides against the final instar larvae of A. albistriga. The $LC_{50}$ values of DFB attained by them for different instars of A.albistriga at 48 and 60 hours post treatment, revealed an increase with the stage of the larvae, indicating that the older larvae were less susceptible than the younger ones. They recorded a marked decline in $LC_{50}$ values at 60 hours which indicated that the effect of DFB is better expressed at 60 hours.

Rajasekaran and Kumaraswami (1984) investigated a possibility of using DFB and triflumeron (Bay SIR 8514) as seed protectants and observed an effective check of S. oryzae at 200 ppm concentration of DFB. They recorded only 22 weevils in this treatment as against 1442 in the untreated check after three months of inoculation. They found the other concentrations viz., 100 ppm and 50 ppm as ineffective.

Retnakaran et al. (1985) discussed in their review on insect growth regulators about the field use-strategies of benzoylphenyl ureas for the control of foliage feeding insects, plant burrowing insects, flies of medical importance and pests of veterinary importance. They described how the biotic and abiotic factors influence field efficacy. On reviewing a number of studies they suggested that late application would be more efficacious for control of Eastern spruce budworm, Choristoneura occidentalis. They further described the three basic characteristics of these chemicals, viz., toxic effect on ingestion; activity occuring at distinct times in insect development; and slowness of the process of death after a toxic dose is acquired by an insect.

*******

# MATERIALS AND METHODS

To investigate the effects of diflubenzuron, a chitin inhibitor, a number of laboratory and field experiments were conducted on the test insects - Spodoptera litura Fab. and Euproctis virgincula Wlk.

## Rearing of test insects

### (a) Spodoptera litura :

The gravid adults were collected on light traps from agricultural research farms of Rajasthan College of Agriculture, Udaipur in the month of October-November in 1982. They were released in glass jars, 20x15 cm, having the inner surface fitted with blotting paper and their tops covered with muslin cloth. The cotton swab soaked in 10% sucrose solution was provided as food for adults. The egg masses laid on the blotting paper were obtained by cutting the paper containing the egg masses. These egg masses were kept in petriplates in an incubator at a temperature of 26 ± 2°C and RH 80% for hatching. The newly hatched larvae were transferred in sterilized petriplates having sacculant castor leaves as food. The second larval instars were also maintained like wise, but, the third larval and onward instars were reared individually to avoid cannibalism and contamination from all sources. The fifth larval instar, aging 1 to 24 hours, were used throughout these studies.

(b) Euproctis virgincula Wlk. :

The larvae of E. virgincula were collected from sugarcane field at Sahelion-ki-Bari farm, Udaipur in the month of April 1982. These larvae were reared in cages, (60x30x45 cms); castor leaves were given ad libitum as food. The pupae obtained were kept on moist sand in glass jars in an incubator at a temperature 30 ± 2°C and RH 90%. The adults so emerged were transferred in separate glass jars (20x15 cms dia), having moist sand layer of 5 cm at the bottom. The fresh green leaves of castor were affixed in the moist sand for egg laying. The sucrose soaked cotton swabs were provided as food for the adults. Three pairs of adults were released in a jar. The eggs laid on the leaves were recovered by cutting the leaves around the egg masses. These eggs were kept for hatching in an incubator at a temperature 30 ± 2°C and RH 90%.

The newly hatched larvae were reared in the similar manner as described for S. litura. The fifth larval instars aging 1 to 24 hrs, were used in various experiments.

On the onset of winter, from late October onward, most of the pupae in the culture of E. virgincula could not develop into adults and they diapaused in pupal stage. The pupal diapause could be successfully terminated using the method of Srivastava (per . com., 1982). This facilitated the availability of desired stage of larvae for experimentations. The details of the procedure followed for termination of diapause was as under :

The diapausing pupae were kept at 4°C in refrigerator for 3-4 weeks. Thereafter these chilled therapied pupae were transferred at a temperature of 35°C for 2 weeks. About 50 per cent of the diapausing pupae emerged as adults. These adults were quite active, mated and laid fertile eggs; however, the fecundity was drastically reduced.

Test chemical :

Diflubenzuron (DFB), chemically known as 1-(4-chlorophenyl)-3-(2,6-diflurobenzoyl)urea, a product of Duphar B.V. Amsterdam, Holland, was supplied by Mysore Insecticides Co. Pvt. Ltd., Lotus Court, 165, Thambu Chetty St. Madras-600 001. The formulation product, in the trade name of Dimilin 25% WP was procured and used in the present investigations.

Preparation of different stock solutions :

Following stock solutions of diflubenzuron were made using Dimilin 25 WP. The details of each stock solution used in different experimental techniques are given below :

(i) In a volumetric flask following ingradients were taken and a solution was made by continuous stirring:

Acetone AR .. 100 ml
Dimilin 25 WP .. 4 g

This solution (of 10,000 ppm) was considered as - stock solution I

(ii) Another solution of same strength was prepared using ordinary water in place of acetone. It was considered as - stock solution II

The stock solutions were stored in refrigerator. The desired concentrations of DFB were prepared freshly from different aliquates of stock solutions prior to use. The solvents used for dilution were the same as used in different aliquates. All the solutions were invariably stirred briskly before use.

Experimental procedures :

I. Toxicological studies :-

The bioefficacy of diflubenzuron was investigated on two test insects, S. litura and E. virgincula larvae using the following techniques.

(i) Dermal toxicity - (a) Dry film method
(b) Dipping method

(ii) Oral toxicity - Feeding method

Dry film method : The treatment dosages selected for both the test insects were 50, 40, 30, 25, 10 and 5 mg/90 $cm^2$ surface area. To obtain these treatment dosages, six different concentrations 5000, 4000, 3000, 2500, 1000 and 500 ppm were prepared from the stock solution I. Ten ml from each prepared concentration was drawn and poured in both the halves of a petriplate (CA 10 cm diameter). The solutions were swirled in the petriplate to get a thorough and uniform coating of the chemical on

the inner surface of the dish. The solution coated petriplates were dried under the electric fan at room temperature. After complete drying of the film 5 fifth instar larvae of the test insect (S. litura / E. virgincula) were released for 12 hrs. and 24 hrs. in treated petriplates, separately. At least 20 larvae were exposed in each treatment which were replicated 3 times. In control the petridishes were treated with acetone. The mortality counts were recorded after the expiry of required exposure periods. Since, no mortality occurred at 24 hours after the treatment the larvae, after the exposure to DFB film, were transferred to sterilized petriplates having sacculant castor leaves as food. The observations were further recorded at 48 hrs. after the treatment. These data were used for calculating $LD_{25}$ and $LD_{50}$ values.

Dipping method :

The treatment concentrations of 10,000, 5,000, 2,000, 1,000, 500, 200, 100 and 50 ppm were prepared from stock solution No.I. Fifteen fifth instar larvae of S. litura and E.virgincula, (1 to 24 hours old) were dipped in different concentrations for 5 seconds in each replicate. Each experiment had 3 replicates. After the treatment the larvae were kept under the fan on glass surface for solvent evaporation. The treated larvae were then maintained individually in petriplates and castor leaves were

provided as food ad libitum. The mortality data was recorded after an interval of 24 hours. The moribund larvae were counted as dead. The data was subjected to probit analysis using Finney's (1971) method to calculate $LC_{50}$.

A check was also run in which larvae were dipped in the respective solvent.

Feeding method :

The fifth instar larval stage of S. litura and E. virgincula was chosen for this experiment. These larvae were starved for 6 hours prior to giving any treatment. The doses of DFB selected were 5.0, 1.0, 0.5 and 0.1 µg/larva. To derive the required treatment doses, different concentrations of DFB were prepared from the stock solution No.II as per details given below :

| | | | |
|---|---|---|---|
| A. | Stock solution II 5 ml | + water 95 ml = | 500 ppm |
| B. | Solution A 20 ml | + water 80 ml = | 100 ppm |
| C. | Solution B 50 ml | + water 50 ml = | 50 ppm |
| D. | Solution C 20 ml | + water 80 ml = | 10 ppm |

The solutions were well stirred prior to use. One hundredth ml of the appropriate concentration was poured on 10 $cm^2$ castor leaf bit to administer the desired dose. Individual larva was provided with one piece of these treated leaf bits. In all 60 larvae were exposed in each treatment. When the treated leaf bits were consumed by the larvae, they were immediately given fresh (DFB free) leaves. A few larvae

failed to consume the treated leaves in 24 hours. They were not included in the experiment.

After the treatment the larvae were maintained individually in plastic containers (5 cm x 5 cm) at a temperature of 26°C ± 2°C and RH 80% and were provided with untreated castor leaves ad libitum.

Since, no mortality was obtained at 24 hrs after the treatment, it was felt desirable to use the total larval mortality before pupation, for the determination of $LD_{25}$ and $LD_{50}$ values.

II. Symptomatic and behavioural investigations :-

S. litura and E. virgincula larvae of fifth instar stage were fed with different DFB doses viz., 5.0, 1.0, 0.5 and 0.1 µg/larva as described in previous pages. They were observed at regular intervals of 24 hrs after the treatment until mortality or adult emergence. The observations were recorded on the appearance of abnormal symptoms like, behavioural changes; the morphological and moulting abnormalities during the larval development till adult formation. The cumulative mortality at different developmental stages, pupation and adult emergence were also recorded. The mortality data alongwith the prominent developmental abnormalities were used for drawing inferences for both the test insects. The details of abnormal symptoms considered for observation had been presented in Chart-I.

## Chart - I

Parameters of abnormal symptoms recorded were as under :-

1. Behavioural changes (in larva) :
   - (i) Sluggishness
   - (ii) Unable to crawl
   - (iii) Unable to feed
   - (iv) Wetting
   - (v) Oozing
2. Morphological deformities :
   - (i) Shrinkage of body length
   - (ii) Discolouration of body and scar formation
   - (iii) Rupturing of cuticle
   - (iv) Rectal prolapse (hind gut everted through anus)
   - (v) Moult just started (frayed or dispersed cuticle in larva)
   - (vi) Mosaic condition of cuticle (pre-pupa)
   - (vii) Incomplete moulting
     - (a) Head capsule remains
     - (b) Thoracic skin remains
       - x - Partially
       - y - Completely
     - (c) Abdominal skin remains
   - (viii) Rupture of old cuticle (larva)
     - (a) Thorax region
     - (b) Abdominal region
   - (ix) Pre-pupae with only part of ventral pupal skin (sclerotized or unsclerotized)
3. Formation of abnormal stages :
   - (i) Larvaeform pupae (larval-pupal intermediates) with
     - (a) Pupal body completely trapped in larval skin

(b) Anterior portion completely larval
- having head and three pairs of thoracic legs larval and posterior region pupal

(c) Dorsal pupal thorax in hump shape
- remaining characters being larval

(d) Larval head and 2 pairs of thoracic legs
- remaining portion pupal

(e) Larval head only
- remaining body pupal

(f) Deformed in shape
- forwardly bent LPI
- twisted LPI
- curved LPI

(ii) Pupal deformities and normal pupation

(x) Abnormal pupae with
(a) Short wing pads on one or both sides
(b) Ventral skin unsclerotized
(c) Forwardly bent in shape
(d) Curved in shape

(y) Normal pupation

(iii) Pupal-adult intermediates normal and abnormal adults

(x) Morphological abnormalities
(a) Pupal-adult intermediates
(b) Adults with abnormal/deformed wings

(y) Normal adult emergence

4. Cumulative mortality during :

(i) Larval stage

(ii) Pre-pupal stage

(iii) Intermediate stages
- Larval-pupal
- Pupal-adult

(iv) Pupal stage
- Abnormal
- Normal

5. Longevity and fecundity of adults; viability and hatching of eggs

Smear Examination -

A thin film was prepared from the smear obtained from the body fluids oozing out at different body regions. The smear film was dried in air and stained with Leishman's stain. After a mimute the stain was diluted with double amount of distilled water and the smear film was allowed to stain for 5 to 10 mimutes more. Thereafter, the stain was drained off and the film was washed for 10 seconds or more with distilled water till it became rose pink in colour.

III. Field evaluation of DFB and its persistence :-

The field experiments were conducted separately to test the bioefficacy of DFB against S. litura and E. virgincula. These were taken at farmer's field situated across the Aayad river, about 4 km away from Udaipur city.

Layout of the experiments :

Experiment 1.

Standing crop of 1 month old cabbage was selected for S. litura for the purpose. The experiment was laid out in randomized block design having the plot size of 5m x 3m. The plant to plant distance was 20 cm and row to row was 40 cm. Five treatment plots and a control (untreated check) plot was kept. Each treatment as well as control was replicated thrice.

Experiment 2.

Sixty days old crop of cowpea was chosen for field evaluation of DFB against E. virgincula. The experiment was carried out in randomized block design having plot size of 4m x 3m. The plant to plant distance was 10 cm and row to row was 45 cm. Five treatment plots and a control (untreated check) plot was kept. Three replications of the experiments were taken.

Selection of dosages and method of application -

Five concentrations of DFB i.e. 0.01, 0.02, 0.05, 0.1 and 0.2 per cent were selected for field application in both the experiments. The required concentrations of DFB were prepared from the formulated product "Dimilin" 25 WP in ordinary well water. The quantity of the spray solution used per plot was 1 litre for both the crops, which was pre-caliberated by spraying water in a test plot with a Ganesh hand sprayer. The amount of formulated product required per plot as well as per hectare had been mentioned in Table 1.

The spraying on cabbage crop was carried out on 22nd November 1984 whereas on cowpea crop it was done on 24th September 1983 during the early hours of morning to avoid drifting due to wind. A thorough coverage was given to each crop. The WP suspension used was continuously stirred during

spraying. In control plot (untreated check) water was sprayed.

Sampling:

Three middle leaves were plucked from randomly selected 5 cabbage plants in each plot. Similarly 25 leaves from mid of the twigs were plucked from 5 randomly selected cowpea plants in each plot. The plucking of leaves was done at an interval of 0, 1, 5 and 10 days after the spray. These leaves were brought to the laboratory and the respective test insects were fed on them.

Treatment to the test insect :

Fifth instar larvae of S. litura and E. virgincula (aged 1-24 hrs) were used to evaluate the bioefficacy of the chemical on the field treated leaves. Twenty larvae of the test insects, pre-starved for 6 hours, were released on the leaves of the respective crops plucked from each treated and the untreated plot. The larvae were kept in cylindrical glass jars of size 15 cm x 10 cm diameter covered with muslin top and were maintained in an incubator at 26 ± 2°C temperature and RH 80%. The treated food was provided ad libitum for 24 hours. Thereafter, the test larvae were given fresh untreated leaves of their respective

Table 1 : Amount of Dimilin 25% WP required for one spray

| Concentration % | Quantity of Dimilin WP kg/ha | | | Amount of DFB a.i. (kg/ha) | |
|---|---|---|---|---|---|
| | g/plot | Cabbage | Cowpea | Cabbage | Cowpea |
| 0.2 | 8.000 | 5.280 | 6.667 | 1.320 | 1.665 |
| 0.1 | 4.000 | 2.640 | 3.333 | 0.660 | 0.832 |
| 0.05 | 2.000 | 1.320 | 1.556 | 0.330 | 0.416 |
| 0.02 | 0.800 | 0.528 | 0.667 | 0.132 | 0.166 |
| 0.01 | 0.400 | 0.264 | 0.333 | 0.066 | 0.083 |

host crops until pupation or death. In control, untreated leaves were supplied to the larvae.

Observations :

The observations were recorded regularly at an interval of 24 hrs after the treatment for larval mortality and pupation in both the test insects.

Statistical Analysis

(a) Calculation of $LD_{50}/LC_{50}$ :-

The mortality data so obtained in different laboratory experiments against the treatment dosages/ concentrations were converted into corrected percentage mortality using Abbott's formula (1925). These data were subjected to probit analysis (Finney, 1971) for calculating $LD_{25}/LC_{25}$ and $LD_{50}/LC_{50}$ (i.e. that dosage/concentration

to give 25 and 50 per cent kill respectively). Analysis for test of heterogenity was also done for these experiments.

(b) Analysis of variance :

The data on total larval mortality prior to pupation in the field evaluation experiments was considered as pupal inhibition. These data were put to statistical analysis for determination of critical difference (C.D.) among the treatments at 5 per cent level.

IV. Histopathological investigations :

The treatment dose of 5.0 µg/larva was given by feeding method to the test insect, the fifth instar larvae of S. litura. These larvae were pre-starved for 6 hours individually in petriplates. The treatment dose was prepared by mixing 5 ml of stock solution II with 95 ml of water. The treatment procedure was similar to that used in bioefficacy experiment (oral toxicity) by feeding method.

Five treated and 5 untreated larvae were taken out randomly at 48, 72 and 96 hrs after treatment and were sacrificed. They were cut alive into four to five pieces of almost equal size and were directly fixed in alcoholic bouins fluid.

The histopathological changes in the cuticle were investigated in DFB treated insects using the Mallory's triple stain technique as described by M.J. Gijswijt (personal communication, 30.1.1985).

Mallory's triple stain technique -

(a) Reagents and apparatus :

1. Bouins fixative

| | | |
|---|---|---|
| Glacial acetic acid | .. | 1 part |
| 40% formaldehyde | .. | 5 parts |
| Saturated alcoholic solution of picric acid mixed immediately prior to use | | 15 parts |

2. Ethanol (different grades viz. 30%, 70%, 80%, 90%, 96% and absolute)

3. Methyle benzoate

4. Xylene

5. Paraffine (B.P. 60°C)

6. $H_2SO_4/K_2Cr_2O_7$ mixture

| | | |
|---|---|---|
| Stock | - $K_2Cr_2O_7$ 5% | 1 part |
| | $H_2SO_4$ 1% | 1 part |
| Dilution | - stock mixture | 1 part |
| | Distilled water | 6 parts |

Note- dilution made prior to use.

7. Sodium bisulfite ($NaHSO_4$)

8. Mallory's triple stain

| | |
|---|---|
| Biebrich red | 0.5 g |
| Orange G | 0.1 g |
| Glacial acetic acid | 0.2 g |
| Aqua dest | 100.0 ml |

9. Phosphotungsten acid (5% aquous solution)

10. Aniline blue stain

| | |
|---|---|
| Aniline blue | 0.25 g |
| HCl 36% | 0.25 ml |
| Aqua dest | 100.00 ml |

11. DPX mountant
12. Hot plate
13. Ice bath
14. 'L' pieces for block preparation
15. Rotary microtome
16. Microscope with sliding stage, adequate power and focus light arrangements

(b) **Procedure** : The procedure involved two stages

(i) Preparation of the blocks

1. Immediately killed test larvae were cut into pieces not more than 1 cm in thickness and were fixed in Bouins fixative
2. Washed in 70% ethanol, 3x1 hr or more, Lithium chloride was used to fasten the process
3. Passed through alcohol series
   80%, 90% alcohol - 1 hr each
   96% alcohol (two washings) - ½ hr each
   Methyl benzoate 2 hrs (two washings)
4. Passed through xylene - two times - ½ hr each time
5. Kept in paraffine medium on a hot plate at 60°C for 10 minutes - paraffine changed 3 times.

6. During latter steps the material was moved by means of a needle for better escape of the enclosed xylene
7. Embedded in paraffine medium with the use of 'L' pieces
8. The blocks were cooled quickly in ice bath to avoid crystallisation of paraffine

(ii) Section cutting, stainning and mounting of the material

1. Sections were cut in 6-8 $\mu$ thickness
2. They were put on slides freshly coated with thin layer of egg albumin
3. Spreading of the sections was done on hot plate using water as media
4. The sections on the slide were dried in an incubator at 30°C for overnight
5. The paraffine wax was removed with xylene - ½ hour treatment
6. Passed through alcohol series (downward) 96% → 90% → 80% → 70% → water for 2 minutes at each stage
7. Oxidised the sections in oxidant ($H_2SO_2/K_2Cr_2O_7$ mixture) for 90 minutes
8. Rinsed in water for 2 times - 2 minutes each time
9. Reduced with 0.125% aquous sodium bisulfite for 3 minutes
10. Rinsed in tap water
11. Stained in Mallory's Triple Stain for 10 minutes

12. Differentiated in alcohol 30% for 10 mimutes
13. Passed the sections through mordant (phosphotungsten acid 5%) for 10 mimutes
14. Rinsed in distilled water then counter stained in analine blue for 5-10 mimutes
15. Rinsed in distilled water for 5 seconds
16. Dehydrated in alcoholic series - 70% → 90% → 96% for 15 seconds each
17. Cleared in xylene - 2 times - for 5 mimutes or little longer
18. Mounted in DPX
19. Covered with coverlip

The histological preparations were ready for examination. The slides were stored after drying at 40°C for 2 days.

Examination of the histological sections :

The histopathological changes in DFB treated cuticle in comparison to control preparations were critically examined under light microscopy.

V. Biochemical investigations :

The fifth instar stage larvae of S. litura were starved for 6 hours individually in petriplates. The treatment doses of 5.0 and 1.0 µg/larva were given by feeding method to the test insects. This was achieved by preparing various concentrations of Dimilin 25 WP in water using stock

solution II as per details mentioned in oral toxicity experiment (feeding method). A definite amount of DFB solutions from these concentrations was pipetted on the surface of castor leaf bits roughly measuring 10 $cm^2$.

These leaf bits were given to the starved test larvae. In most of the cases the larvae consumed the treated leaf bits completely. However, in a very few cases the larvae failed to consume them; such larvae were not included in the treatment. In all, fifty larvae were exposed to each treatment dose. They were then given fresh food ad libitum after being fed on treated leaf bits. A control was also run simultaneously in which DFB free leaf bits were fed.

Effect of DFB on haemolymph and cuticular proteins :

(A) Haemolymph protein pattern

The treated larvae were sacrificed after 24, 48 and 72 hrs in both the treatments. The effect of DFB on the haemolymph protein picture was investigated using gel electrophoresis technique of Maurer (1968) as described by Srivastava (1970).

1. Preparation of samples

The haemolymph of the treated and untreated larvae were extracted at different time intervals by puncturing the insect body at the site of the prolegs. The haemolymph was

collected through glass capillaries. The samples were used freshly for the electrophoretic separation of proteins. Larvae once utilized were not reused.

2. Qualitative estimation of proteins

The Shandon polyacrylamide gel disc electrophoresis equipment (type SAE 2761 Shandon Scientific Co. Ltd. London N.W. 10) was used for the separation of proteins. The details of the gel electrophoresis technique used, was as under :-

(i) Preparation of solutions

Different solutions were prepared by mixing the components as described below. All the solutions were stored in amber coloured reagent bottles in the refrigerator :

Solution : 1

| | |
|---|---|
| 1 N Hydrochloric acid | 48.0 ml |
| Tris (Tris hydroxy methyl amino methane) | 36.6 g |
| Temed (Tetra methyl ethylene diamine) | 0.46 ml |
| Distilled water | 100.0 ml |

Solution : 2

| | |
|---|---|
| Acrylamide | 28.00 g |
| Bis (NN'-Methylene bis acrylamide) | 0.736 g |
| Distilled water | 100.00 ml |

Solution : 3

| | |
|---|---|
| Per (ammonium peroxide sulphate) | 0.14 g |
| Distilled water | 100.00 ml |

Solution : 4

| | |
|---|---|
| 1 M orthophosphoric acid | 26.6 ml |
| Tris | 5.7 g |
| Distilled water | 100.0 ml |

Solution : 5

| | |
|---|---|
| Acrylamide | 10.0 g |
| Bis | 2.5 g |
| Distilled water | 100.0 ml |

Solution : 6

| | |
|---|---|
| Riboflavin | 0.4 mg |
| Distilled water | 100.0 ml |

Solution : 7

| | |
|---|---|
| Sucrose | 40.0 g |
| Distilled water | 100.0 ml |

Solution : 8

| | |
|---|---|
| Tris-glycine buffer (stock) | 8.3 pH |
| Tris | 6.0 g |
| Glycine | 28.8 g |
| Distilled water | 1000.0 ml |

(ii) Electrophoretic procedure :-

The running gel was prepared by mixing solution 1, 2, distilled water and solution 3 in the ratio 1:2:1:4. The quantity used for 10 gel tubes was 3, 6, 3 and 12 ml respectively. The pH of the gel was maintained as 8.9 and the pores were of medium size.

The gel tubes were fixed in a stand and were filled with running gel upto two-third of their height. The surface of the gel was covered with 2-3 drops of distilled water to

check the shrinkage of the gels. The gel filled tubes were kept under a day light flourescent tube for an hour or so for polymerization.

The spacer gel was prepared by mixing solutions 4, 5, 6 and 7 in the ratio 1:2:1:4. The pH of the spacer gel was 6.9. After removing the water from the gel tubes, 2-3 ml of spacer gel was poured on the top of running gel in each tube. Then again, 2-3 drops of distilled water were added on the surface of spacer gels. Polymerization of spacer gel was done by exposing each side of the tubes for half an hour to a U.V. lamp.

After removing excess water from the surface of the gel with the help of glass capillary tube/a syringe, the gel tubes, were fixed in the upper tank of the electrophoretic equipment keeping sufficient length on the lower side so as to reach in the lower tank. Both, the upper as well the lower tanks were filled with 10 per cent buffer solution to the level in such a way that the ends of the gel tubes remained dipped in the buffer.

In each gel tube 0.05 ml of the sample (haemolymph) was poured on the top of the spacer gels from where excess water was removed earlier. The remaining part of the tubes were filled with 10 per cent buffer solution prepared from the solution 8. Thereafter 0.001% aqueous bromophenol blue was

added to the buffer in the upper chamber which served as a marker to indicate appropriate time to terminate the electrophoresis. It was also used to calculate the Rm values of the protein bands.

The electrophoresis was carried out at a constant current of 4 m A per tube. The current was discontimued when the marker rings reached the ends of the gel columns. The gels were removed from the tubes by pushing the distilled water on their sides with the help of a syringe. Gel columns were fixed and stained in 1 per cent Amido Black B (E. Merck) in 7 per cent acetic acid for half an hour. Excess stain was removed by washing the columns with 7 per cent acetic acid till the bands were clearly visible. The gel columns were stored at room temperature in the same solution .

(iii) Interpretation of gel columns :

The relative electrophoretic mobility (Rm) values of the separated protein bands were calculated by using the following formula :

$$Rm = \frac{\text{Distance travelled by protein bands from origin}}{\text{Distance travelled by tracking dye from origin}}$$

The junction of spacer and the running gels was considered as point of origin.

(B) Cuticular protein pattern

The treated larvae were sacrificed after 24, 48 and 72 hours. The effect on the cuticular protein picture was investigated by using the technique described earlier. However, the preparation and extraction of the samples were different.

(i) Preparation of cuticles

The cuticles of the treated and untreated larvae were obtained by the method used by Hackman (1953) with modifications described by Srivastava (1970). The details of the method were as under :

To obtain the cuticles the larvae were cut open longitudinally and the body contents were removed in a washing medium of 65% (v/v) aqueous ethanol. The cuticles were then transferred to fresh aqueous ethanol and the remaining tissues, muscles and hypodermal cells were removed by scraping with a blunt scalpel and a match stick. The last stage of removal being carried out under a binocular microscope. All possible care was taken to remove the muscles and the hypodermis. The cuticles were rinsed for several times with fresh aqueous ethanol (65%). This helped in denaturing the muscles and their subsequent removal; further it also avoided the loss of cuticular proteins. The cuticles were then kept in petrol-ether (B.P. 50-60°C) for 24 to 48 hrs at room temperature to remove lipid material. Finally

they were dried in a vacuum dessiccator over phosphorous penta-oxide and potassium hydroxide. Cuticles of 25 to 30 individuals were prepared at one time.

(ii) Extraction of proteins

The dried cuticles were grounded with glass powder in a mortar. For the electrophoretic investigation the water soluble fractions of cuticular proteins were extracted through borax-boric acid buffer solution at pH 9.2 for 2 days at 50°C. following one of the methods of Hackman (1953). The Borax-boric acid buffer was prepared as under :

Borax-boric acid buffer (pH 9.2)

Solution A - 0.2 m sol. of Boric acid by mixing 12.4 g of the chemical in 1 litre distilled water

Solution B - 0.5 m sol. of Borax by mixing 19.05 g of the chemical in 1 litre distilled water

Stock Buffer - 50 ml of A + 114 ml of B. The volume was made upto 200 ml.

The pH 9.2 was adjusted with the help of 1N HCl or 1N NaOH

Final Buffer - Prepared by mixing stock buffer, distilled water, ethanol and diethyl ether in the ratio of 5:5:4:1

Distilled water - double or glass distilled water was used

The quantity of buffer used was 30-50 times of the material.

The solution of extracted protein was filtered, acidified to pH between 3 to 4 with 1N HCl and the protein precipitate was obtained. After maintaining the required pH the protein solution was further precipitated by one-third saturation with $(NH_4)_2SO_4$. It was shaken well with the help of test tube shaker until all the $(NH_4)_2SO_4$ was dissolved.

The protein was collected by centrifugation at 6500 rpm. The supernatant was discarded and the residue was resuspended in distilled water. It was dialysed in a cellophan sac against distilled water for two days at 10-15°C in refrigerator. Completion of dialysis was tested by way of precipitation of sulphate ions in a sample of the water by mixing $BaCl_2$.

To obtain the water-soluble fraction, dialysed solution was evaported in Vacuo in a rotavapour. The dried residue protein was weighed.

(iii) Electrophoretic separation and interpretation of gel columns :

The dried protein was disolved in electrophoretic buffer making proper graded solutions. The electrophoretic analysis and the interpretation of the gel columns were done in the similar way as described earlier.

# EXPERIMENTAL FINDINGS

In order to find out the physiotoxicological effects of diflubenzuron (DFB), a chitin inhibitor, both the test insects i.e. Spodoptera litura Fab. and Euproctis virgincula Wlk. were subjected to intensive investigation. However, histopathological and bio-chemical experiments were carried out only on S. litura.

## A. Spodoptera litura Fab.

### I. TOXICOLOGICAL INVESTIGATIONS :

The bioefficacy of DFB was investigated by evaluating its dermal and oral toxicity.

(i) Dermal toxicity : ($LD_{25}/LD_{50}$)

The dermal toxicity had been worked out by two methods viz. dry film and dipping method.

(a) Dry film method :

The fifth instar larvae were exposed to different treatment dosages for 12 and 24 hrs to determine the $LD_{25}$. The results were given in Table 2. There was no mortality during both the exposure periods. The cumulative mortality

Table 2. Dermal toxicity of diflubenzuron against the larvae of S. litura (Dry-film method)

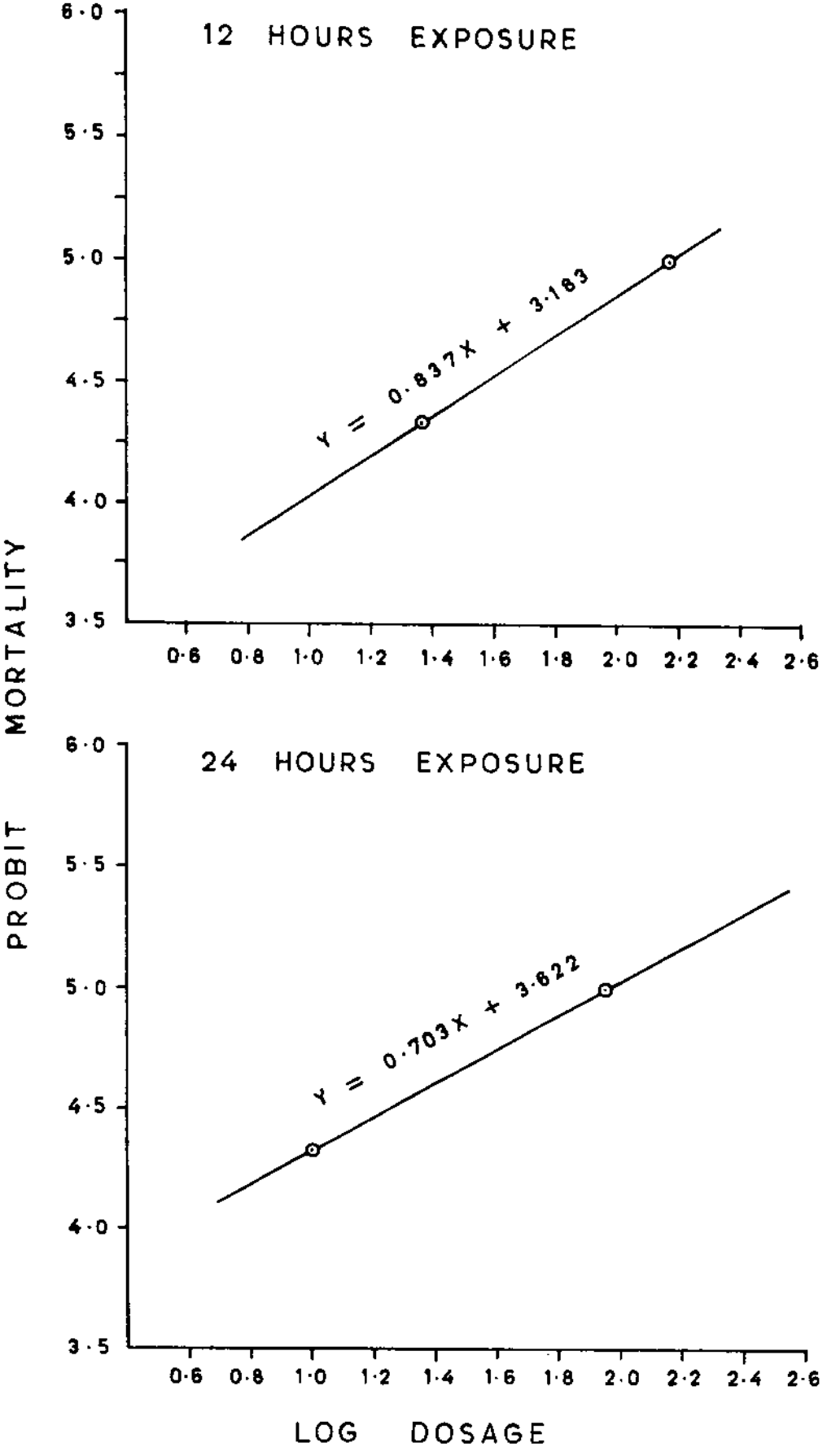
| Exposure periods | Heterogenity ** | Regression equation | $LD_{25}$* | Ratio $\frac{12\ hr}{24\ hr}$ | $LD_{50}$* | Ratio $\frac{12\ hr}{24\ hr}$ | Fiducial limits* of $LD_{25}$ |
|---|---|---|---|---|---|---|---|
| 12 hours | $X^2(5)=1.7965$ | $Y = 0.837x+3.183$ | 23.21 | 2.31:1 | 148.60 | 1.63:1 | 16.43 to 33.66 |
| 24 hours | $X^2(5)=1.2958$ | $Y = 0.703x+3.622$ | 10.03 | | 91.28 | | 5.34 to 18.82 |

$LD_{25}$ and $LD_{50}$ = Lethal dosages to give 25 and 50 per cent mortality

* = mg/90 $cm^2$ surface area; Y = probit kill; X = log concentration

**= In none of these cases, the data were found to be significantly heterogenous at P =0.05

DIFLUBENZURON AGAINST S. LITURA LARVAE (DRY FILM METHOD)



was recorded after 48 hours of the treatment. The data so obtained were used for calculating $LD_{25}$, which were 23.21 and 10.03 mg/90 $cm^2$ surface area for 12 and 24 hrs exposure periods respectively. The regression equations derived for $LD_{25}$ were also used to determine the $LD_{50}$ for these exposure periods. The slopes of regression were 0.837 and 0.703 for the corresponding treated periods (Table 2).

When compared, the $LD_{25}$ value at 24 hrs exposure was found 2.31 times less than that of 12 hrs exposure. A similar sequence was observed in the case of $LD_{50}$ values where the ratio was 1.63. The standard dosage mortality curves of DFB film treatment against S. litura had been given in Fig. 1.

(b) Dipping method :

The $LC_{25}$ and $LC_{50}$ values obtained were 85.33 and 629.80 ppm respectively. The slope value was 0.777. The Chi-square value at P = 0.05 was significantly homogenous (Table 3). The standard dosage mortality curve for dipping treatment had been depicted in Fig. 2.

(ii) Oral toxicity : ($LD_{25}/LD_{50}$)

No mortality was obtained 24 hrs after the treatment. The $LD_{25}$ and $LD_{50}$ values calculated on the basis of total

Table 3. Dermal toxicity of diflubenzuron against S. litura Fab. larvae (Dipping method)

| Heterogenity | Regression equation | $LC_{25}$ (ppm) | $LC_{50}$ (ppm) | Fiducial limits of $LC_{50}$ (ppm) |
|---|---|---|---|---|
| $X^2$ (6) 0.1754 | Y = 0.777 x + 2.825 | 85.33 | 629.80 | 488.80 - 809.30 |

$LC_{25}$; $LC_{50}$ = Lethal concentration to give 25 or 50 per cent mortality respectively

$X^2$ = Chi-square value at P = 0.05 was significant

Y = Probit kill, x = log concentration

Table 4. Oral toxicity of diflubenzuron against S. litura larvae (feeding method)

| Heterogenity | Regression equation | $LD_{25}$ /ug/larva | $LD_{50}$ /ug/larva | Fiducial limits of $LD_{50}$ (/ug/larva) |
|---|---|---|---|---|
| $X^2_{(2)}$ 0.5494 | Y = 0.619 x + 4.526 | 0.41 | 5.83 | 1.72 - 19.79 |

$LD_{25}$; $LD_{50}$ = Lethal dose to give 25 or 50 per cent mortality respectively

$X^2$ = Chi-square value at P = 0.05 was significant

Y = Probit kill, x = log dose

mortality before pupation were 0.41 and 5.83 /ug/larva respectively. The slope value was 0.619. The Chi-square value at P = 0.05 was found significantly homogenous (Table 4). The standard dosage mortality curve had been given in Fig. 3.

II. SYMPTOMATIC AND BEHAVIOURAL INVESTIGATIONS :

1. Behavioural changes

(i) Sluggishness

Sluggishness was considered as the initiation of behavioural changes exhibited by the larvae after consuming the treated food. This effect was observed from next day of the feeding of the treated food i.e. 24 hours after eating of DFB mixed food. Sluggishness was triggered with unwillingness of the larva to move from the place of their stay. The larvae did not stir their body even after they were subjected to pricks.

The sluggishness was observed in 14.03 per cent larvae in 5.0 /ug dose of DFB per larva. The percentages of larvae showing this symptom in 1.0 and 0.5 /ug doses were 3.38 and 3.51 respectively. The lowest dose chosen was 0.1 /ug/larva. Here the treated larvae were almost like the larvae in control and showed no differential symptoms (Table 5).

FIG. 2 STANDARD DOSAGE MORTALITY CURVE OF DIFLUBENZURON TREATMENT AGAINST S. LITURA LARVAE BY DIPPING METHOD

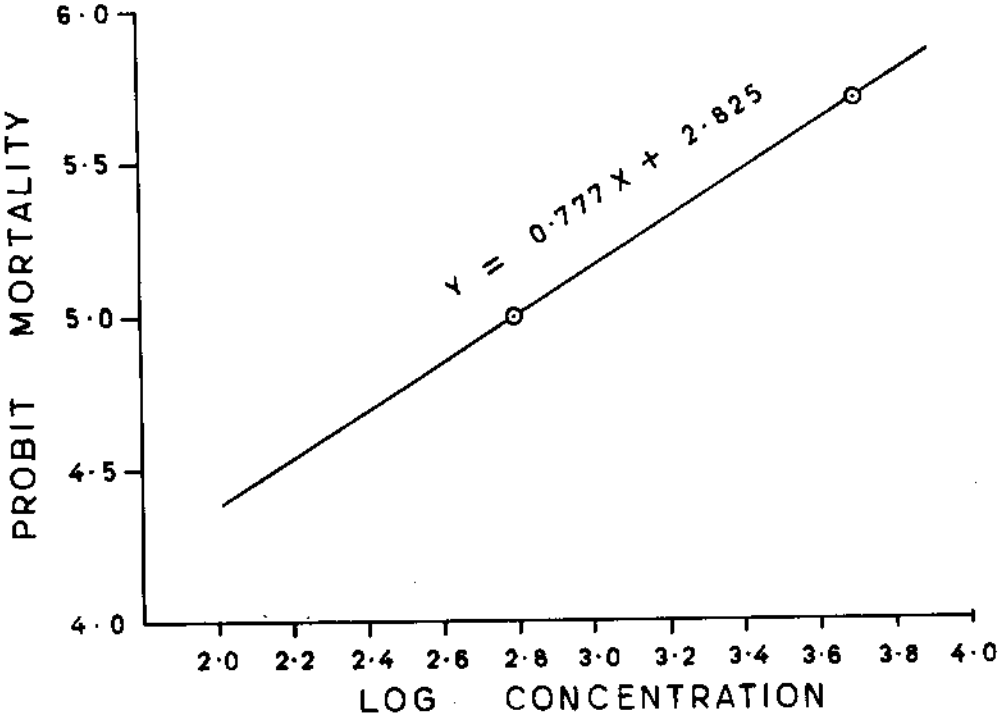
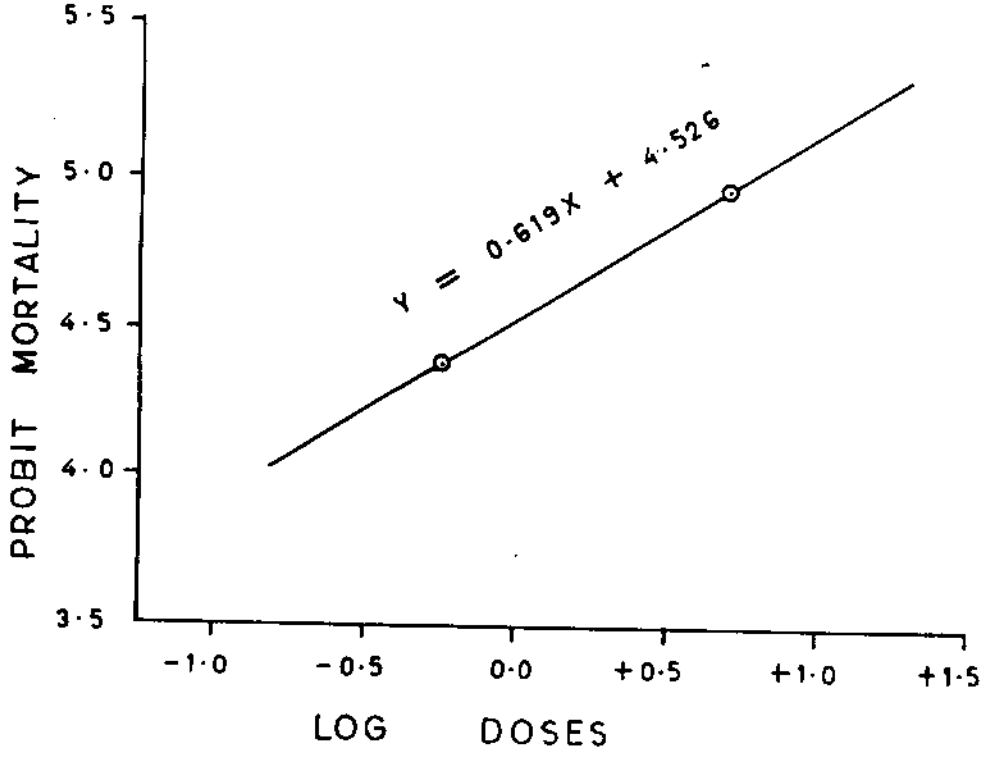



FIG. 3 STANDARD DOSE MORTALITY CURVE OF DIFLUBENZURON AGAINST S. LITURA LARVAE BY FEEDING METHOD



(ii) Cessation of locomotion

Sluggishness was culminated to its highest order when the larvae ceased to move from the place where they were lying for the last 24 hours after consuming the treated food. The larvae showing such symptoms were 10.53 per cent in 5.0 /ug/larva dose and 10.17 per cent in 1.0 /ug dose whereas in the lower dose of 0.5 /ug, only 1.75 per cent larvae showed cessation of locomotion. In the lowest dose (0.1 /ug/larva) and in control none of the larvae exhibited this symptom (Table 5).

(iii) Cessation of feeding

An inevitable effect following the sluggishness and cessation of locomotion was the ceasing of feeding. It was observed that inspite of large amount of food available to the larvae in the petridishes the feeding stopped from 24 to 48 hours after consuming the treated food. These behavioural changes led to a sort of total ceasing of physiological activities within the larval body. The percentages of larvae exhibiting these symptoms in different treatment doses had been presented in Table 5. In 5.0 /ug/larva dose 28.07 per cent larvae ceased feeding. The per cent larvae affected in 1.0 /ug and 0.5 /ug doses were 13.58 and 8.77 respectively. In the lowest dose (0.1 /ug) and in control no larva was observed who ceased feeding.

Table 5. Behavioural abnormalities in S. litura larvae fed with different doses of DFB

| Behavioural parameters | Per cent insects showing abnormal symptoms Doses in µg/larva | | | | |
|---|---|---|---|---|---|
| | 5.0 | 1.0 | 0.5 | 0.1 | 0.0(control) |
| 1. Sluggishness | 14.03 | 3.38 | 3.51 | 0.00 | 0.00 |
| 2. Cessation of locomotion | 10.53 | 10.17 | 1.75 | 0.00 | 0.00 |
| 3. Cessation of feeding | 28.07 | 13.58 | 8.77 | 0.00 | 0.00 |
| 4. Wetting | 68.42 | 45.76 | 36.83 | 20.00 | 0.00 |
| 5. Oozing | 80.70 | 49.15 | 35.08 | 15.00 | 0.00 |
| Number of larvae exposed to treatment | 57 | 59 | 57 | 40 | 60 |

(iv) Wetting

The wetting was recorded in 68.42, 45.76, 36.83 and 20.00 per cent larvae in the treatment doses of 5.0, 1.0, 0.5 and 0.1 /ug (Table 5). In the cases of excessive liquid coming out of the body the larvae had to be dried with blotting paper twice a day for recording the observations. In animals treated with higher doses of DFB, the wetting was invariably heavy.

(v) Oozing

It was difficult to differentiate between wetting and oozing. However, under this caption liquid coming out from definite sources such as mouth, anus etc. had been incorporated. The oozing of the body fluid was observed in 80.70, 49.15, 35.08 and 15.00 per cent larvae in 5.0 /ug, 1.0/ug, 0.5 /ug and 0.1 /ug/larva doses respectively (Table 5).

The site of oozing of body fluid was examined with the help of stereoscopic microscope. When constantly observed the oozing of body fluid could be seen from mouth, anus and through intersegmental membranes which caused complete wetting of the body. In animals treated with higher doses of DFB, the oozing was invariably heavy.

Generally clear light green or dark green coloured or occasionally pale green fluid was oozed out from the mouth.

Whereas from anus mostly brown or dirty coloured liquid came out. However, in some cases it was a clear green liquid. In a few heavily DFB dosed animals the oozed fluid was green with fine suspended particles.

The smear examination of the fluids obtained from mouth and anus mostly showed the presence of undigested food particles. The haemocytes were invariably seen in all the smear preparations obtained from different body regions.

2. Morphological deformities

(i) Shrinkage in larval body

Normally the larval body shrinks when it tends to undergo pupation. Abnormal shrinkage of the body was observed in treated larvae to an extent of 1/5th of the normal one, further most of these larvae remained straight, and no signs of pre-pupation was observed (Plate 1; Fig. 1, 2, 3). The texture of such larvae was also not soft and they lost their natural colouration. Further, the behaviour of such larvae was also different from those of normal larvae; they were not sensitive to touch.

Data given in Table 6a shows that in 5.0/ug treatment, 35.09 per cent larvae were shrunken, whereas in 1.0 /ug and 0.5 /ug doses the affected larval percentages were 20.34 and 17.54 respectively. The shrunken larvae were observed least

Table 6a. Morphological symptoms in S. litura larvae fed with DFB

| Morphological symptoms | Per cent larvae with the abnormal symptoms (doses /ug/larva) | | | | |
|---|---|---|---|---|---|
| | 5.0 | 1.0 | 0.5 | 0.1 | 0.0 (Control) |
| 1. Shrinkage of body | 35.09 | 20.34 | 17.54 | 2.50 | 0.00 |
| 2. (a) Discolouration | 66.67 | 54.24 | 28.07 | 5.00 | 0.00 |
| (b) Scar formation | 1.75 | 6.78 | 0.00 | 0.00 | 0.00 |
| 3. Rupturing of cuticle | 31.58 | 15.25 | 14.03 | 2.50 | 0.00 |
| 4. Rectal papillae | 5.26 | 1.69 | 0.00 | 0.00 | 0.00 |
| Number of larvae treated | 57 | 59 | 57 | 40 | 60 |

in 0.1 /ug (upto 2.5 per cent). There was no such abnormal shrinkage of the body in control.

(ii) Discolouration and scar formation

It was invariably associated with oozing of liquid from the body of treated insect. Dark brown spots were formed at the site of fluid oozing. In some cases, where treated larvae did not feed, their body was shrunked and the cuticle colour changed to dark brown or dark black. Whereas, in a few cases, where the cuticle was not properly sclerotized, it did not retain its natural texture and colour. It showed somewhat mosaic pattern.

The discolouration of cuticle was found in 66.67, 54.24, 28.07 and 5.0 per cent larvae in the treatment doses of 5.0, 1.0, 0.5 and 0.1 /ug respectively. Such variations in colour pattern were wanting in the control larvae (Table 6a).

In some of the larvae scars were seen. This symptom was observed only in the larvae fed with 5.0 and 1.0 /ug doses; the larvae showing scar formation symptoms were 1.75 and 6.78 per cent in these treatments respectively. The colour of scars was usually blackish and were found on lateral sides of the thoracic region in both the treatments (Table 6a).

(iii) Cuticular rupture

The cuticle rupture of the larvae was maximum (31.58 per cent) in 5.0 /ug DFB dose, which declined to 15.25 and 14.03 per cent in treatment doses of 1.0 and 0.5 /ug/larva respectively. In the lowest treatment dose (0.1 /ug) this effect was only in 2.5 per cent animals (Table 6a). Cuticular rupturing was totally absent in control. In the treated larvae the rupture of cuticle was found with variable degrees and loci. The symptom usually appeared 48 hours after the treatment and the rupturing process continued gradually at different sites, followed by oozing of body fluid until the larvae were dead. Splitting was invariably observed at the intersegmental membranes. However, in a few cases it was also observed on dorsal as well lateral thoracic and the lateral abdominal regions.

(iv) Rectal prolapse

It was observed that in some of the DFB fed larvae, which had body fluid leakage from the anus, a baloon like structure was seen protruding from the anus. This structure was membranous and filled with clear green or yellowish fluid. These rectal bulging became darker within 24 hours and finally bursted leading to the leakage of fluid from it. Such individuals died within 2 to 3 days after appearance of the rectal bulgings. Rectal prolapse percentages were 5.26 and 1.69 in 5.0/ug and 1.0/ug treatments respectively. These symptoms could not be observed at the doses lower than 1.0 /ug (Table 6a).

(v) Frayed condition of cuticle

Frayed conditions of the cuticle were met with when the process of moulting was to start. Although, the cuticle was not ruptured but fragments of cuticle got removed from the body at scattered regions.

Frayed condition was observed in all the treatments doses. It was seen to the tune of 10.52, 3.39, 5.26 and 5.0 per cent cases when the larvae were fed with 5.0, 1.0, 0.5 and 0.1 /ug/larva doses respectively. In control (untreated) larvae such condition was totally absent (Table 6b).

(vi) Mosaic condition of cuticle

In the mosaic condition of cuticle in pre-pupae several patches of cuticle were completely sclerotised. There were dark-brown spots at different parts of the body.

The appearance of mosaic like conditions on the outer covering of the body was predominent in the treated animals. Presence of mosaic condition differed in degrees with the doses administered to the larvae; it was observed in 42.11, 47.46, 28.07 and 30.00 per cent larvae supplied with 5.0, 1.0, 0.5, and 0.1 /ug DFB doses respectively (Table 6b).

(vii) Incomplete shedding of exuviae

The incomplete shedding of exuviae was observed in 10.52, 6.78 and 3.51 per cent larvae at the head capsule region; in 3.50, 5.08 and 3.51 per cent larvae at thoracic

Table 6b. Morphological abnormalities observed at larval and prepupal stages of S. litura fed with DFB treated food

| Treatment doses /ug/ larva | Number of larvae treated | Frayed condition of cuticle in larva | Mosaic condition of cuticle in prepupa | Incomplete shedding of exuviae at different body regions | | | | Rupture of old cuticle at | | Larvae with partial pupal cuticle in ventral abdominal region |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Head capsule | Thoracic region partial | Thoracic region complete | Abdominal region | Thorax | Abdomen | |
| 5.0 | 57 | 10.52 | 42.11 | 10.52 | 1.75 | 1.75 | 14.03 | 5.26 | 3.51 | 21.05 |
| 1.0 | 59 | 3.39 | 47.46 | 6.78 | 5.08 | 0.00 | 5.08 | 0.00 | 0.00 | 35.59 |
| 0.5 | 57 | 5.26 | 28.07 | 3.51 | 3.51 | 0.00 | 5.26 | 0.00 | 0.00 | 12.28 |
| 0.1 | 40 | 5.00 | 30.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 10.00 |
| 0.0 (Control) | 60 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

region and in 14.03, 5.08 and 5.26 per cent cases at abdominal region in the treatment doses of 5.0, 1.0 and 0.5 /ug/larva respectively. Whereas in 0.1 /ug and the control, no larva was observed with incomplete shedding of the old cuticle (Table 6b).

In 5.0 /ug dosage some of the larvae (1.75 per cent) had shedding of the cuticle in a part of the thorax only. Whereas some others (1.75 per cent cases) shedded cuticle in whole of the thoracic region. In subsequent lower doses none of the larvae had cuticle shedding in whole of the thorax. Cases with shedding of the cuticle in part of the thorax were 5.08 per cent in 1.0 /ug dosage and 3.51 per cent in 0.5 /ug dosage.

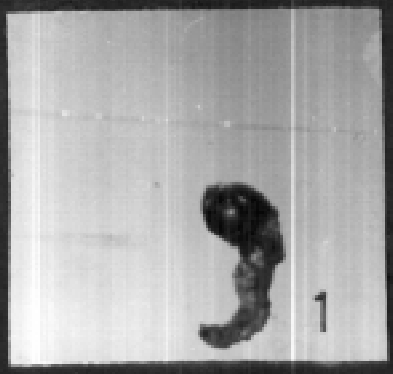
(viii) Rupturing of old cuticle

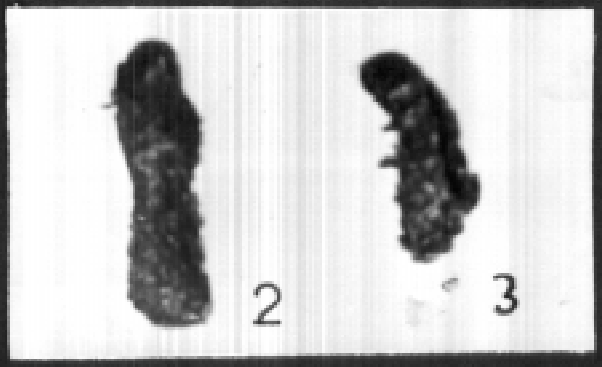
The rupture of old cuticle at pre-pupal stages was met with in 5.0 /ug dose only; it was found that 5.26 per cent animals ruptured at thoracic region while 3.51 per cent at the abdominal region (Table 6b).

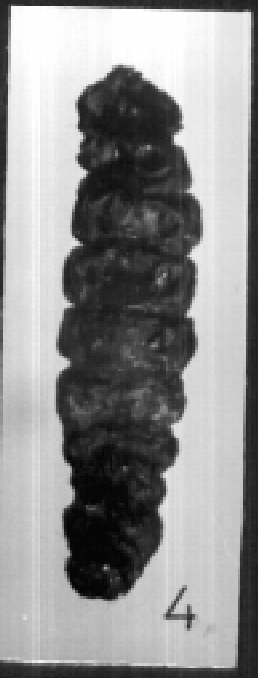
(ix) Presence of pupal cuticle in parts (Fig 4, 5, 6 of Plate 1)

In some of the DFB fed larvae only the ventral part of the thorax developed pupal cuticle, which was clearly visible below the old cuticle. There was sporadic sclerotization of the cuticle. (In some cases it was sclerotized,

# PLATE 1

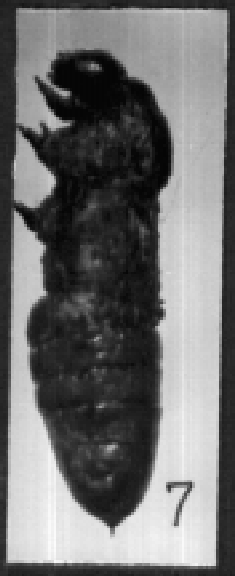

1


2 3

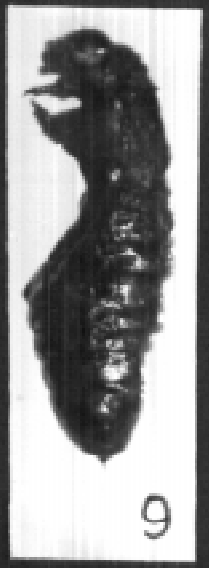

4


5


6


7


8


9


10

whereas, in others it was not). However, rest of their body retained larval cuticle and characters. Such cases were 21.05, 35.59, 12.28 and 10.00 per cent among the DFB fed larvae with 5.0, 1.0, 0.5 and 0.1 /ug doses respectively. Such symptoms did not occur among the control larvae (Table 6b).

3. Formation of abnormal stages

The treated larvae, which could survive upto pre-pupal stage and beyond, also depicted a number of morphological abnormalities like the formation of larval-pupal intermediates (LPI), abnormal pupae and abnormal adults (Tables 7 and 8; Plates 1 and 2).

(i) Larval-Pupal Intermediates

Data given in Table 7 and 8 showed that the LPI formed in 5.0 /ug dose were 22.81 per cent of the treated larvae; among them 8.77 per cent remained completely trapped in the larval skin (Plate 2; Fig 1 ), 3.51 per cent were with anterior portion of their body completely larval (Plate 1; Fig. 7 ), 8.77 per cent showed a cuticular pupal hump on the dorsal region of the thorax (Plate 1 ; Fig. 8 ). Some of the LPI (1.75 per cent) were twisted in shape. No larva could reach upto the stage of pupation at this dose.

In 1.0 /ug dose 37.29 per cent LPI were formed; among them 6.78 per cent remained completely trapped in larval skin whereas 11.86 per cent LPI had their posterior portion pupal

Table 7. Formation of abnormal stages in S. litura fed with DFB treated food at fifth instar stage

| Abnormalities | Doses in /ug/larva | | | | |
|---|---|---|---|---|---|
| | 5.0 | 1.0 | 0.5 | 0.1 | 0.0 (Control) |
| 1. Larval-Pupal Intermediates (LPI) showing partial moulting : | | | | | |
| a. Pupal body completely trapped in larval skin | 8.77 | 6.78 | 3.51 | 0.00 | 0.00 |
| b. Anterior portion completely larval | 3.51 | 11.86 | 12.28 | 27.50 | 0.00 |
| c. Dorsal pupal thorax humped out of split larval skin | 8.77 | 8.47 | 3.51 | 7.50 | 0.00 |
| d. Larval head + 2 thoracic legs | 0.00 | 3.39 | 1.75 | 0.00 | 0.00 |
| e. Larval head remains | 0.00 | 0.00 | 5.26 | 7.50 | 0.00 |
| f. Deformed LPI | | | | | |
| i. Twisted | 1.75 | 1.69 | 1.75 | 0.00 | 0.00 |
| ii. Curved | 0.00 | 5.08 | 3.51 | 0.00 | 0.00 |
| iii. Anteriorly bent | 0.00 | 0.00 | 3.51 | 0.00 | 0.00 |
| 2. Pupal deformities | | | | | |
| a. With short wing pads | 0.00 | 0.00 | 1.75 | 5.00 | 0.00 |
| b. With only ventral abdominal skin sclerotized | 0.00 | 0.00 | 0.00 | 2.50 | 0.00 |
| c. Forwardly bent pupae | 0.00 | 0.00 | 1.75 | 0.00 | 0.00 |
| d. Curved pupae | 0.00 | 3.39 | 0.00 | 0.00 | 0.00 |
| 3. Normal pupae | 0.00 | 8.47 | 19.30 | 32.50 | 95.00 |
| Number of larvae released | 57 | 59 | 57 | 40 | 60 |

and anterior portion completely larval. LPI showing a cuticular pupal hump on the back of their thorax were 8.47 per cent while 3.39 per cent were having larval head and 2 pairs of larval thoracic legs (Plate 1 ; Fig.9,10); remaining portion transformed into pupal. Twisted LPI were 1.69 per cent while curved LPI were found 5.08 per cent (Table 7 and 8).

In 0.5 /ug dose the percentages of LPI trapped in larval skin, the LPI with anterior half region retaining larval characters, humped LPI, the LPI with larval head and 2 pairs of thoracic legs, the LPI with only larval head (Plate 2; Fig. 5 ), and those with deformed shape were 3.51, 12.28, 3.51, 1.75, 5.26 and 8.77 respectively. Among the deformed LPI, 1.75 per cent were twisted, 3.51 per cent were curved (Plate 2 Fig. 4) 3.51 per cent were anteriorly bent (Plate 2 ; Fig.2,3).

In 0.1 /ug dosed animals none of the LPI was found trapped in larval skin. The LPI with anterior portion of body completely larval were 27.5 per cent whereas humped LPI and those with larval head were 7.5 per cent each. There was no deformed LPI observed at this dose. Such abnormal symptoms were not found in control individuals also.

(ii) Pupal deformities

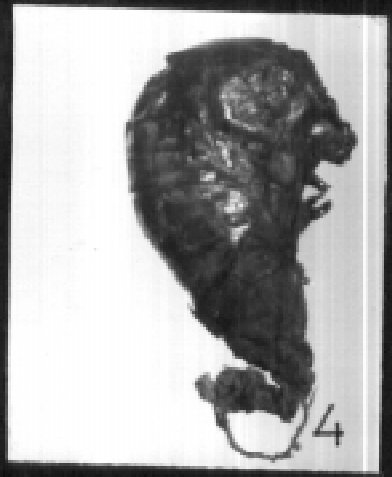
No larva could reach upto the stage of pupation in the highest tested dose of 5.0 /ug/larva. Some of the treated larvae reached pupation in 1.0 /ug dose. Among these 3.39

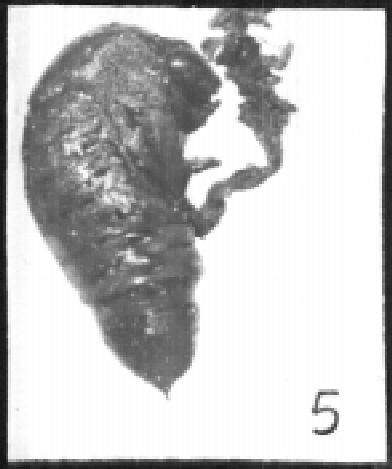
# PLATE 2

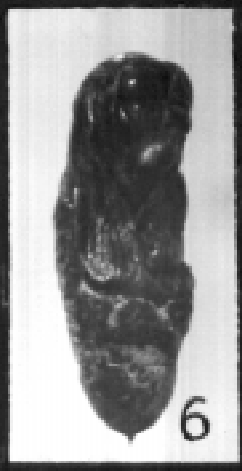

1

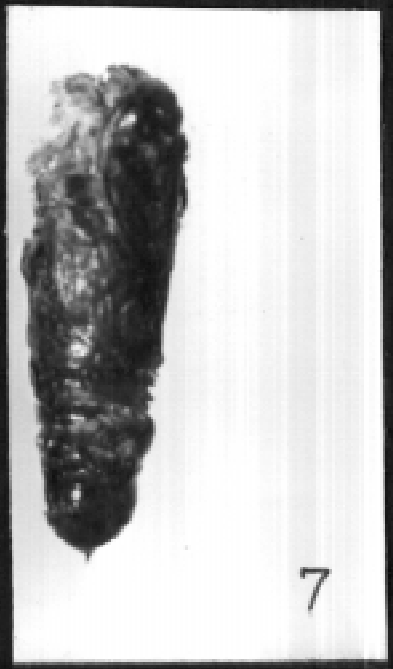

2

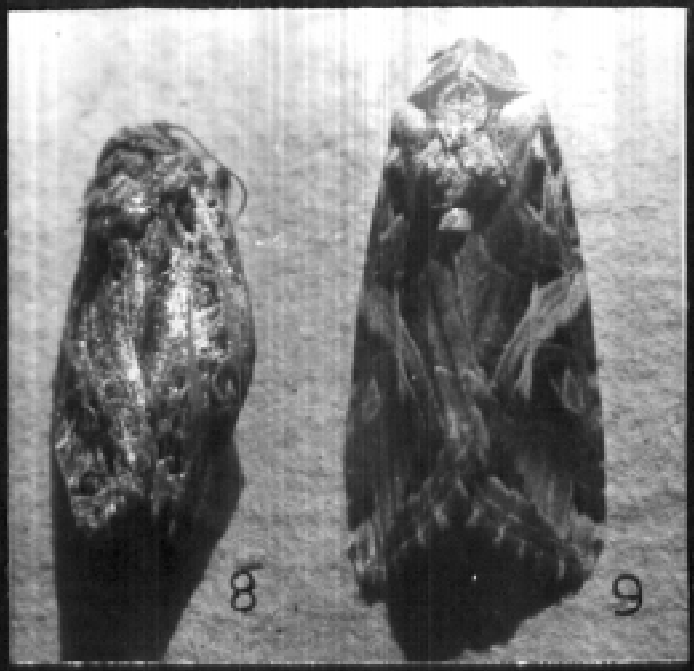

3


4


5


6


7


8 9

per cent were curved pupae and 8.47 per cent developed into normal pupae as shown in Table 7.

Deformed pupae observed in 0.5 /ug dose were 3.50 per cent (Table 7). Among these 1.75 per cent had short wing pads (Plate 2 ; Fig. 6 ) and 1.75 per cent were anteriorly bent. About 19 per cent pupae were looking normal in shape, texture and structure.

In 0.1 /ug dose 5 per cent pupae obtained from the DFB fed larvae were having short wing pads and 2.5 per cent had ventral skin unsclerotized ( Table 7 ) . Normal pupation at this dose was 32.5 per cent. In none of the control individuals such abnormal symptoms were ever observed .

(iii) Pupal-adult intermediates, abnormal and normal adults

No adult was emerged in the highest treatment dose of 5.0 /ug/larva where all the pupae were abnormal. At 1.0 /ug dose also none of the normal pupae could emerge as adult; they remained completely trapped within the pupal shell (Plate 2; Fig. 7 ). However, in 0.5 /ug dose 8.77 per cent adults were emerged, out of which only 1.75 per cent were normal; 7.02 per cent were pupal-adult intermediates or abnormal adults. Such adults were either remained attached to the pupal shell, or partly exposed from the pupal shell,

or when emerged, had their wings crippled or nonfunctional and were regarded as abnormal adults (Plate 2; Fig. 8 ). These abnormal stages were observed 12.50 per cent in 0.1 /ug dose. No such abnormality was recorded in control insects (Table 8).

4. Cumulative mortality at larval, pupal and adult stages

It was observed that mortality of the treated larvae occured irrespective of the developmental stage. The treatment doses of DFB were not fatal to the larvae immediately. Mortality was usually recorded 2 to 3 days after the treatment. However, most of the larvae died at the time of moulting with the development of abnormal symptoms described earlier.

The cumulative mortality data for each treatment dose had been summarized in Table 8. The mortality was different in different developmental stages. During larval and pre-pupal stages it ranged from 17.5 to 77.19 per cent. There was only 5 per cent mortality in untreated control. Percentages of LPI formed were 22.81, 37.29, 37.09 and 42.50 at the treatment doses of 5.0, 1.0, 0.5 and 0.1 /ug/larva respectively. Most of the LPI died within 2 days; in a few cases they could survive upto a period of 4 to 6 days but remained in moribund stage and were considered as dead. Rest of the surviving larvae were further subjected to examination.

Table 8. Effect on metamorphosis and cumulative mortality of S. litura in DFB fed larvae

| Treatments /ug/larvae | Number of insects treated | Mortality | | | LPI | Pupation | | Pupal mortality | | Adult emergence | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Larval | Prepupal | Total | | Abnormal | Normal | Among normal | Total | Abnormal | Normal |
| 5.0 | 57 | 49.12 | 28.07 | 77.19 | 22.81 | 0.00 | 0.00 | - | - | 0.00 | 0.00 |
| 1.0 | 59 | 35.59 | 15.25 | 50.85 | 37.29 | 3.39 | 8.47 | 8.47 | 11.86 | 0.00 | 0.00 |
| 0.5 | 57 | 31.58 | 10.52 | 42.10 | 35.09 | 3.51 | 19.30 | 10.52 | 14.04 | 7.02 | 1.75 |
| 0.1 | 40 | 15.00 | 2.50 | 17.50 | 42.50 | 7.50 | 32.50 | 12.50 | 20.00 | 12.50 | 7.50 |
| 0.0 (Control) | 60 | 5.00 | 0.00 | 5.00 | 0.00 | 0.00 | 95.00 | 5.00 | 5.00 | 0.00 | 90.00 |

There was no pupation in 5.0 /ug dose, whereas, in 1.0 /ug dose 8.47 per cent normal and 3.39 per cent abnormal pupation occured. In subsequent lower doses of 0.5 /ug and 0.1 /ug, the normal pupation was 19.30 and 32.50 per cent whereas, the abnormal pupae formed were 3.51 and 7.5 per cent respectively. Among the abnormal ones no pupa survived for adult emergence. Out of normal looking pupae also, 8.47, 10.53 and 12.50 per cent pupae died. Among adults those which did not come out of pupal shell were found dead.

5. Longevity and fecundity of adults : viability and hatchability of eggs

Longevity of adult moths emerged in 0.5 and 0.1 /ug ranged from 6 to 8 days. None of the normal looking adults which emerged from these treatment doses could mate and lay fertile eggs. In control the adults lived for 8-10 days. Their fecundity ranged from 230 to 380 eggs per female.

III. FIELD EVALUATION OF DFB AND ITS PERSISTENCE :

The field treated cabbage leaves sprayed with different concentrations of DFB i.e. 0.2, 0.1, 0.05, 0.02 and 0.01 per cent ; and plucked at different time intervals viz. 0, 1, 5 and 10 days after the spray, were fed to S. litura larvae for 24 hrs in lab.

Two aspects viz. larval mortality and ultimate number of larvae which failed to become pupae were estimated for further inferences.

1. Larval mortality

Although it was experienced that some per cent of the larvae would be knocked down with some higher concentrations of DFB but it was observed that this chemical hardly had any effect in bringing about the quick knock down of the larval population. During the entire period of experimentation not a single larva was found dead or moribund within 24 hours of the administration of DFB treated food.

2. Pupal inhibition

The pupal inhibition was calculated by recording the mortality prior to pupation in different treatments. The data were presented in Table 6. The results of this experiment revealed that all the concentrations of DFB were found significantly effective over control at all the time intervals tested viz. 0, 1, 5 and 10 days. The pupal inhibition of S. litura larvae fed with the spray deposits of 0.2, 0.1 and 0.05 per cent DFB was 100 per cent (Table 9).

One day old DFB treated leaves gave an inhibition of pupation by 97.76, 96.71, 94.32, 87.81 and 76.89 per cent in respect to treatment concentrations of 0.2, 0.1, 0.05, 0.02

Table 9. Pupal inhibition in the fifth instar larvae of S. litura fed with DFB treated cabbage leaves in field at different time intervals

| Concentrations | Per cent pupal inhibition*, days after treatment | | | |
|---|---|---|---|---|
| | 0 | 1 | 5 | 10 |
| 0.2 | 100.00 (90.00) | 97.76 (81.39) | 93.30 (75.00) | 77.14 (61.46) |
| 0.1 | 100.00 (90.00) | 96.71 (79.55) | 89.05 (70.69) | 70.31 (57.00) |
| 0.05 | 100.00 (90.00) | 94.32 (76.26) | 81.81 (64.76) | 61.76 (51.81) |
| 0.02 | 89.04 (70.67) | 87.81 (69.55) | 76.03 (60.69) | 55.07 (47.91) |
| 0.01 | 73.56 (59.06) | 76.89 (61.26) | 63.63 (52.91) | 39.87 (39.15) |
| 0.00 (Control) | 9.60 (18.05) | 13.24 (21.34) | 9.60 (18.05) | 11.57 (19.89) |
| S.Em. ± | 1.66 | 4.91 | 4.93 | 3.50 |
| C.D. at 5% | 5.30 | 15.70 | 15.77 | 11.18 |

* Figures in parentheses are arc-sine angles of percentages of pupal inhibition
Figures out side parentheses are back transformed values
Data based on three replicates

and 0.01 per cent. Here again, all the treatments were statistically superior over control. However, 0.2, 0.1, 0.05 and 0.02 per cent concentrations were statistically at par. The latter concentrations of 0.05 and 0.02 per cent in turn were statistically equal to the subsequent lower concentration i.e. 0.01 per cent.

Similar significance was observed in 5 day old treated leaves. Here again, all the treatments were statistically superior over control. The treatment concentrations of 0.2, 0.1, 0.05 and 0.02 per cent had no statistical difference among themselves, however, the former two concentrations (0.2 and 0.1 per cent) were significantly better over the lowest concentration of 0.01 per cent. The range of pupal inhibition obtained at this time interval was quite high (63.63 to 93.30 per cent) as is seen in Table 9.

Ten days old leaves also exhibited the residual effect of DFB to a great extent i.e. upto 77.14 per cent in the highest concentration of 0.2 per cent. There was more than 55 per cent mortality even in the dose of 0.02 per cent concentration. The highest concentration (0.2 per cent) was significantly better than 0.01 and 0.02 per cent concentrations and was simultaneously at par with 0.1 and 0.05 per cent concentrations. All the treatment concentrations were superior over the control (Table 9).

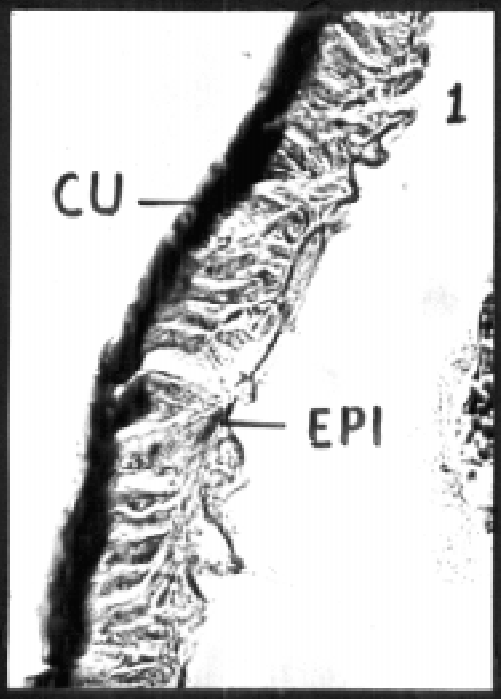
IV. HISTOPATHOLOGICAL INVESTIGATIONS :

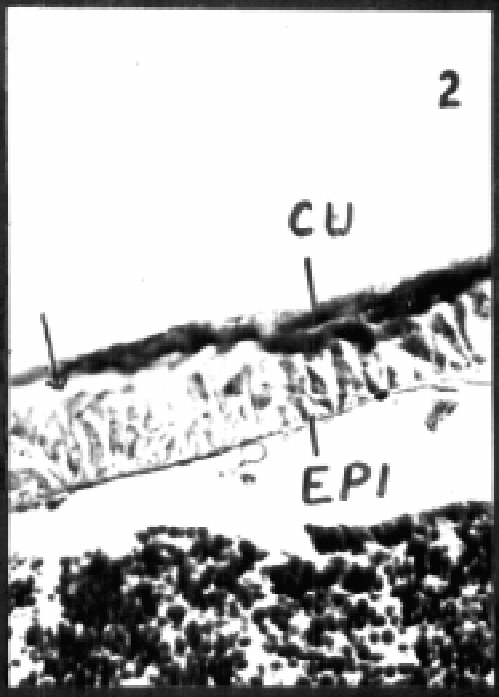
The oral dose of diflubenzuron 5.0/ug/larva was not lethal to the fifth instar stage of S. litura immediately. The treated larvae showed no fatal symptoms initially but the abnormal symptoms, described earlier, were invariably seen at the time of apolytic stage proceeding the shedding of the exuviae. Such treated animals failed in shedding the old cuticle and finally died. The histopathological changes in the cuticle of treated larvae were examined at 48, 72 and 96 hours after the treatment.

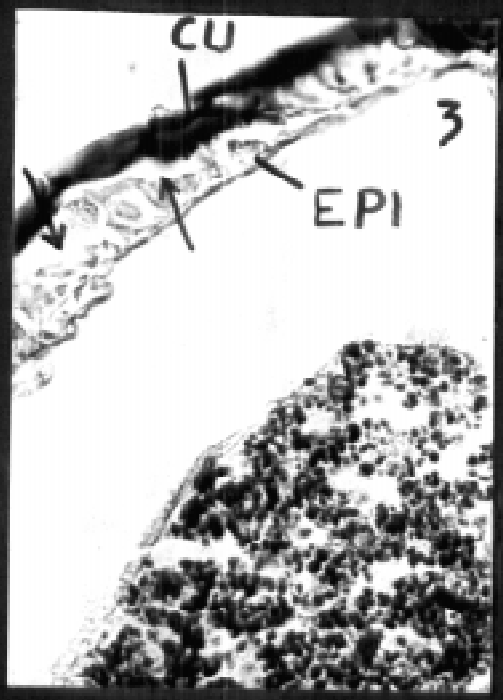
The integument of untreated larvae consisted of non cellular cuticular layer which was divisible into epicuticular, exocuticular and endocuticular layers. These cuticular layers were connected to the epidermis which consisted of columner epithelial cells with centrally placed nucleus. These cells were compactly packed with no or very little space between them (Plate 3; Fig. 1).

In spite of the normal appearance in treated larvae, 48 hours after the treatment, the cuticle showed severe lesions and the process of disintigration in endocuticular zone with the development of a space between the cuticle and the epidermis. The epithelial layer of the epidermis also got affected and developed intercellular spaces (Plate 3; Fig. 2).

# PLATE 3




The histological preparations of 72 hours old treated animals gave more advanced cuticular lesions as can be seen in the photomicrograph (Plate 3; Fig. 3). Increasing gaps between the epidermal cells and non-cellular cuticular layer were clearly visible. The space contained coagulated material which got stained red by the barbiturate scarlet of Mellory's triple stain. These red globules were completely wanting in untreated animals.

The cuticle of treated animals, sectioned 96 hours after the treatment, gave more advanced stage of cuticular lesions. The endocuticle was completely obliterated and the epi-and exocuticular layer thus lost the attachment with epithelial cells of the epidermis. A well marked space was seen between these two layers (Place 3; Fig. 4).

V. BIOCHEMICAL INVESTIGATIONS :

The effect of DFB on the haemolymph and the cuticular proteins of S. litura larvae was investigated through electrophoresis. For haemolymph protein studies the blood samples were drawn from treated animals at 24, 48 and 72 hours, similarly for cuticular proteins 24, 48 and 72 hours old treated cuticles were processed.

The results of haemolymph and cuticular proteins had been presented in figures 4 to 7 and Tables 10 to 14 for different treatment doses at different intervals. The sequence followed in the presentation of results was :-

1. The total number of protein bands involved in the whole operation, including the control and the diflubenzuron treated larvae.
2. Evaluation of numerical number of protein bands in different treatments in relation to control.
3. Evaluation of common protein bands in all the treatments alongwith control.
4. Qualitative changes, as depicted by the absence of protein bands in the treated larvae but presence in control.
5. Qualitative changes as depicted by the appearance of new bands in all the treated larvae which were otherwise absent in control.
6. Evaluation of such protein bands which were present or absent in different treatments but present in control.
7. Evaluation of such other bands which were absent in control but appeared in treated larvae with either of the different dosages.

1. <u>Haemolymph protein pattern</u> :

The protein bands of the larvae obtained in one treatment did not telly with the protein bands obtained in other treatments. Thus differed in their behaviour, for

example band number 28 with Rm value 0.77 was present in the control as well as in 1.0 /ug dose level but it got obliterated in 5.0 /ug dose level. Such a diversified behaviour was observed in a number of protein bands at different treatment dosages and time intervals as depicted in Table 10 to 12.

(i) Twenty four hours after treatment

Actual number of protein bands involved during the whole operation after 24 hours of treatment were 16 whose Rm values fall between 0.05 and 0.95 (Table 10).

In control, there were 10 bands, while in 1.0 /ug and 5.0 /ug treatments, there were 12 and 7 bands respectively. (Table 10; Fig. 4).

There were some bands present in the control and simultaneously in different treatment doses. The number of these bands were 6 having Rm values 0.05, 0.14, 0.21, 0.34, 0.38 and 0.48.

Three protein bands (Rm values 0.60, 0.67 and 0.85) were present in the control but absent in both the treatments whereas band having Rm value 0.77 was present in control as well as in 1.0/ug treatment but was disappeared in 5.0/ug treatment.

Table 10. Relative mobility values of haemolymph proteins of S. litura larvae at 24 hours after feeding DFB

| Band No. | Rm values | Control | Doses per larvae 1.0 /ug | 5.0 /ug |
|---|---|---|---|---|
| 1 | 0.03 | - | - | - |
| 2 | 0.05 | + | + | + |
| 3 | 0.08 | - | - | - |
| 4 | 0.11 | - | + | - |
| 5 | 0.14 | + | + | + |
| 6 | 0.16 | - | - | - |
| 7 | 0.20 | - | - | - |
| 8 | 0.21 | + | + | + |
| 9 | 0.22 | - | - | - |
| 10 | 0.23 | - | - | - |
| 11 | 0.28 | - | - | - |
| 12 | 0.31 | - | - | - |
| 13 | 0.34 | + | + | + |
| 14 | 0.38 | + | + | + |
| 15 | 0.40 | - | - | - |
| 16 | 0.43 | - | - | - |
| 17 | 0.45 | - | - | - |
| 18 | 0.48 | + | + | + |
| 19 | 0.54 | - | - | - |
| 20 | 0.60 | + | - | - |

Contd....

Contd. Table 10

| Band No. | Rm values | Control | 1.0 /ug | 5.0 /ug |
|---|---|---|---|---|
| 21 | 0.63 | - | - | - |
| 22 | 0.65 | - | - | - |
| 23 | 0.67 | + | - | - |
| 24 | 0.68 | - | - | - |
| 25 | 0.70 | - | - | - |
| 26 | 0.72 | - | - | - |
| 27 | 0.74 | - | + | - |
| 28 | 0.77 | + | + | - |
| 29 | 0.81 | - | - | - |
| 30 | 0.83 | - | - | - |
| 31 | 0.85 | + | - | - |
| 32 | 0.87 | - | + | - |
| 33 | 0.90 | - | + | - |
| 34 | 0.92 | - | - | + |
| 35 | 0.95 | - | + | - |
| Total number of bands | | 10 | 12 | 7 |

+ Present

- Absent

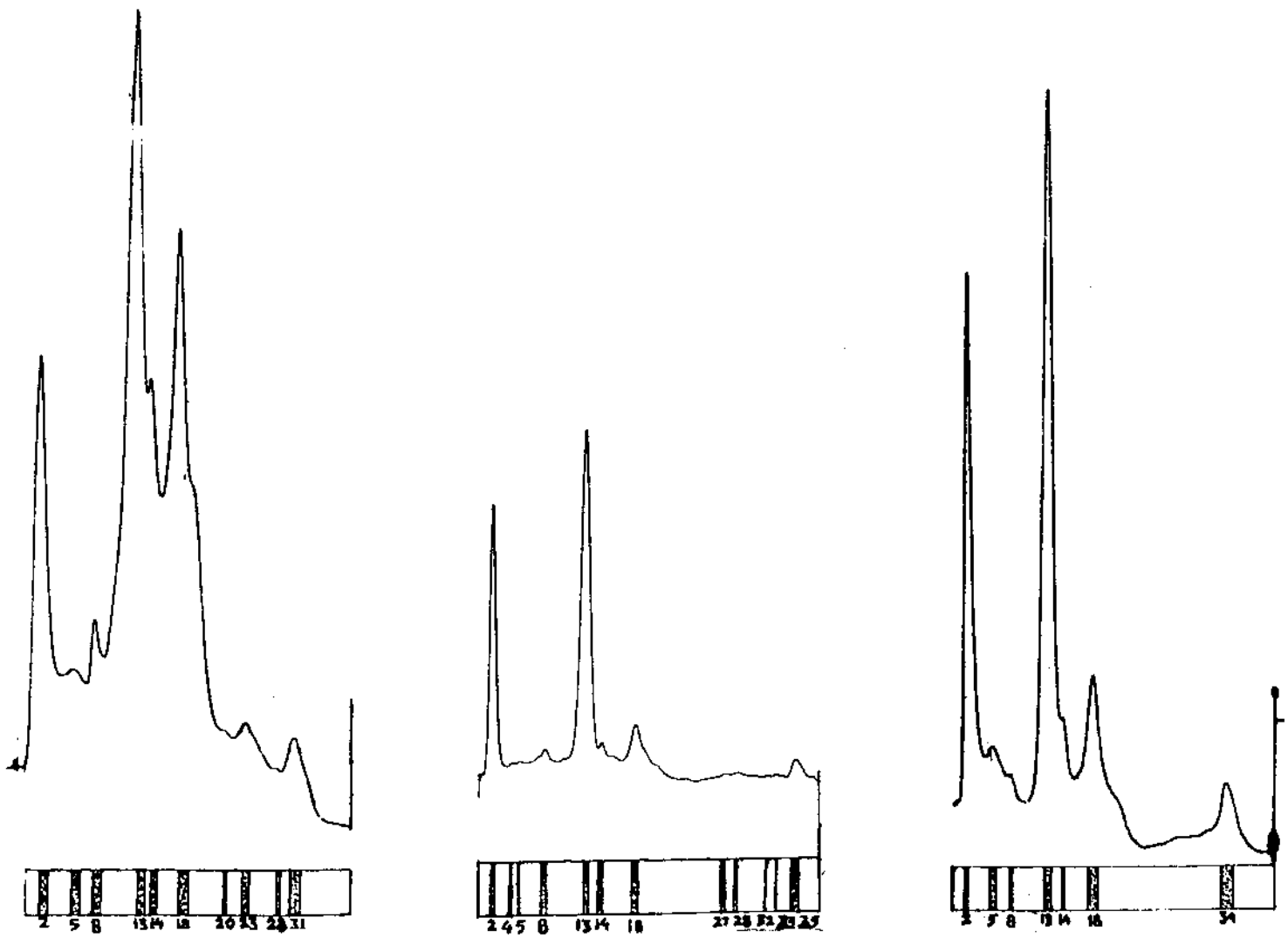


Fig. 4. Scanning of the haemolymph proteins of S. litura larvae at 24 hours after fed with different doses of diflubenzuron

A - Untreated (Control) B - 1.0 /ug/larva C - 5.0 /ug/larva

Six bands having Rm value 0.11, 0.74, 0.87, 0.90, 0.92 and 0.95 appeared as new bands either in the first treatment or in the second treatment, they were however, absent in the control. Out of these protein bands only one band with Rm value 0.92 was present in treated larvae with 5.0 /ug dose and rest of the new bands appeared in the haemolymph of the larvae fed with 1.0 /ug of DFB.

(ii) Forty eight hours after treatment

The protein bands involved during the whole operation after 48 hours of the treatment were 20 whose Rm values fall between 0.5 to 0.95 (Table 11).

In control, there were 15 bands and in treatments of 1.0 /ug and 5.0 /ug dosages of DFB they were 11 and 9 respectively (Fig. 5). The protein bands with Rm values 0.20, 0.38, 0.70, 0.83 and 0.90 were present in control but found absent in both the treatments.

Bands having Rm values 0.16,0.54, 0.77 and 0.95 were present in the control as well as in 1.0 /ug treatment, whereas they were absent in 5.0 /ug treatment. Similarly, the protein bands with Rm values 0.63 and 0.92 were present in control and in higher dose (5.0 /ug) but absent in lower dose (1.0 /ug).

Table 11. Relative mobility values of haemolymph proteins of S. litura larvae at 48 hours after feeding DFB

| Band No. | Rm value | Control | Doses per larvae 1.0 /ug | 5.0 /ug |
|---|---|---|---|---|
| 1 | 0.03 | - | - | - |
| 2 | 0.05 | + | + | + |
| 3 | 0.08 | + | + | + |
| 4 | 0.11 | - | - | - |
| 5 | 0.14 | - | - | - |
| 6 | 0.16 | + | + | - |
| 7 | 0.20 | + | - | - |
| 8 | 0.21 | - | - | - |
| 9 | 0.22 | - | - | + |
| 10 | 0.23 | - | - | - |
| 11 | 0.28 | - | + | - |
| 12 | 0.31 | - | - | - |
| 13 | 0.34 | + | + | + |
| 14 | 0.38 | + | - | - |
| 15 | 0.40 | - | - | - |
| 16 | 0.43 | - | - | - |
| 17 | 0.45 | - | - | - |
| 18 | 0.48 | + | + | + |
| 19 | 0.54 | + | + | - |
| 20 | 0.60 | - | - | - |

Contd....

Contd. Table 11

| Band No. | Rm value | Control | 1.0 /ug | 5.0 /ug |
|---|---|---|---|---|
| 21 | 0.63 | + | - | + |
| 22 | 0.65 | - | - | - |
| 23 | 0.67 | - | - | - |
| 24 | 0.68 | - | + | + |
| 25 | 0.70 | + | - | - |
| 26 | 0.72 | - | - | - |
| 27 | 0.74 | - | + | - |
| 28 | 0.77 | + | + | - |
| 29 | 0.81 | - | - | - |
| 30 | 0.83 | + | - | - |
| 31 | 0.85 | - | - | - |
| 32 | 0.87 | - | - | + |
| 33 | 0.90 | + | - | - |
| 34 | 0.92 | + | - | + |
| 35 | 0.95 | + | + | - |
| Total number of bands | | 15 | 11 | 9 |

+ Present

- Absent

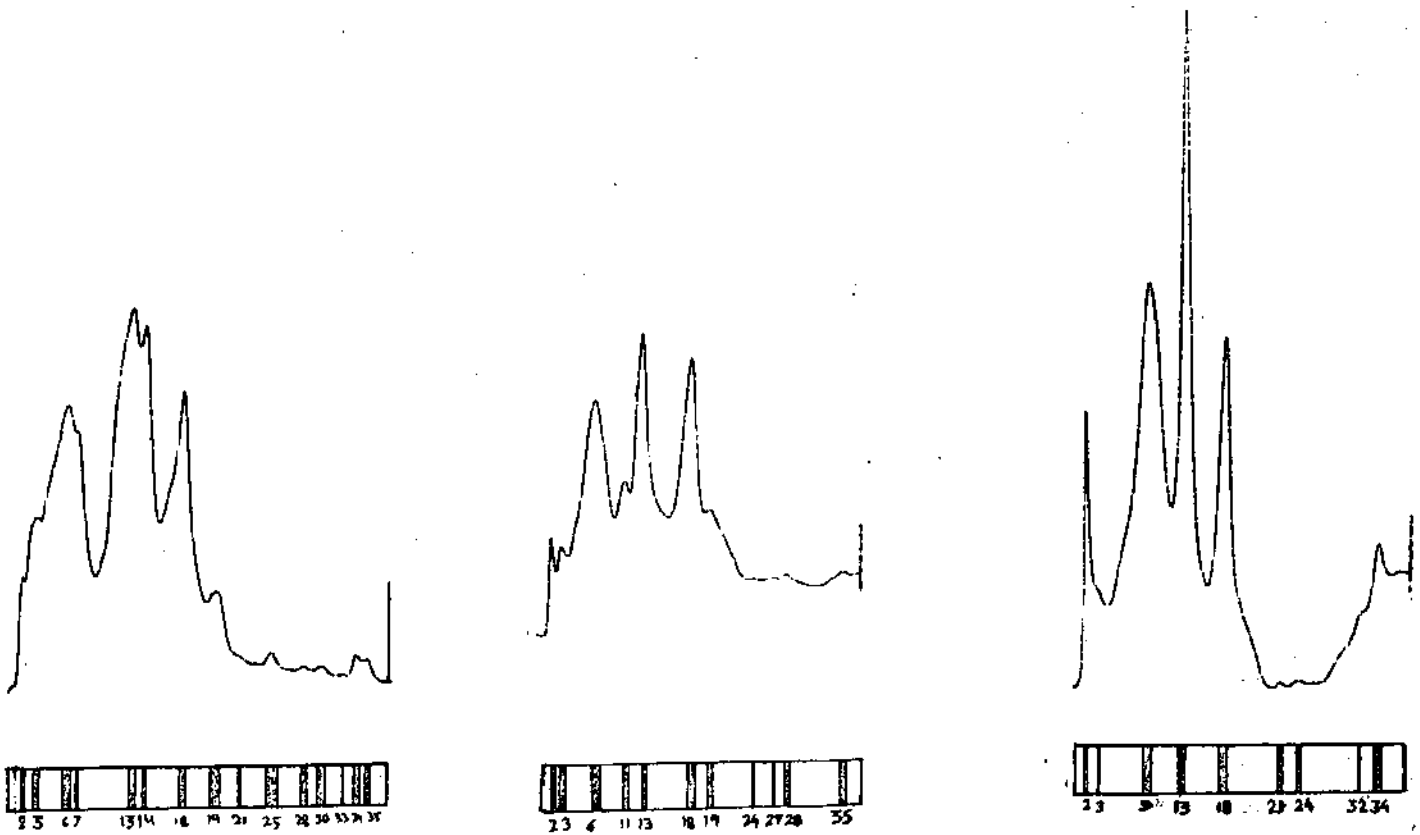


Fig. 5. Scanning of the haemolymph proteins of S. litura larvae at 48 hours after fed with different doses of diflubenzuron

A - Untreated (Control) B - 1.0 /ug/larva C - 5.0 /ug/larva

Among the new protein bands appeared in 48 hours after DFB treatment, only one band with Rm value 0.68 was available in both the treatments and was evidently absent in control. Some other newly formed bands were found in either of the treatments but were absent in control; they were having Rm values 0.28, 0.74 (1.0 /ug), 0.22 and 0.87 (5.0 /ug).

(iii) Seventy two hours after treatment

The actual number of protein bands involved during the whole operation after 72 hours of the treatment was 23 whose Rm values fall between 0.03 and 0.95 (Table 11).

In control, there were 13 bands available while in 5.0 /ug and 1.0 /ug treatment doses 11 and 10 bands appeared respectively (Fig. 6).

Only one band remained constantly in the control as well as in the treatment, which was band number 28 having Rm value 0.77.

Three bands appeared as new were having Rm values 0.03, 0.43 and 0.65 which were present in both the treatments but absent in control. While 5 new bands appeared in 1.0 /ug treatment having Rm values 0.31, 0.45, 0.54, 0.81 and 0.92 and 2 bands with Rm value 0.34 and 0.70 appeared as new in 5.0 /ug dose.

Table 12. Relative mobility values of haemolymph proteins of S. litura larvae at 72 hours after feeding DFB

| Band No. | Rm values | Control | Doses per larva 1.0 /ug | 5.0 /ug |
|---|---|---|---|---|
| 1 | 0.03 | - | + | + |
| 2 | 0.05 | - | - | - |
| 3 | 0.08 | + | - | - |
| 4 | 0.11 | - | - | - |
| 5 | 0.14 | - | - | - |
| 6 | 0.16 | + | - | + |
| 7 | 0.20 | - | - | - |
| 8 | 0.21 | - | - | - |
| 9 | 0.22 | - | - | - |
| 10 | 0.23 | + | - | - |
| 11 | 0.28 | + | - | + |
| 12 | 0.31 | - | + | - |
| 13 | 0.34 | - | - | + |
| 14 | 0.38 | - | - | - |
| 15 | 0.40 | + | - | - |
| 16 | 0.43 | - | + | + |
| 17 | 0.45 | - | + | - |
| 18 | 0.48 | + | + | - |
| 19 | 0.54 | - | + | - |
| 20 | 0.60 | + | - | - |

Contd....

Contd. Table 12

| Band No. | Rm values | Control | 1.0 /ug | 5.0 /ug |
|---|---|---|---|---|
| 21 | 0.63 | - | - | - |
| 22 | 0.65 | - | + | + |
| 23 | 0.67 | - | - | - |
| 24 | 0.68 | + | - | - |
| 25 | 0.70 | - | - | + |
| 26 | 0.72 | + | + | - |
| 27 | 0.74 | - | - | - |
| 28 | 0.77 | + | + | + |
| 29 | 0.81 | - | + | - |
| 30 | 0.83 | + | - | + |
| 31 | 0.85 | - | - | - |
| 32 | 0.87 | + | - | - |
| 33 | 0.90 | - | - | - |
| 34 | 0.92 | - | + | - |
| 35 | 0.95 | + | - | + |
| Total number of bands | | 13 | 11 | 10 |

+ Present

- Absent

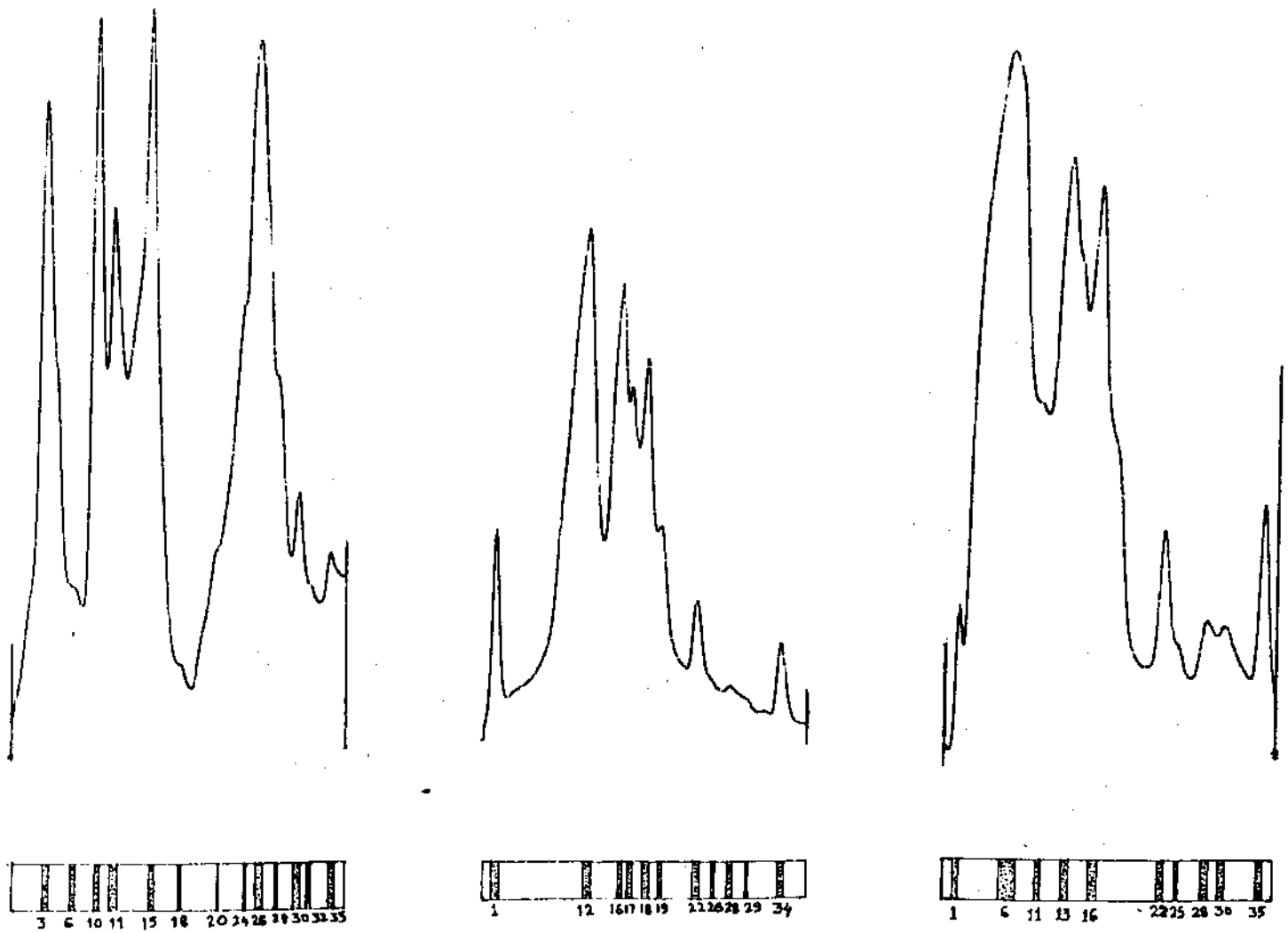



Fig. 6. Scanning of the haemolymph proteins of S. litura larvae at 72 hours after fed with different doses of diflubenzuron

A - Untreated (Control) B - 1.0 /ug/larva C - 5.0 /ug/larva

A large number of protein bands (six) disappeared altogether from the haemolymph of the larvae fed with 1.0 /ug or 5.0 /ug dose of DFB which were present in control. These bands were with Rm values 0.08, 0.23, 0.40, 0.60, 0.68 and 0.87. Two bands with 0.48 and 0.72 Rm values were although present in control as well as in 1.0 /ug treatment but were missing in 5.0 /ug dose. The protein bands with Rm values 0.16, 0.28, 0.83 and 0.95 were present in 5.0 /ug treatment alongwith control but were absent in 1.0 /ug treatment.

2. Cuticular protein pattern :

(i) Twenty four hours after treatment

Actual number of protein bands involved during the whole operation after 24 hours of treatment were 8 whose Rm values fall between 0.02 and 0.87 (Table 13).

In the control all the 8 bands having Rm values 0.02, 0.04, 0.10, 0.13, 0.18, 0.77, 0.81 and 0.87 were present (Fig. 7A) which were reduced to 6 at the dose level of 1.0 /ug/larva (Fig. 7B). Two bands with Rm values of 0.18 and 0.77, which were present in the control, however, disappeared in the treatment.

A similar trend of decrease in protein bands was also observed at the highest dose level of 5.0 /ug/larva (Fig. 7C) wherein the same two bands (Rm values 0.18 and 0.77) remained absent.

Table 13. Relative mobility values of cuticular proteins of S. litura larvae at 24 hours after feeding DFB

| Band No. | Rm values | Control | Doses per larva 1.0 /ug | 5.0 /ug |
|---|---|---|---|---|
| 1 | 0.02 | + | + | + |
| 2 | 0.04 | + | + | + |
| 3 | 0.08 | - | - | - |
| 4 | 0.10 | + | + | + |
| 5 | 0.13 | + | + | + |
| 6 | 0.18 | + | - | - |
| 7 | 0.65 | - | - | - |
| 8 | 0.73 | - | - | - |
| 9 | 0.77 | + | - | - |
| 10 | 0.81 | + | + | + |
| 11 | 0.87 | + | + | + |
| Total number of bands | | 8 | 6 | 6 |

+ Present

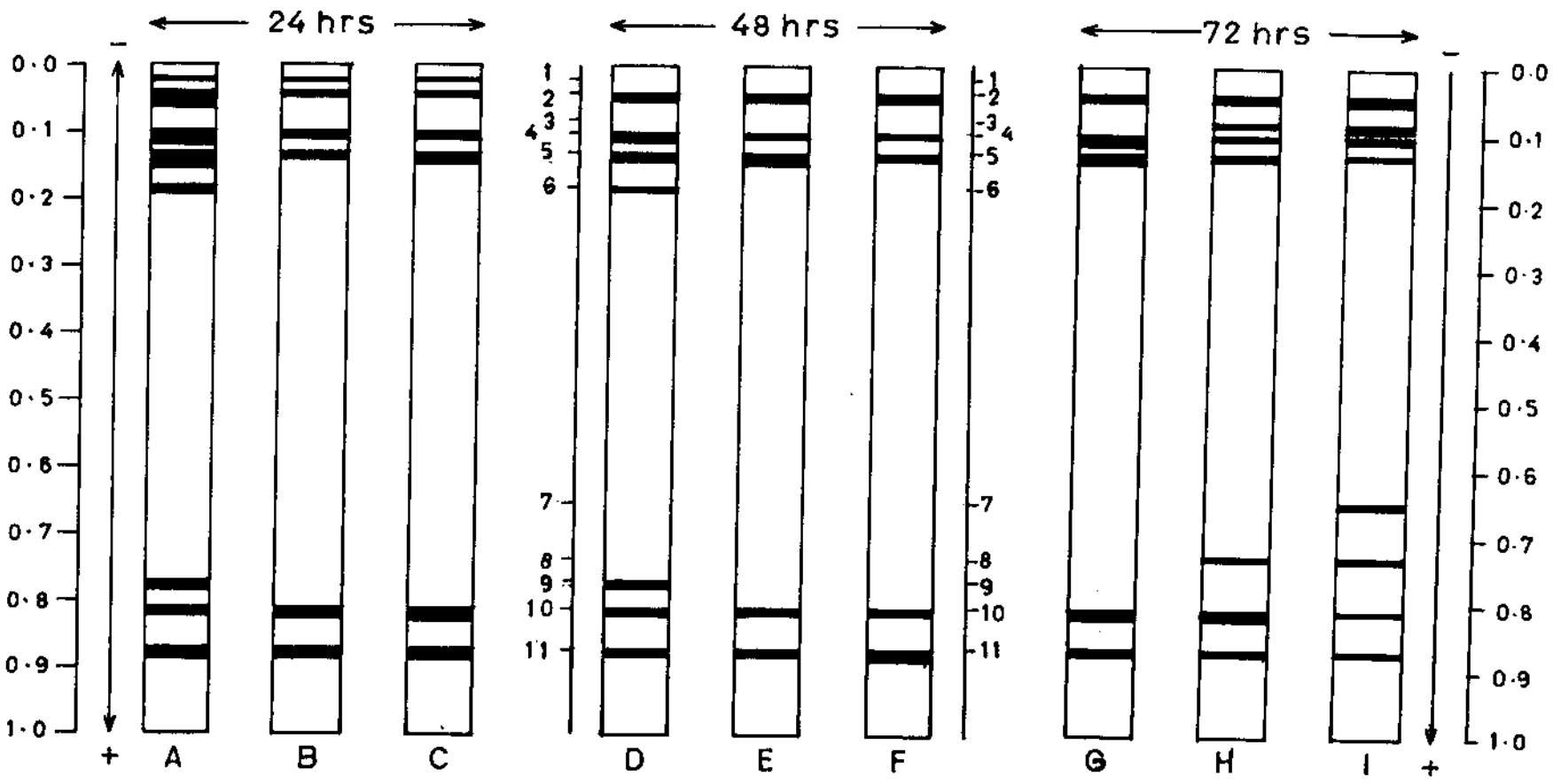
- Absent

FIG. 7 THE ELECTROPHOROGRAMS OF THE CUTICULAR PROTEINS OF S. LITURA LARVAE AT DIFFERENT TIME INTERVALS AFTER FEEDING WITH DFB

ADG = CONTROL

BEH = 1 µg / LARVA

CFI = 5 µg / LARVA



No new band appeared in any of the treatment doses (Fig. 7).

(ii) Forty eight hours after treatment

At this period total number of protein bands obtained were 7 whose Rm values fall between 0.04 and 0.87 (Table 14).

The number of bands observed in control were 7 (Fig 7D) with Rm values 0.04, 0.10, 0.13, 0.18, 0.77, 0.81 and 0.87; which were reduced to 5 in both the treatment doses viz. 1.0/ug and 5.0/ug/larvae (Fig. 7E & F).

Two protein bands having Rm values 0.18 and 0.77, which were present in control, disappeared in both the treatment doses.

No new band appeared in the cuticle of any of the treatments.

(iii) Seventy two hours after treatment

The number of protein bands involved at 72 hours after treatment were 8. Whose Rm values fall between 0.04 and 0.87 (Table 15).

In control only 5 bands were present (Fig.7G) whose Rm values were 0.04, 0.10, 0.13, 0.81 and 0.87. The number

Table 14. Relative mobility values of cuticular proteinsof S. litura larvae at 48 hours after feeding DFB

| Band No. | Rm values | Control | Doses per larva | |
|---|---|---|---|---|
| | | | 1.0 /ug | 5.0 /ug |
| 1 | 0.02 | - | - | - |
| 2 | 0.04 | + | + | + |
| 3 | 0.08 | - | - | - |
| 4 | 0.10 | + | + | + |
| 5 | 0.13 | + | + | + |
| 6 | 0.18 | + | - | - |
| 7 | 0.65 | - | - | - |
| 8 | 0.73 | - | - | - |
| 9 | 0.77 | + | - | - |
| 10 | 0.81 | + | + | + |
| 11 | 0.87 | + | + | + |
| Total number of bands | | 7 | 5 | 5 |

+ Present

- Absent

Table 15. Relative mobility values of cuticular proteins of S. litura larvae at 72 hours after feeding DFB

| Band No. | Rm values | Control | Doses per larva 1.0 /ug | 5.0 /ug |
|---|---|---|---|---|
| 1 | 0.02 | - | - | - |
| 2 | 0.04 | + | + | + |
| 3 | 0.08 | - | + | + |
| 4 | 0.10 | + | + | + |
| 5 | 0.13 | + | + | + |
| 6 | 0.18 | - | - | - |
| 7 | 0.65 | - | - | + |
| 8 | 0.73 | - | + | + |
| 9 | 0.77 | - | - | - |
| 10 | 0.81 | + | + | + |
| 11 | 0.87 | + | + | + |
| Total number of bands | | 5 | 7 | 8 |

+ Present

- Absent

increased to 7 in 1 /ug dose level. Which further increased upto 8 in the highest dose 5.0 /ug.

The 5 bands which were present in control were also available in the cuticle of DFB fed larvae at both the dose levels (Fig. 4E & F).

Some bands which were altogether absent in control, appeared in both the treatments. These were 2 in number having their Rm values were - 0.08 and 0.73. One band having Rm value 0.65 was present only in 5.0 /ug dose/larva.

None of the band available in control was found to be absent in any treatment.

## B. Euproctis virgincula

### I. TOXICOLOGICAL INVESTIGATIONS :

The bioefficacy of DFB was evaluated by dermal and oral toxicity.

(i) Dermal toxicity : ($LD_{25}/LD_{50}$)

The dermal toxicity had been determined by two methods viz. dry-film and dipping method.

(a) Dry-film method

The fifth instar larvae of the test insect were exposed to different doses of dry film treatments for different periods viz. 12 and 24 hours. There was no mortality to the larvae during the exposure periods. The $LD_{25}$ values calculated from the mortality data after 48 hours, were 35.28 and 21.88 mg/90 $cm^2$ surface area for 12 and 24 hours exposure respectively. The regression equations derived for $LD_{25}$ were also used to determine the $LD_{50}$ values for these periods which were 116.20 and 95.10 mg/unit surface area for 12 and 24 hrs exposure periods respectively. The slopes of regression were 1.302 and 1.056 for these periods. The fiducial limits for $LD_{25}$ and the chi-square values at P = 0.05 had been presented in Table 16.

Table 16. Dermal toxicity of diflubenzuron against the larvae of *E. virgincula* (Dry-film method)

| Exposure periods | Heterogenity ** | Regression equation | $LD_{25}$* | Ratio $\frac{12\ hr}{24\ hr}$ | $LD_{50}$* | Ratio $\frac{12\ hr}{24\ hr}$ | Fiducial limits* of $LD_{25}$ |
|---|---|---|---|---|---|---|---|
| 12 hours | $X^2$ (5) = 0.0092 | Y = 1.302 x + 2.310 | 35.28 | 1.61:1 | 116.20 | 1.22:1 | 24.76 to 50.27 |
| 24 hours | $X^2$ (5) = 0.1903 | Y = 1.056 x + 2.911 | 21.88 | | 95.10 | | 11.86 to 40.35 |

$LD_{25}$ and $LD_{50}$ = Lethal dosages to give 25 and 50 per cent mortality respectively

* = mg/90 $cm^2$ surface area; Y = probit kill; x =log concentration

**= In none of these cases, the data were found to be significantly heterogenous at P = 0.05

The standard dosage mortality curves for both the exposure periods of DFB film treatment against E. virgincula had been presented in Fig. 8.

The $LD_{25}$ value at 24 hrs exposure was found to be 1.61 times less than that of 12 hrs exposure. A similar sequence was obtained in the case of $LD_{50}$ values where the ratio was 1.22 (Table 16).

(b) Dipping method

No mortality was obtained 24 hrs after the treatment. The $LC_{25}$ and $LC_{50}$ values obtained after 48 hrs of treatment were 430.13 and 2590.48 ppm respectively. The slope value was 0.856. It could be seen from the data in Table 18 that the Chi-square at P = 0.05 was significant. The regression equation and the fiducial limits for $LC_{50}$ had been presented in Table 17. The standard dose mortality curve of DFB dipping treatment against E. virgincula had been depicted in Fig. 9.

(ii) Oral toxicity : ($LD_{25}/LD_{50}$)

The $LD_{25}$ and $LD_{50}$ values calculated for E.virgincula after feeding DFB treated food to 5th instar larvae were 0.95 and 16.82 /ug/larva. The slope value was 0.541 (Table 18). The standard dose mortality curve had been given in Fig. 10.

DIFLUBENZURON AGAINST E. VIRGINCULA LARVAE (DRY FILM METHOD)

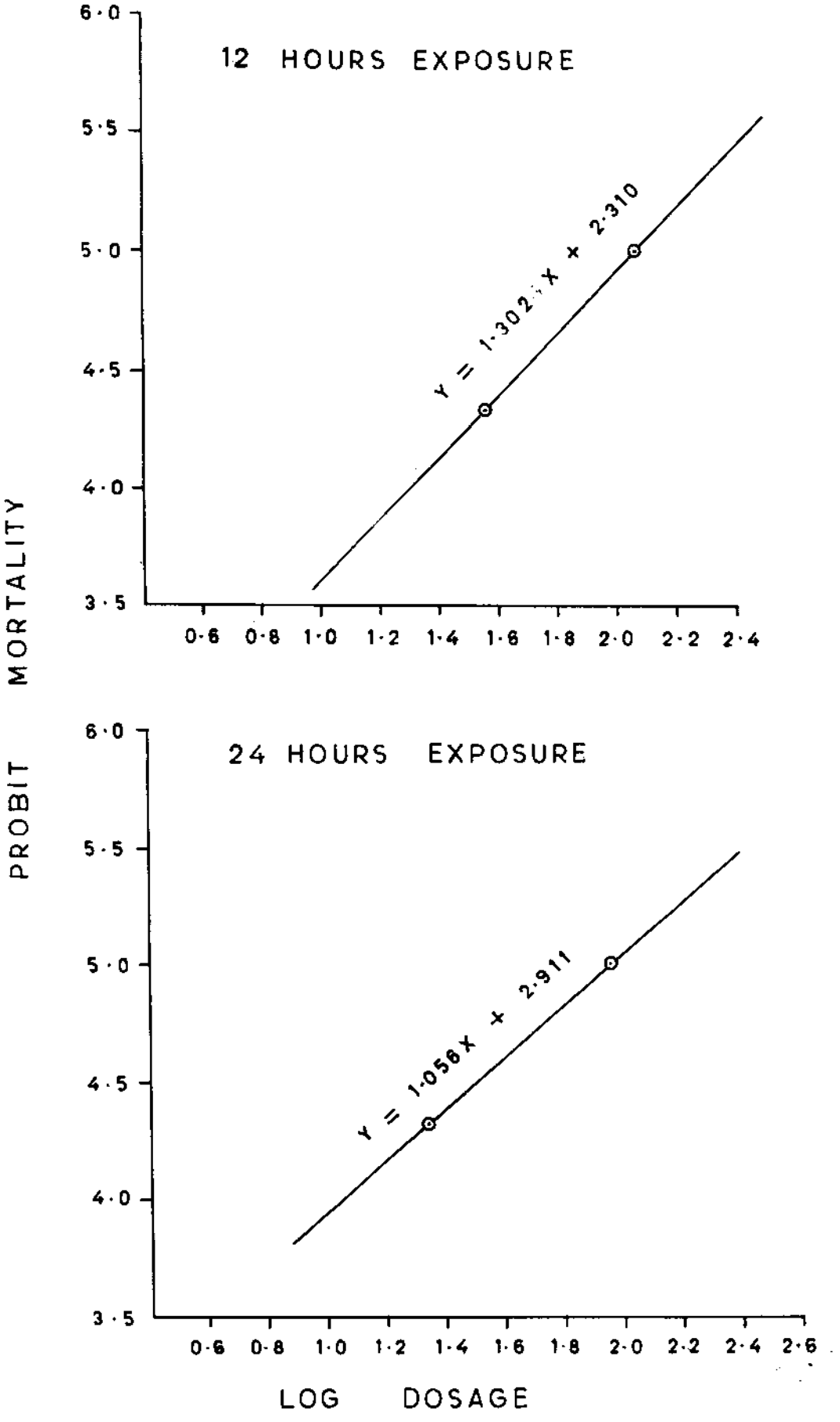


Table 17. Dermal toxicity of diflubenzuron against E. virgincula larvae (Dipping method)

| Heterogenity | Regression equation | $LC_{25}$ (ppm) | $LC_{50}$ (ppm) | Fiducial limits of $LC_{50}$ (ppm) |
|---|---|---|---|---|
| $X^2$ (6) 3.5749 | Y = 0.856 x + 2.047 | 430.13 | 2590.48 | 1567.73 - 4280.26 |

$LC_{25}$; $LC_{50}$ = Lethal concentration to give 25 or 50 per cent mortality respectively

$X^2$ = Chi-square value of P = 0.05 was significant

Y = Probit kill, x = log concentration

Table 18. Oral toxicity of diflubenzuron against *E. virgincula* larvae (feeding method)

| Heterogenity | Regression equation | $LD_{25}$ /ug/larva | $LD_{50}$ /ug/larva | Fiducial limits of $LD_{50}$ (/ug/larva) |
|---|---|---|---|---|
| $X^2_{(2)}$ 0.3688 | Y = 0.541 x + 4.336 | 0.95 | 16.82 | 9.48 - 31.25 |

$LD_{25}$; $LD_{50}$ = Lethal dose to give 25 or 50 per cent mortality respectively

$X^2$ = Chi-square value at P = 0.05 was significant

Y = Probit kill, x = log dose

FIG. 9 STANDARD DOSAGE MORTALITY CURVE OF DIFLUBENZURON TREATMENT AGAINST E. VIRGINCULA LARVAE BY DIPPING METHOD

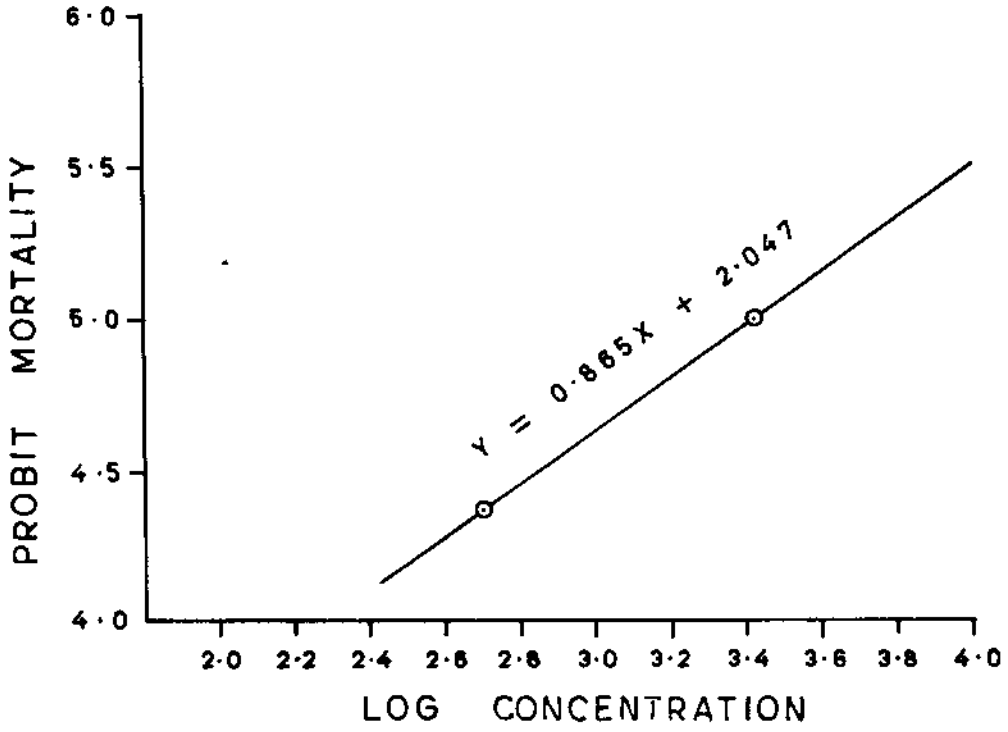
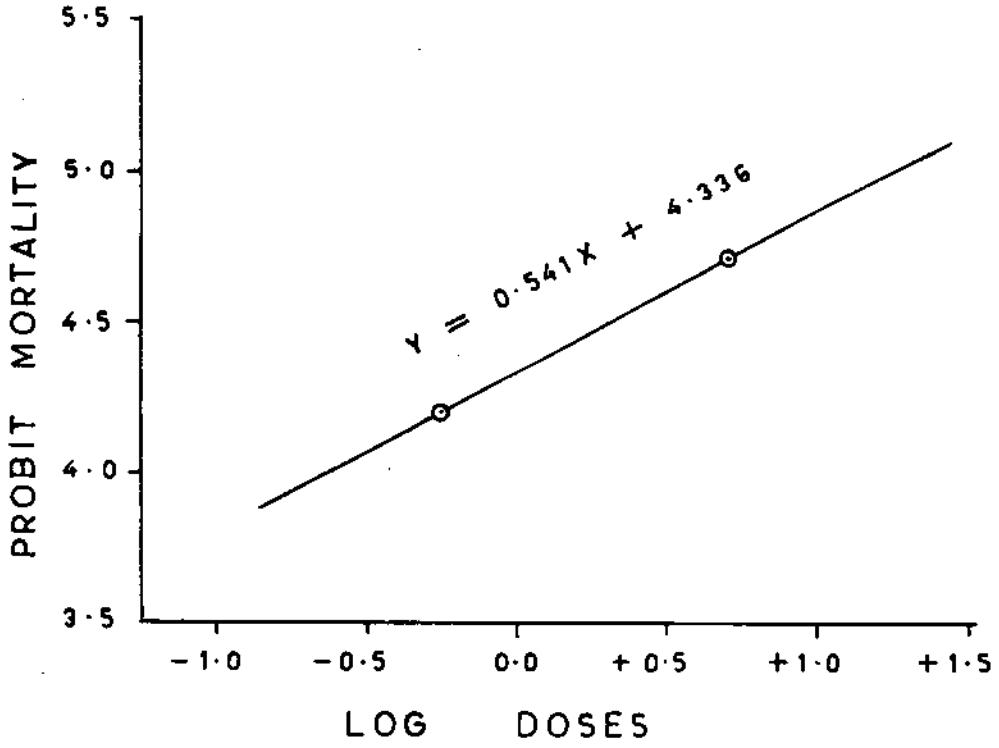



FIG. 10 STANDARD DOSE MORTALITY CURVE OF DIFLUBENZURON AGAINST E. VIRGINCULA LARVAE BY FEEDING METHOD



It may be pointed out that this chemical, DFB, had not yet been evaluated against E. virgincula. However, one other species of hairy caterpillar, Amsacta moorei, was selected by Srivastava et al. (1984) for conducting bioassay tests evaluating dermal toxicity of DFB through topical application; they obtained median mortality at a dose of 0.25 /ug/larva. The latter results were not comparable with those of the present results since the methodology adopted for evaluating dermal toxicity was different. Yet a common inference which could be derived on the basis of the available observations was that DFB acted as a dermal poison against hairy caterpillar also inspite of their outer hairy coverings.

In dipping treatment the $LC_{25}$ and $LC_{50}$ values were 430.13 and 2590.48ppm respectively which indicated again the contact action of DFB. Here again, there was no quick knock down effect of DFB as not a single larva died in first 24 hrs observation after the treatment.

When compared with S. litura, the $LD_{50}$ as well as $LD_{25}$ values were higher for E. virgincula which indicated that the former insect was more susceptible to DFB.

Conclusively it could be said that present findings gave an indication of low cuticular absorption of DFB and as such had a lesser degree of contact toxicity. This inference confirmed with the contention of Retnakaran et al. (1985).

II. SYMPTOMATIC AND BEHAVIOURAL INVESTIGATIONS :

1. Behavioural changes

(i) Sluggishness

The behavioural changes in the treated larvae after feeding with DFB doses initiated with sluggishness. This effect was observed from 48 hrs after the treatment. The affected larvae either did not move from the place they were kept in previous observation or they showed a lithargic or very slow movement. They did not give a positive response to prickings with a needle.

This symptom was recorded in 12.73, 5.17 and 3.39 per cent larvae in 5.0, 1.0 and 0.5 /ug doses respectively. However, sluggishness was not observed in the lowest dose of 0.1 /ug/larva as well as in the control (Table 19).

(ii) Cessation of locomotion

The sluggish effect reached its hight when the affected larvae did not move at all from the place of their stay. The larvae showing cessation of locomotion were 9.09 per cent in 5.0 /ug/larva dose. The effect was almost similar in 1.0 /ug dose where 8.62 per cent larvae ceased locomotion, whereas in 0.5 /ug dose 1.69 per cent larvae showed this symptom. However, in lowest dose (0.1 /ug) and in control all the larvae had a normal movement (Table 19).

(iii) Cessation of feeding

An enhanced effect of DFB feeding following the sluggishness and cessation of locomotion was observed as stopping of feeding. The larvae ceased feeding gradually from 48 to 72 hours after consuming the treated food. The affected larvae did not feed at all or just nibbled the food even when they were put directly over the fresh leaves. These behavioural changes led to a sort of total ceasing of physiological activities within the larval body. The percentages of larvae showing cessation of feeding were 27.27, 12.06 and 6.78 in the treatment doses of 5.0, 1.0 and 0.5 /ug/larva. However, this symptom was not observed in any of the larva in the lowest dose of 0.1 /ug/larva and in the control as shown in Table 19.

(iv) Wetting

The wetting of larval body was observed in all the treatments to a tune of 72.72, 46.56, 38.98 and 22.22 per cent cases in 5.0, 1.0, 0.5 and 0.1 /ug doses per larva respectively (Table 19). In the larvae which extruded large amount of body fluid their body hairs got detached; such larvae were found completely bathed in their body fluid when observed even at a short interval of 6 to 8 hours. Such cases were invariably found in higher doses.

Table 19. Behavioural abnormalities in E. virgincula larvae fed with different DFB doses

| Behavioural parameters | Per cent insects showing abnormal symptoms Doses in ug/larva | | | | |
|---|---|---|---|---|---|
| | 5.0 | 1.0 | 0.5 | 0.1 | 0.0 (Control) |
| 1.Sluggishness | 12.73 | 5.17 | 3.39 | 0.00 | 0.00 |
| 2.Cessation of locomotion | 9.09 | 8.62 | 1.69 | 0.00 | 0.00 |
| 3.Cessation of feeding | 27.27 | 12.06 | 6.78 | 0.00 | 0.00 |
| 4.Wetting | 72.72 | 46.55 | 38.98 | 22.22 | 0.00 |
| 5.Oozing | 81.82 | 44.82 | 30.51 | 14.81 | 0.00 |
| Number of larvae exposed to treatment | 55 | 58 | 59 | 54 | 60 |

(v) Oozing

Differentiation between wetting and oozing was difficult. However, the cases with extruding of body fluid from different sources such as mouth, anus etc. had been incorporated here. Data presented in Table 19 showed that the oozing was recorded in 81.82, 44.82, 31.58 and 14.81 per cent larvae in treatment doses of 5.0, 1.0, 0.5 and 0.1 µg/larva respectively (Table 19).

When the affected larvae were constantly observed under stereoscopic microscope, the body fluid was seen oozing from mouth, anus and the intersegmental membranes specifically on the lateral sides. In the larvae fed with higher doses of DFB, the oozing was invariably heavy.

The colour of the oozed liquid from mouth was clear light or dark green in most of the cases or was pale green in few larvae, while a clear green liquid was extruded from anus alongwith fine suspended particles. In a few treated larvae brown or dirty liquid came out.

The smear examination of the fluid obtained from oral and anal regions of the body exhibited undigested food particles. The blood cells were constantly seen in all the smear preparations of different body regions.

2. Morphological deformities

(i) Shrinkage in larval body

It was observed that body of some of the DFB fed larvae was shrinking when examined after every 24 hours. This effect was seen 2 to 3 days after consuming the treated food by test larvae. Such shrinkage was different from the normal contraction of the body which occurs when the larva tends to undergo pupation. Here, the larval characters were retained but the body length was reduced to an extent of 1/5th of the normal one. Such larvae did not feed or gradually reduced feeding and became lithargic; they lost their natural body colour and the texture. Percentages of such cases were 32.73, 22.41 and 16.95 in the doses of 5.0, 1.0 and 0.5 /ug/ larvae respectively. This effect was found least (5.55 per cent) in the lowest dose of 0.1 /ug and absent in the untreated larvae.

(ii) Discolouration and scar formation

Change in body colouration tending towards darkening or with dark spots was observed in quite a large population of the treated larvae as shown in Table 20a. With 5.0 /ug and 1.0 /ug doses 60.91 and 56.90 per cent cases were seen. This effect was observed reduced to an extent of 28.81 and further to 3.70 per cent individuals fed with 0.5 and 0.1 /ug DFB per

Table 20a. Early morphological symptoms observed in *E. virgincula* larvae fed with DFB doses

| Abnormal symptoms | Per cent insects showing abnormal symptoms Doses in µg/larva | | | | |
|---|---|---|---|---|---|
| | 5.0 | 1.0 | 0.5 | 0.1 | 0.0 (Control) |
| 1. Shrinkage of body | 32.73 | 22.41 | 16.95 | 5.55 | 0.00 |
| 2. (a) Discolouration | 61.82 | 56.90 | 28.81 | 3.70 | 0.00 |
| (b) Scar formation | 1.82 | 0.00 | 0.00 | 0.00 | 0.00 |
| 3. Rupturing of cuticle | 25.45 | 10.34 | 8.47 | 3.70 | 0.00 |
| 4. Rectal prolapse | 3.64 | 1.72 | 1.69 | 0.00 | 0.00 |
| Number of larvae released | 55 | 58 | 59 | 54 | 60 |

larva respectively. No colour change was observed in any larvae in the control.

The scar formation was recorded only in 5.0 /ug dose; 1.82 per cent larvae showing this symptom.

(iii) Cuticular rupture

The rupture of cuticle in the treated larvae was found with variable degrees and loci. The symptom was seen 48 to 72 hours after the treatment. The rupturing process contimued at different locations in the body of affected larvae followed by oozing of body fluid until the larvae were dead. Generally, the splitting was observed at the intersegmental regions.

Maximum percentage of larvae showing this symptom were in 5.0 /ug treatment (25.45), which was reduced to a tune of 3.70 per cent cases in the lowest dose level (0.1 /ug/larva), whereas, this symptom was recorded in 10.34 and 8.47 per cent larvae fed with 1.0 and 0.5 /ug doses respectively. However, in control (untreated larvae) such cuticular ruptures were not seen.

(iv) Rectal prolapse

In some of the DFB fed larvae a baloon like structure protruding from the amus, was seen. These rectal bulgings were membranous and filled with clear green or yellowish fluid.

These bulgings were found darker when examined 24 hours after their appearance, in most of the cases and finally bursted leading to leakage of body fluid. The larvae depicting this symptom did not survive for more than 3 days. Their percentage was maximum (3.64) in the highest dose of 5.0 /ug/larva, while in subsequent doses per cent occurance was 1.72 and 1.69 in 1.0 /ug and 0.5 /ug respectively. Rectal prolapse was not seen in the lowest dose (0.1 /ug) and the control (Table 20a).

(vi) Frayed condition of cuticle

It was seen just before the onset of the moulting process. In such cases showing frayed condition of cuticle, the rupturing was not initiated but the cuticle got removed as very small bits from the scattered areas of the body wall. This symptom was seen in all the treatment doses except the lowest one (0.1 /ug/larva), upto an extent of 10.91, 5.17 and 3.39 per cent cases in 5.0, 1.0 and 0.5 /ug DFB/larva respectively. In untreated larvae the frayed condition of larval skin was not found (Table 20b).

(vi) Mosaic condition of cuticle

In prepupal stage some of the individuals showed dark brown and well sclerotised pupal skin in patches, seen below the larval skin. This symptom was predominant in the

Table 20b. Advanced morphological abnormalities observed at the larval and pre-pupal stages of *E. virgincula* fed with DFB treated food

| Treatment doses /ug/ larva | Number of larvae treated | Frayed condition of cuticle in larva | Mosaic condition of cuticle in prepupa | Incomplete shedding of exuviae at different body regions | | | | Rupture of old cuticle at | | Larvae with partial pupal cuticle in ventral abdominal region |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Head capsule | Thoracic region partial | Thoracic region complete | Abdominal region | Thorax | Abdomen | |
| 5.0 | 55 | 10.91 | 45.45 | 9.09 | 3.64 | 1.82 | 14.54 | 7.27 | 1.82 | 21.81 |
| 1.0 | 58 | 5.17 | 44.83 | 5.17 | 3.45 | 0.00 | 6.90 | 1.72 | 1.72 | 31.03 |
| 0.5 | 59 | 3.39 | 20.34 | 6.78 | 3.39 | 0.00 | 5.08 | 0.00 | 0.00 | 11.86 |
| 0.1 | 54 | 0.00 | 18.52 | 1.85 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5.56 |
| 0.0 (Control) | 60 | 0.00 | 00.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 00.00 |

treated animals. Such cases were maximum as 45.45 and 44.83 per cent in the higher doses viz. 5.0 and 1.0 /ug/larva. While in the lower doses i.e. 0.5 and 0.1 /ug/larva their existence were recorded as 20.34 and 18.52 per cent. No individual from the untreated lot exhibited the mosaic pattern of cuticle (Table 20a).

(vii) Incomplete shedding of exuviae

As the moulting started, it was seen that in some individuals shedding of the exuviae was incomplete. The ecdysis at head capsule region was seen in 9.09, 5.17, 6.78 and 1.85 per cent pre-pupae in 5.0, 1.0, 0.5 and 0.1 /ug/larva doses respectively; while at thoracic region only, it was seen in 3.64, 3.45, 3.39 and 0.00 per cent cases in the corresponding doses. Among these some of the prepupae (1.82 per cent in 5.0 /ug dose) shedded exuviae in whole of the thoracic region; others ecdysed in part of this region. Similarly, there were cases with ecdysis performed only at abdominal region. Such symptoms appeared in 14.54, 6.90 and 5.08 per cent individuals in the treatment doses viz. 5.0, 1.0 and 0.5 /ug/larva respectively.

(viii) Rupturing of old cuticle

This symptom also appeared at pre-pupal stage where the ecdysis was initiated only. This condition was seen in 7.27 and 1.72 per cent individuals in 5.0 and 1.0 /ug doses;

no such case was observed in subsequent lower doses and in control (Table 20b).

(ix) Presence of pupal cuticle in parts

The pupal cuticle developed only in the ventral part of the thorax in some of the DFB fed larvae; remaining skin was larval. This pupal cuticle was clearly visible below the old larval skin and was sclerotised in some and non sclerotised in other cases. The individuals with these symptoms were 21.81, 31.03, 11.86 and 5.56 per cent in the treatment doses of 5.0, 1.0, 0.5 and 0.1 /ug/larva respectively. None of the untreated larva could be seen showing this symptom (Table 20b).

3. Formation of abnormal stages

(i) Larval-Pupal Intermediates

In all the treatments DFB fed larvae exhibited different types of larval-pupal intermediates (LPI) as shown in Table 21. The LPI developed in 5.0 /ug dose were 34.55 per cent of the treated larvae (Table 22). Of these 12.73 per cent remained trapped in larval skin; another 9.09 per cent had their anterior portion completely larval and the posterior portion developed into pupa. Individuals with dorasl pupal thoracic hump, those with larval head and 2 pairs of thoracic legs, the LPIs with larval head only and anteriorly bent (deformed) LPI were also

Table 21. Formation of abnormal stages in *E. virgincula* fed with DFB treated food at the fifth instar stage

| Abnormalities | Doses in µg/larva | | | | |
|---|---|---|---|---|---|
| | 5.0 | 1.0 | 0.5 | 0.1 | 0.00 (Control) |
| 1. Larval-pupal Intermediates (LPI) showing partial moulting : | | | | | |
| a. Pupal body completely trapped in larval skin | 12.73 | 8.62 | 6.78 | 7.41 | 0.00 |
| b. Anterior portion completely larval | 9.09 | 13.79 | 13.56 | 14.81 | 0.00 |
| c. Dorsal pupal thorax humped out of split larval skin | 3.64 | 3.45 | 5.08 | 3.70 | 0.00 |
| d. Larval head + 2 thoracic legs | 1.82 | 5.17 | 5.08 | 9.26 | 0.00 |
| e. Larval head remains | 5.45 | 5.17 | 6.78 | 7.41 | 0.00 |
| f. Deformed LPI | | | | | |
| i. Twisted | 0.00 | 1.72 | 3.39 | 0.00 | 0.00 |
| ii. Curved | 0.00 | 1.72 | 0.00 | 0.00 | 0.00 |
| iii. Anteriorly bent | 1.82 | 3.45 | 3.39 | 1.85 | 0.00 |
| 2. Pupal stages : | | | | | |
| a. Deformed pupae | | | | | |
| i. With short wing pads | 1.82 | 3.45 | 5.08 | 3.70 | 0.00 |
| ii. With only ventral abdominal skin sclerotised | 1.82 | 1.72 | 1.69 | 1.85 | 0.00 |
| iii. Forwardly bent pupae | 0.00 | 1.72 | 1.69 | 0.00 | 0.00 |
| iv. Curved pupae | 0.00 | 1.72 | 0.00 | 1.85 | 0.00 |
| b. Normal pupae | 0.00 | 10.34 | 18.64 | 33.33 | 98.33 |
| Number of larvae released | 55 | 58 | 59 | 54 | 60 |

observed in this dose. Their percentages were 3.64, 1.82, 5.45 and 1.82 respectively (Table 21).

Similarly, in 1.0 /ug dose 43.10 per cent LPI were formed (Table 22); among them 8.62 per cent were trapped in larval skin, 13.79 per cent were having their posterior part only developed into pupa, 3.45 per cent developed pupal hump, 5.17 per cent were having larval head with 2 thoracic legs and an equal number had only larval head while 6.89 per cent individuals were deformed LPI with twisted curved or anteriorly bent in shape as shown in Table 21.

In 0.5 /ug dose the LPI formed were 44.07 per cent (Table 22); among these, LPI trapped in larval skin were 6.78 per cent, those with posterior pupal region were 13.56 per cent, humped LPI and those with larval head and 2 pairs of thoracic legs were 5.08 per cent each, while LPI with only larval head retained were 6.78 per cent. Some of the LPI were deformed with twisted or bent in shape (3.39 per cent each) as shown in Table 21.

In the lowest dose of 0.1 /ug/larva LPI trapped in larval skin were 7.41 per cent whereas those with posterior part developed into pupal were 14.81 per cent. LPI showing hump, LPI with larval head and 2 pairs of thoracic legs and those with larval head retained were 3.70, 9.26 and 7.41 per cent respectively. Deformed LPI were 1.85 per cent.

Such intermediate stages were not obtained among the untreated larvae.

(ii) Pupal deformities

None of the larva fed with 5.0 /ug dose transformed into normal pupa. The deformed pupae developed were only 3.64 per cent; of which 1.82 per cent had one their wing pads short; remaining 1.82 per cent had their skin partly sclerotised in ventral abdominal region.

In 1.0 /ug dose level 10.34 per cent individuals developed into normal looking pupae (Table 21, 22); while rest of the pupae formed were abnormal (8.62 per cent); of these 3.45 per cent had short wing pads and 1.72 per cent each were curved, bent or having only abdominal skin sclerotised (Table 21).

In 0.5 /ug dose abnormal pupae were 8.47 per cent (Table 22); among these 5.08 per cent had short wing pads. Whereas abnormal pupae having ventral skin only sclerotised or bent in shape were found 1.69 per cent each.

Deformed pupae developed in 0.1 /ug were 7.41 per cent of the treated larvae, among these 3.70 per cent had short wing pads; the curved pupae or those had only ventral skin sclerotised were 1.85 per cent each as given in Table 21). No deformity was observed in the pupae developed in control.

Table 22. Effect on metamorphosis and cumulative mortality of *E. virgincula* in DFB fed larvae

| Treatments /ug/larvae | Number of insects treated | Mortality | | | LPI | Pupation | | Pupal mortality | | Adult emergence | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Larval | Prepupal | Total | | Abnormal | Normal | Among normal | Total | Abnormal | Normal |
| 5.0 | 55 | 38.18 | 23.64 | 61.82 | 34.55 | 3.64 | 0.00 | - | 3.64 | 0.00 | 0.00 |
| 1.0 | 58 | 27.59 | 10.34 | 37.93 | 43.10 | 8.62 | 10.34 | 6.89 | 15.51 | 3.45 | 0.00 |
| 0.5 | 59 | 22.03 | 8.47 | 30.50 | 44.07 | 8.47 | 18.64 | 10.17 | 18.64 | 8.47 | 3.39 |
| 0.1 | 54 | 9.26 | 5.55 | 14.81 | 44.44 | 7.41 | 33.33 | 11.11 | 18.52 | 12.96 | 9.26 |
| 0.0 (Control) | 60 | 1.67 | 0.00 | 1.67 | 0.00 | 0.00 | 98.33 | 1.67 | 1.67 | 0.00 | 96.67 |

(iii) Pupal-Adult Intermediate, abnormal and normal adult emergence

In the highest treatment dose no adult was emerged. However, in the subsequent dose of 1.0 /ug/larva 3.45 per cent abnormal adults were developed but no normal adult was obtained. These remained trapped in pupal shell. In 0.5 /ug dose the percentages of abnormal and normal adults were 8.47 and 3.39 respectively. In lowest dose of 0.1 /ug/larva their percentages were 12.96 and 9.26 respectively. In control there was no abnormality observed in adults (Table 22).

4. Cumulative mortality at larval, pupal and adult stage

The mortality was observed usually 2-3 days after consuming the DFB treated food by the larvae. But most of the larval mortality was found during moulting irrespective of the developmental stage.

The cumulative mortality data for each treatment dose had been summarised in Table 22. It was ranging from 9.26 to 38.18 per cent in larval stage and 5.55 to 23.64 per cent in pre-pupal stage. Most of the LPI formed did not survive for a longer period. They died within 2-3 days. Some of them continued to survive for 4-7 days in moribund stage, hence were considered as dead. Similarly abnormal pupae did not result into adult and hence were treated as dead. These were 3.64,

8.62, 8.47 and 7.41 per cent in 5.0, 1.0, 0.5 and 0.1 /ug doses respectively. Among the normal pupae also, those from which no adult was emerged, were counted as dead. These were 6.89, 10.17 and 11.11 per cent in 1.0, 0.5 and 0.1 /ug doses respectively. In control, however, 1.67 per cent larval mortality and an equal percentage of pupal mortality was recorded.

5. Longevity and fecundity of adults; viability and hatchability of eggs

The adults emerged from the larvae fed with 1.0, 0.5 and 0.1 /ug DFB doses lived for 5 to 6 days only. Since the adults emerged in 1.0 /ug dose were trapped in pupal skin they could not mate; and the normal looking adults in 0.5 /ug dose also did not mate and lay eggs. However, the single female emerged among the adults in 0.1 /ug mated with the male emerged in the same dose but there was no egg laying.

## III. FIELD EVALUATION OF DFB AND ITS PERSISTENCE

Leaves treated with different concentrations of DFB in the field were brought to laboratory at different time intervals viz. 0, 1, 5 and 10 days after the treatment and fed to E. virgincula larvae. In this experiment two criteria were undertaken i.e. the larval mortality and the cumulative number of individuals which could not reach pupation.

1. Larval mortality

There was no immediate knock down of the treated larvae in 24 hours of the experimentation; neither any larva was found in moribund state. Hence these larvae after being fed treated food for 24 hours, were kept under observation to determine pupal inhibition which was considered for interpretation and deriving the inferences.

2. Pupal inhibition

The cumulative mortality data obtained in different concentrations at different time intervals were used for calculating pupal inhibition. The results had been presented in Table 23, which revealed that all the DFB concentrations used were proved significantly superior over control at all the time intervals i.e. 0, 1, 5 and 10 days.

The pupal inhibition obtained in the larvae fed with the treated leaves plucked immediately after DFB spray (i.e. 0 day) was 100 per cent in the higher concentrations viz. 0.2 and 0.1 per cent. Minimum inhibition was 65.05 per cent observed in the lowest concentration of 0.01 per cent.

After 1 day of treatment, the field sprayed leaves resulted pupal inhibition ranging from 63.63 to 93.51 per cent. The highest concentration was significantly superior to all the

Table 23. Pupal inhibition in fifth instar stage of *E. virgincula* fed with DFB treated cowpea leaves in field at different time intervals

| Concentrations % | Per cent pupal inhibition*, days after treatment | | | |
|---|---|---|---|---|
| | 0 | 1 | 5 | 10 |
| 0.2 | 100.00(90.00) | 93.51(75.24) | 90.40(71.95) | 73.56(59.06) |
| 0.1 | 100.00(90.00) | 87.79(69.55) | 83.79(66.26) | 66.81(54.83) |
| 0.05 | 91.85(73.41) | 81.87(64.80) | 75.10(60.07) | 56.75(48.88) |
| 0.02 | 76.70(61.14) | 76.99(61.33) | 65.23(53.87) | 41.66(40.20) |
| 0.01 | 65.05(53.76) | 63.63(52.91) | 55.18(47.97) | 26.47(30.94) |
| 0.00 (Control) | 8.15(16.59) | 6.49(14.76) | 9.60(18.05) | 11.57(19.89) |
| S.Em.± | 1.398 | 3.568 | 3.423 | 2.414 |
| C.D. at 5% | 4.47 | 11.43 | 10.95 | 7.72 |

* Figures in parentheses are arc-sine angles of percentages of pupal inhibition
Figures outside parentheses are back transformed values
Data based on three replicates

subsequent concentrations except 0.1 per cent to which it did not show any statistical difference. In its turn 0.1 per cent concentration was at par with the next lower concentrations i.e. 0.05 and 0.02 per cent.

Similarly, 0.2 and 0.1 per cent concentrations in 5 days interval were alike statistically. Also 0.1 per cent concentration was equal to its next lower concentration. Maximum pupal inhibition was as high as 90.40 per cent attained in the highest concentration and the minimum of 55 per cent was obtained in the lowest concentration (0.01 per cent).

Ten days after the treatment, the pupal inhibition was decreased to an extent of 26.47 per cent in the lowest concentration of 0.01 per cent; it was very high i.e. 73.56 per cent in 0.2 per cent concentration. This treatment was found statistically at par with 0.1 per cent concentration (Table 23).

*****

# DISCUSSION

Although the use of chitin inhibitors for pest control was initiated for the first time by Vaan Daalen et al. (1972) and subsequently supplemented by a host of workers (Busschbach, 1975; Bijloo, 1975; Retnakaran, 1978; Westigard, 1979; Rabindra and Balasubramanian, 1984 and Retnakaran et al., 1985), yet a comparative evaluation of this upcoming pesticide alongwith its economic aspects had not been studied so far.

In view of this an assessment of the effectiveness of the chitin inhibitor (DFB), as a pesticide, was warranted on the basis of the information already known and the new data generated during the present investigation.

Consequently the results obtained during the present studies had been discussed in order to draw the inferences thereof. The discussion had been oriented for both the pests viz. S. litura and E. virgincula, separately; comparative evaluation evolved during the process of drawing the inferences had also been incorporated wherever found necessary.

## A. Spodoptera litura Fab.

### I. TOXICOLOGICAL INVESTIGATIONS

#### (1) Dermal toxicity of DFB

The $LD_{25}$ values obtained after 12 and 24 hrs exposure through dry-film method were 23.21 and 10.03 mg/unit surface area respectively. Curiously enough the median lethal dosages ($LD_{50}$), derived for the same exposure periods i.e. 12 and 24 hrs, were found to be fairly high than the former values; the values being 148.60 and 91.28 mg/unit surface area respectively. These results indicated firstly that DFB was having contact action against S. litura and secondly that sufficiently high amount of the chemical was required to achieve fifty per cent kill of the test population within the limits of exposure periods of 12 and 24 hrs.

Yet another inference derived was, that large amount of DFB was required for the desired kill in shorter periods. The ratio obtained between the treatment periods (12 vs 24 hrs) for $LD_{25}$ and $LD_{50}$ were 2.31:1 and 1.63:1 respectively (Table 2). This showed that for achieving 25 per cent kill or 50 per cent kill of the test insects larger amount of DFB was required in 12 hrs exposure while

for the same quantum of the kill (i.e. 25% and 50%) in 24 hrs exposure lesser amount of DFB was necessary.

A very important and noteworthy observation which needed to be discussed, was, that the initiation of the mortality started only after 48 hrs of the exposure period. This clearly indicated that the chemical had a slow contact action.

Summarising the inferences drawn on the basis of the discussion made on the data generated on dermal toxicity it could be inferred that :

1. DFB was a slow contact poison apparently much slower in action than the other contact insecticides currently in vogue.
2. That larger quantity of DFB was required for a desired kill in a stipulated time.

The above inferences found support in the earlier contribution of Moore and Taft (1975) who did not find any mortality using a dose of 10 mg/unit surface area. Das et al. (1984) recorded 30.7 per cent mortality in potato weevil when treated with 0.2 per cent dry film of DFB. The contribution of Abo Elghar et al. (1978) on S. litura, although strikes a parallelism with the present investigation, yet had a little relevance with the present finding due to differences in methodology. They broadcasted DFB gramules as soil surface

treatment in pots and irrigated. They found that the value of $LR_{25}$ (lethal rate for 25 per cent kill of test larvae) came out to be 3 kg ai/feddan after 4 days of exposure. When this quantity was converted into mg/unit surface area it worked out to be 6.5 mg for 91 $cm^2$ surface area. This value apparently was too low in comparison to the $LD_{25}$ value obtained during the present investigation. This anamoly may be explained that they have exposed the test animals for a longer duration i.e. 4 days.

The $LC_{25}$ and $LC_{50}$ values obtained by dipping method (85.33 and 629.80 ppm respectively) further confirmed the previous results by dry-film method. For $LC_{50}$ the amount of DFB required was much more than was required for $LC_{25}$. Early contributions of Moore and Tuft (1975), Fytizas (1976) and Earle et al. (1978) on different insects reported a little or no mortality by DFB through dipping method.

2. Oral toxicity

The data on oral toxicity, for $LD_{25}$ and $LD_{50}$ revealed three important aspects which needed a careful analysis. The first and foremost aspect was the long range of variation in $LD_{50}$ values obtained by various workers. El Sayed (1978) recorded the median mortality at 96 hrs through contimuous feeding of treated food at

a dose of 7.6 ppm. Radwan et al. (1978) found that the median lethal dose was 0.11 /ug/larva at 48 hrs and 0.007 /ug/larva at 96 hrs. Saad et al. (1981) used two resistant strains (Alexandria and Menifia) of this pest in their experiments with DFB. They found that 50 per cent kill of the population was attained at 1400 and 1600 ppm concentrations for both the strains respectively.

A scrutiny of these data showed that a little relationship existed between one another due to sharp variations. Further they did not have any comparative significance also with the results obtained during the present investigation. The probable explanation for such variations was the use of different methodologies by different workers. In some of the cases the poison was poured in the buccal cavity of the test insect in a single shot. Evidently this got quickly absorbed in the digestive system resulting in higher mortality with lesser quantity of the chemical. Whereas in the present investigation the poison administered was through the leaves poured with known quantity of DFB. The latter technique was adopted since the ultimate aim of this investigation was to assess the efficacy of DFB in the fields. Consequently, it could be safely inferred that the value of $LD_{50}$, obtained during the present investigation, was of practical utility and significance.

The other aspect that needed a consideration was the delayed mortality caused by the use of DFB in the test animals. Delay in mortality had already been recorded by a number of workers viz. Mulder and Gijswijt (1973), Ascher and Nemny (1976b), Radwan et al. (1978), El Sayeed (1978), Reed and Bass (1980), Subramanyam et al. (1980) and Rabindra and Balasubramanian (1981). In all these cases the initiation of mortality started from 24 to 96 hrs. Such a wide range of variation evidently needed a further probe to monitor the use of DFB as an insecticide. In the present investigation no mortality, whatsoever, was observed upto 24 hrs of consuming the DFB treated food by the larva. Evidently this indicated that DFB was a slow stomach poison.

Yet another aspect was the method of death observed in the larvae after consuming treated food with DFB. There were reports of deaths through inhibition of moulting, preponderance of unusual stages, rupturing of cuticle and so on. This aspect had already been taken care of under appropriate heading in this very manuscript.

## II. SYMPTOMATIC AND BEHAVIOURAL INVESTIGATIONS

The DFB fed larvae exhibited several abnormal symptoms (Chart I; page 59). These were catagorised as behavioural changes and morphological deformities.

1. Behavioural changes

The initial poison symptoms developed were sluggishness, cessation of locomotion and cessation of feeding in S. litura larvae after feeding DFB treated food. These symptoms appeared in 5.0, 1.0 and 0.5 /ug DFB dosed larvae. Their intensity decreased with the decrease in dose, indicating a dose dependency. These symptoms had also been repoted by Mulder and Gijswijt (1973) in Colarado potato beetle, Buschwein and Granett (1977) in eastern spruce bud worm, Sahota and Shepherd (1975) in Western hemlock looper, Reed and Bass (1980) in Soybean looper, Rupes et al(1977) in housefly larvae and Subramanyam et al. (1980) in A. janata larvae.

The sluggishness, cessation of locomotion and cessation of feeding, all these factors are related to muscular traction (Wigglesworth, 1965 and Chapman, 1969). It appeared that DFB was having direct effect on the muscular system of the test animals, thus revealing these abnormal behavioural symptoms.

Wetting and oozing were closely associated to each other. Both the symptoms were found in all the treatments. Wetting was recorded in 68.42, 45.76, 36.83 and 20.00 per cent of treated insects with 5.0, 1.0, 0.5 and 0.1 /ug/larva doses respectively. Whereas oozing was observed in 80.7, 49.15, 35.08 and 15.00 per cent cases respectively in the corresponding treatment doses.

These symptoms were noticed by Mulder and Gijswijt (1973) in P. brassicae larvae, Sahota and Shepherd (1975) in western hemlock looper, Bushwein and Granett (1977) in Eastern spruce budworm after consuming DFB treated food. They observed that these symptoms were associated with rupture of the cuticle. While Salama et al. (1976) and Reed and Bass (1980) described that bursting of rectal prolapse caused haemolymph leakage. During the present investigation also, oozing of body fluid was observed from the anus, mouth and from intersegmental membranes. Possible reason for oozing from mouth might be due to regurgitation by the insect in the state of unwillingness. Oozing through intersegmental membrane could be attributed to breakages of the membranes as a result of increased intrinsic pressure of the body preparing itself for ecdysis.

2. Morphological deformities

Shrinkage of the larval body had invariably been observed in all the treatment doses which was followed by cessation of feeding. In higher dose, 5.0 µg DFB/larva, as many as 35 per cent larvae exhibited these symptoms but in lower doses appearance of these symptoms were reduced. Sundarmurthy (1977) also observed that DFB fed larvae became short and did not complete their moulting. They also found the symptoms of cessation of feeding and reduction in weight

in the larvae. Neal (1974) went a step further; he reported reduction in size of the adults developed from DFB treated larvae.

In all, probability of the shrinkage of larval body was due to the loss of liquid content which oozed o ut of the body. Wigglesworth (1965) added a few more parameters; he was of the opinion that cessation of feeding restricted the growth resulting in loss of locomotion and an overall shrinkage of body length.

Discolouration of larval cuticle was also observed in a number of cases in higher doses which was reduced with the decrease in the dose. Such a symptom was also observed by Mulder and Gijswijt (1973), Deul et al. (1978) and Neal (1974). It appeared that DFB treatment decreased the activity of carbohydrate degrading enzymes which reduced the amount of carbohydrates available for chitin synthesis. This suggestion was supported by Ishaaya & Ascher (1977) who estimated the trehlase, amylase and invertase activity in DFB treated insects. Further an increased phenyloxidase activity in DFB treated animals was observed by Ishaaya and Casida (1974). This enzyme was responsible for the darkening and hardening of the cuticle. Yu and Terriere (1975, 1977) tried to explain this factor on the basis of ecdysone and chitinase activity which was refuted by O'Neill et al., 1977.

In DFB treated S. litura larvae, rupturing of the cuticle followed by oozing and wetting was invariably seen in all the treatment doses. These symptoms were also observed in P. brassicae (Mulder and Gijswijt, 1973): Soybean looper (Reed and Bass, 1980) and western hemlock looper (Sahota and Shepherd, 1975).

The expression of these symptoms may be explained by the fact that larvae when treated with DFB shortly after ecdysis ceased to strengthen their endocuticle as it is a known chitin inhibitor. Simultaneously the larval body grows for the next moult, a stage will arive when the cuticle will no longer resist the intrinsic haemolymph pressure or the muscular pressure of the body or both, leading to the rupture of the cuticle, followed by leakage of the body fluid due to the intrinsic pressure of the blood (Chapman, 1969 and Wigglesworth, 1965).

About 5.26 and 1.69 per cent of the larvae in treatment doses of 5.0 and 1.0 /ug respectively, had haemolymph leakage from anus. Subramanyam et al. (1980) also recorded 6 per cent larvae of A. janata with this symptom in 1 ppm treatment. Reed and Bass (1980) too, reported 2.5 per cent larvae showing rectal papillae. In such individuals pieces of membranes were often

seen protruding from the anus. In these instances probably the hind gut was broken due to the effect of DFB. This might have allowed the haemolymph to enter the hind gut which ultimately escaped through the anus. In a few cases the membrane was complete in the form of a sac filled with yellowish fluid. Such symptoms of anal papillae had also been reported in DFB & IGR treated animals by Salama et al. (1976) and Tsutomu et al. (1976). Reed and Bass (1980) termed this symptom as hindgut everted at anus in his investigations, indicating protruding of sac like structure through the anus, as was observed in present findings. Negishi et al. (1976) observed similar rectal prolapse in S. litura treated with sub-optimal doses of JH analogues indicating juvenomimetic effect of DFB.

Frayed and mosaic condition of cuticle was observed in the larvae and prepupae in all the treatment doses ranging from 0.1 to 5.0 /ug/larva. Such symptoms had also been reported in gypsy moth (Granett and Dunbar, 1975), in S. littoralis (Sundarmurthy, 1977) and in A. janata (Rabindra and Balasubramanian, 1981).

Both, in the larvae and prepupae, the patches of old cuticle were casted off and at these places well sclerotised pupal skin was observed thus giving mosaic appearance in the integument texture. This was most likely due to inhibition of the moulting of the larvae on one hand and the irregular deposition of the new cuticle on the other hand, as DFB inhibits ecdysone metabolism (Yu and Terriere, 1977).

The incomplete shedding of cuticle in DFB fed animals may be explained on the basis of histopathological studies. The newly formed cuticle in the treated animal consisted only of epicuticle and exocuticular tissues, which were not properly attached to epidermis through endocuticle. However, the newly formed cuticle was very delicate and as such could not have resisted the muscular traction and the increased turger pressure during moulting.

There was inconsistency in shedding of the cuticle. Such inconsistency were also observed by a number of workers in different insects (Mulder and Gijswijt, 1973, P. brassicae; Sundarmurthy, 1977, S. littoralis; Subramanyam et al., 1980 and Rabindra and Balasubramanian, 1981, A. janata). However, at this stage nothing conclusive could be said about this phenomenon and consequently more data on this aspect was warranted.

3. Formation of abnormal stages

All the treatments resulted in the formation of larval-pupal-intermediates, thus mimicing the affect of juvenile hormone (JH) and their analogues. It is an established fact that insect metamorphosis is influenced by varying titres of morphogenetic hormones viz. ecdysone and JH. Under the influence of a falling titre of JH the ecdysone promotes metamorphosis i.e.

larva to pupa and pupa to adult. Hence the left over exogenous dose of DFB probably acted as a JH mimic and at the last larval instar caused the development of LP (Subramanium et al., 1980). The retention of larval cuticle and larval features in pupating S. litura after DFB treatment suggested that besides interfering with the synthesis of chitin, the hormonal titre of the body was also influenced by it.

The influence of the chemical was evident both on the pupae and the emergence of adults. The per cent normal pupation and the adult emergence also decreased with the increase in the doses. No adults emerged in 1.0 /ug and higher dose, while those emerged in lower dose were mostly abnormal. Sundarmurthy (1977) reported less than 10 per cent adult emergence even in the highest dose 0.01 per cent DFB. He also observed that the adults emerged in 0.04 per cent and lower dose were non-functional with deformities. Present findings were in agreement with these results.

The incomplete moulting, and mosaic pattern of deformities observed in the present study might be due to the blockage of deposition of chitin in the endocuticle as had been reported in Pieris brassicae (Post and Vincent, 1973 and Gijswijt et al., 1979) and in housefly larvae (Ishaaya and Casida, 1974). However, it was possible that the diflubenzuron molecules might have interacted with neuro-endocrine systems

affecting the biosynthesis of steroids which were responsible for producing larval-pupal intermediates. It had also been shown that the disturbances in the metabolism of steroids reflected on the metamorphosis of larvae by way of affecting the growth and development as some of the steroids appeared to be likely precursors of ecdysone (Svoboda et al., 1975).

III. FIELD EVALUATION OF DFB AND ITS PERSISTENCE

The field testing of DFB had been investigated by a number of workers against a variety of insect pests viz. Pieris brassicae (Post and Vincent, 1973), Zebra caterpillar (Tamaki and Turner, 1974), pest faunal complex of cotton (Rizk and Radwan, 1975), S. littoralis (Abo-Elghar et al., 1977; Sundarmurthy, 1977 Natesan and Balasubramanian, 1979 and Watson et al., 1981) and A. alibistriga (Rabindra and Balasubramanian, 1984). In all the experiments Dimilin had been found to be quite effective against chewing type of insects as stomach poison.

In the present field trials the treatment concentrations of 0.2, 0.1, 0.05, 0.02 and 0.01 per cent were tested and residual toxicity of Dimilin was investigated against S. litura by feeding method. The results revealed that 0.2, 0.1, 0.05 and 0.02 per cent concentrations were effective upto 10 days, giving more than 50 per cent pupal inhibition.

These field findings, reported here, for Dimilin strongly suggest excellent prospect for its practical use against S. litura in cabbage crop. All the treatment dosages were significantly superior than the control to the extended period of 10 days. The present findings agreed with those of Watson et al. (1981) and Abo Alghar et al. (1977) against S. littoralis. They reported that this antimoulting compound Dimilin revealed potentiality as a condidate material in S. littoralis management programme and may also be effective to an extended period of 2 to 4 weeks in cotton crop. Unlike the JH analogues and their mimics the chitin inhibitor "Dimilin" had a long residual toxicity and acted as a long persistant insecticide which was confirmed by the pupal inhibition recorded in the present field trial.

IV. HISTOPATHOLOGICAL INVESTIGATIONS

The histopathological changes in the cuticle had earlier been investigated by a number of workers using the chitin inhibitors like diflubenzuron (Mulder and Gijswijt, 1973; Hunter and Vincent, 1974; Salama et al., 1976; Ker, 1977; Duel et al., 1978; Grosscurt, 1978; Mitsui et al., 1980 and Saxena and Kumar, 1981), Difluron (Saxena and Kumar, 1981), Polyosin-D (Gijswijt et al., 1979). PH 60-38 (Mulder and Gijswijt, 1973), penfluron (Saxena and Kumar, 1981) and Du 19.111 (Post et al., 1974).

The results of present work clearly indicated that the DFB treatment on the fifth instar larva of S. litura caused an obliteration of the connection between the epidermal cells and the cuticular layer and dissolution of endocuticular tissue which was replaced by scattered globular particles. Similar results were obtained by Gijswijt et al.(1979) and Salama et al. (1976). Contrary to our findings Hunter and Vincent (1974), Ker (1977) and Grasscurt (1978) observedthat thickness of the cuticle continued to increase even after DFB treatment. They maintained that protein synthesis was not affected by diflubenzuron. It appeared that the thickness measured by Mulder and Gijswijt (1973) was related to the undamaged layers only reflecting protein deposition in the form of globules.

In the present investigation the endocuticle was obliterated by DFB trea-tment. Thus creating a space in between the epicuticle and epidermal layers (Plate 3; Fig. 4). These findings get support from the observation of Salama et al. (1976).

In the insect cuticle the chitin forms a structural frame work around which the protein molecules arrange themselves (Wigglesworth, 1965 and Chapman, 1969). In this test insect, it was concluded that DFB inhibited the chitin synthesis. The plausibility of this suggestion was supported bythe observation that the endocuticle was completely obliterated. Further, it was presumed that the mesocuticle continued to grow during DFB

treatment and the synthesis of chitin was arrested. This obliteration could only be due to protein synthesis. Such a suggestion was supported by the findings of Gijswijt et al. (1979) in which the incorporation of radio active form of glucose into various fractions examined, depicted the chitin fraction of the cuticle reduced by the treatment of polyoxin D, while the other fractions did not show such a reduction.

V. BIOCHEMICAL INVESTIGATIONS

Since Dimilin is a chitin inhibitor, it was found desirable to assess its impact on the protein make-up of the haemolymph and its correlation with the building up of protein pattern in the cuticle. Results obtained on the haemolymph proteins by administering different doses of Dimilin to the test insects revealed changes having biochemical significance. In this connection two observations pertaining to haemolymph protein pattern needed to be discussed.

In the control, the haemolymph protein pattern obtained from the mature larva in 3 successive stages (i.e. after 24 hrs, 48 hrs and 72 hrs) showed 10, 15 and 13 bands. This evidently showed that biochemical changes were continuing within the metabolic pool of the test insect even in a normal way. Comparing this very trend with the protein make-up obtained after administering 1.0 /ug and 5.0 /ug doses of Dimilin, a different picture was met with. A dose of 1.0

/ug Dimilin initiated a little change in the metabolic activity. The protein bands appeared after 24 hrs were 12 i.e. 2 more bands were found as compared to the control. Later on, i.e. after 48 and 72 hrs there was a fall in the protein bands at 1.0 /ug dose where only 11 bands were left. In 5.0 /ug dose level radical changes appeared at 24 hr stage in the protein bands which were reduced to 7 only as compared to 10 in the control. Interestingly enough in the 48 and 72 hr stage some new bands appeared and consequently their number rose to 9 and 10 respectively. Thus it could be very easily inferred that the introduction of Dimilin in the body of the test insect resulted in creating high metabolic activity. There were new protein bands that appeared, some already known protein bands disappeared resulting thereby formation of new linkages, bands etc. - indicative of internal changes reflecting the mode of action of Dimilin in the protein make-up of the test insect.

The problem was further carried to the level of protein make-up in the cuticular composition of the same stages of the test insect for which haemolymph proteins were scanned.

In the control similar pattern was observed as was seen in the haemolymph proteins. In 1.0 /ug dose the protein bands were reduced to 6 in comparison to 8 in the control (Table 13). This trend was contrary to the observation found in the haemolymph protein wherein after 24 hrs the protein bands rose to 12 as compared to 10 in the control. In 5.0 /ug dose the

protein bands remained more or less the same as in 1.0 /ug dose. Normally changes in the higher dose level should have appeared but a sort of static picture was observed in the 5.0 /ug dose for which no explanation could be given at this juncture. However, a further probe involving different dose levels and different time intervals is warranted.

The protein make-up after 48 hrs of introduction of Dimilin into the metabolic pool of the test insect presented a definite decline in the protein bands; as compared to 7 bands in the control only 5 bands were obtained in 1.0 /ug and 5.0 /ug doses. There was, however, a consistency of the trend of events at both the dose levels at 24 hrs and 48 hrs. A general trend was that the protein bands continued to reduce in number consequent to the introduction of Dimilin.

After 72 hrs of the treatment it was found that the protein bands in 1.0 /ug and 5.0/ug dose rose to 7 and 8 as compared to 5 in the control. This rise of protein bands appeared to be due to building up of new proteins that contribute in the formation of the cuticle. Since after 72 hrs the mature larva invariably changes to pre-pupa, formation of new proteins was evidently inevitable.

A much wider and deeper probe involving many more biochemical parameters is warranted to find an answer to the mode of action of Dimilin at such a level.

*****

## B. *Euproctis virgincula* Wlk.

### I. TOXICOLOGICAL INVESTIGATIONS

#### 1. Dermal toxicity

Inferences drawn in the case of *S. litura* had been fully supported by the data obtained on *E. virgincula*. DFB was having contact action against this pest also and secondly high amount of chemical was needed to achieve 50 per cent kill of the test population. As was the case in *S. litura* in *E. virgincula* also large amount of DFB was required for the desired kill in shorter period. The ratio obtained between the treatment periods i.e. 12 and 24 hrs for $LD_{25}$ and $LD_{50}$ were 1.61:1 and 1.22:1 respectively.

The initiation of mortality also found support in the case of *E. virgincula*; the insects died after 48 hrs of the exposure as was the case in *S. litura*.

The comparison of $LD_{50}$ values among the two test animals indicated that *S. litura* was comparatively more susceptible than *E. virgincula*. This difference in susceptibility to dermal toxicity of DFB might be due to the hairy nature of *E. virgincula* larvae. This hairy covering of the body provided the animal an extra protection against the direct contact with the treated surface, thus resulting in reduced mortality.

2. Oral toxicity

As for S. litura, the $LD_{25}$ and $LD_{50}$ values for E. virgincula (0.95 - 16.82 /ug/larva) also gave a clear indication of stomach toxicity of DFB against this insect. Again, as there was no immediate mortality in present case also, it confirmed that DFB acts very slowly. Such a slow action of this chemical had been reported against a number of insects by several workers in the past (Mulder and Gijswijt, 1973; Radwan et al., 1978; Subramanyam et al., 1980 and Srivastava et al., 1984).

II. SYMPTOMATIC AND BEHAVIOURAL INVESTIGATIONS

1. Behavioural changes

The administration of DFB brought sluggishness, cessation of locomotion and feeding as initial poison symptoms but such effects could be seen only after 48 hrs of the treatment. The latter fact indicated that E. virgincula was less susceptible to DFB in comparison to S. litura.

Similar to S. litura, the symptoms of wetting and oozing of body fluid was prevalent in all the treatments in case of E. virgincula. The percentage of larvae showing wetting was little higher in E. virgincula but oozing was exhibited in lesser degree in them.

2. Morphological deformities

Among the morphological symptoms, discolouration of body was the prominent symptom as was the case in S. litura. However, in E. virgincula a lower percentage of treated larvae showed the symptom of discolouration than found in S. litura.

Shrinkage of the larval body was also exhibited by a lower percentage of larvae as compared to S. litura in 5.0 and 0.5 /ug doses, while in other two doses (1.0 and 0.1 /ug) it was little higher.

With regard to the appearance of rupturing of cuticle and its intensity in E. virgincula in different doses a similar trend was observed as in S. litura. However, considering the overall prevalence of this symptom it could be inferred that DFB affected E. virgincula in lesser intensity as compared to S. litura.

As regards scar formation and rectal prolapse, their percentages were so meagre that no definite comparison could be made between the two test insects.

Rectal prolapse had been considered an important abnormality leading to death (Salama et al., 1976: Subramanyam et al., 1980 and Reed and Bass, 1980). It appeared in 5.0, 1.0 and 0.5 /ug doses in E. virgincula but was seen only in former doses in S. litura larvae. Comparative evaluation of results showed that it affected E. virgincula in lesser intensity at the dose level of 5.0 /ug/larva. The latter fact indicated once again that DFB had a lesser effect on E. virgincula.

The frayed and mosaic conditions of cuticle observed in E. virgincula larvae in higher doses were in accordance with that found in S.litura where more than 40 per cent of treated larvae showed mosaic conditions in 5.0 and 1.0 /ug/larva doses and more than 10 per cent exhibited frayed condition in 5.0 /ug/larva dose. In subsequent doses, however, these symptoms were more in S. litura as compared to E. virgincula, which indicated pronounced effect of DFB on the former test insect.

There was almost similar consistency in the incomplete shedding of cuticle in DFB fed E. virgincula and S. litura larvae. Rupture of old cuticle was, however, observed in more number of E. virgincula larvae than S. litura.

The percentage of larvae exhibiting pupal skin on ventral side of abdomen only, was static in highest dose level (5.0 /ug) in both the test insects. However, the prevalence of this symptom was lowered down at subsequent doses in the case of E. virgincula as compared to S. litura. This again indicated less susceptibility of E. virgincula than S. litura larvae to DFB.

3. Formation of abnormal stages

Development of different forms of Larval-Pupal Intermediates (LPI), from the DFB fed E. virgincula larvae, was more in all the dose levels as compared to S. litura.

The explanation for this could be given on perusal of the larval and pre-pupal mortality data of S. litura (Table 8), which revealed a higher mortality in S. litura as compared to E. virgincula (Table 22) at all the dose levels. This resulted into higher percentage of larval survival in the latter insect; evidently higher number of LPI were formed. However, on reviewing the data on pupation, it was very clear that out of the treated larvae the pupal formation was less in S. litura and more in E. virgincula.

A similar picture was obtained with adult emergence, thereby, suggesting that the chemical was more effective on S. litura than on E. virgincula.

4. Cumulative mortality at larval, pupal and adult stage

Similar to S. litura, in E. virgincula also, the influence of DFB was evident on its mortality at different developmental stages. The mortality occurred 2 to 3 days after the larvae consumed DFB treated food and was observed during moulting stage in most of the cases. These observations were again in accordance with those found in S. litura.

The cumulative mortality data revealed a lower percentage of larval and pre-pupal mortality in E. virgincula as compared to S. litura indicating that DFB exhibited a lesser influence on the former test insect. A similar trend was

observed on pupation and adult emergence in E. virgincula with regard to DFB treatment.

5. Longevity and fecundity of adults; viability and hatchability of eggs

Since a negligible number of adults could emerge from the DFB fed larvae of E. virgincula any inference drawn on the basis of such a meagre data might have led to an over enthusiastic or erroneous conclusion. Consequently the results on longevity, fecundity etc. had not been discussed.

III. Field evaluation of DFB and its persistence

The results revealed that DFB spray @ 0.2, 0.1 and 0.05 per cent remained effective upto 10 days in the field against E. virgincula, giving more than 50 per cent pupal inhibition of the test larvae.

Earlier in the field experiment with another test insect S. litura, in addition to these concentrations, 0.02 per cent concentration also showed effectiveness giving more than 50 per cent pupal inhibition. It clearly indicated that DFB proved more effective against S. litura than E. virgincula.

When overall perusal of the field data was made, it depicted that at 0 day after spray, cent per cent pupal inhibition was obtained with 0.2 and 0.1 per cent concentration

for E. virgincula whereas for S. litura the subsequent lower concentration i.e. 0.05 per cent was equally effective.

After 1 day spray also similar trend was obtained wherein above 90 per cent pupal inhibition could be attained with concentration upto 0.05 per cent in S. litura whereas a little effect could be seen in E. virgincula with 0.2 per cent concentration only.

This comparative evaluation of the effectiveness of DFB on S. litura and E. virgincula led to a conclusion that at all the levels of concentrations and time intervals, DFB proved more effective against S. litura than E. virgincula.

On the basis of the results and inferences drawn thereof, it could be concluded that DFB offered a great potential for being used as a means to control the test insects and other allied species. Since DFB has been found to be slow in action it evidently can not be used as an insecticide desired to bring about a quick knockdown effect. Consequently compatibility studies are required to be carried out wherein the right place of DFB in the sequence of pest management strategy could be ascertained. Further the quality of DFB that it has a long residual effect bringing about 70 per cent pupal inhibition even upto 10 days in present findings with 0.2 per cent concentration needs to be fully exploited.

DFB could also be used as a prophylactic measure in checking the multiplication and building up of residual population of pests into an alarming status to enable it to inflict losses to the extent of economic injury level. Since DFB has a little or no adverse effect to human beings and other domesticated organisms its use either as a sole agent of limiting the population, or as an aid in the sequence of pest management system, or as prophylactic spray to inhibit the development of life stages of various pests, could not be challenged. In nut shell DFB is bound to develope as an asset in one way or the other to limit the pest population without having any adverse effect, whatsoever, on the biosphere.

*****

# SUMMARY

The present study was undertaken to evaluate the effect of diflubenzuron, a chitin inhibitor on Spodoptera litura Fab. and Euproctis virgincula Wlk.

I. TOXICOLOGICAL INVESTIGATIONS

1. Dermal toxicity was evaluated by dry film and dipping methods using 5 to 50 mg/90 $cm^2$ surface area dosages and 50 to 10,000 ppm concentrations respectively for both the test insects. The exposure periods in dry film method were 12 and 24 hrs.

(a) No immediate mortality was obtained in both the methods and both the test larvae. The mortality data recorded at 48 hrs were analysed.

(b) $LD_{25}$ values obtained were lower for S.litura as compared to E. virgincula for both the exposure periods in dry film method.

(c) $LD_{50}$ values were also calculated from the same regression equation which were higher in S. litura after 12 hrs exposure and lower after 24 hrs exposure as compared to E. virgincula.

(d) $LC_{50}$ values obtained by dipping method and $LC_{25}$ values calculated from the same regression equation were lower in case of S. litura than E. virgincula.

2. Oral toxicity was evaluated by feeding method using 0.1 to 5.0 /ug/larva doses of DFB to S. litura and E. virgincula larva.

(a) No immediate mortality was found in both the test insects hence total larval mortality before pupation was subjected to analysis.

(b) $LD_{25}$ and $LD_{50}$ values obtained for S. litura were lower than for E. virgincula.

(c) DFB was more effective against S. litura than E. virgincula.

II. SYMPTOMATIC AND BEHAVIOURAL INVESTIGATIONS

Different doses of DFB were administered by feeding method to the fifth instar larvae of both the test insects viz. S. litura and E. virgincula, which projected the following changes :-

1. Behavioural changes

(i) Behavioural abnormalities like sluggishness, cessation of locomotion and feeding, wetting of larval body and oozing of body fluid were observed in the larvae of both the test insects treated with different doses of DFB.

(ii) Among these symptoms, wetting of larval body and oozing of body fluid were more prominent in both the test larvae.

(iii) The percentage of larvae exhibiting these symptoms increased with an increase in the dose level of DFB.

(iv) Out of the two test insects, S. litura was more vulnerable to DFB as more percentages of the larvae of former insect exhibited these behavioural changes.

2. Morphological deformities

Oral administration of different doses of DFB to the larvae of S. litura and E. virgincula caused several morphological deformities :-

(i) Larvae with shrunken body and discoloured cuticle were produced more in S. litura as compared to E. virgincula and the effect increased with the increase in dose levels of DFB.

(ii) Percentage of larvae exhibiting ruptured cuticle and rectal prolapse were more pronounced in S. litura than in E. virgincula.

(iii) Frayed and mosaic conditions of cuticle also observed at the time of moulting in the treated larvae of both the test insects.

(iv) Incomplete shedding of exuviae was observed in head, thorax and abdominal regions of both the test larvae. The larvae exhibiting such symptoms in abdominal region were more as compared to those showing incomplete shedding in head and thoracic regions.

(v) Rupture of old cuticle at the pre-pupal stage was observed at 5.0 /ug dose level only in S. litura while in E. virgincula; it also appeared in 1.0 and 5.0 /ug doses.

(vi) Larvae developing pupal cuticle in ventral abdominal region were observed in both the test insects.

3. Formation of abnormal stages

Feeding of DFB treated food to 5th instar larvae of S. litura and E. virgincula resulted into the formation of different abnormal stages such as LPIs, abnormal pupae, PAIs and abnormal adults.

(i) LPIs were formed in all the doses in both the test insects. The percentages of LPI formation in different doses were different. It was more in the case of E. virgincula than S. litura.

(ii) No abnormal pupae were formed in S. litura at 5.0 /ug dose.while in E. virgincula a few cases of abnormal pupation were recorded.

(iii) Various deformities in abnormal pupae were observed in both the test insects.

(iv) The formation of normal pupae was more in lower doses and declined with increase in dose level in both the test insects; their percentage was higher in E. virgincula as compared to S. litura.

4. Cumulative mortality at larval, pupal and adult stages

(i) The mortality in treated larvae occured irrespective of the developmental stage; it was however, initiated 2 to 3 days after the treatment in both the test insects.

(ii) The mortality was different in different developmental stages.

(iii) During larval and pre-pupal stages, the mortality was higher in S. litura as compared to E. virgincula; it increased with the increase in dose level of DFB.

(iv) The pupal mortality (among normal looking pupae) was higher in S. litura and lower in E. virgincula; it decreased with the increase in dose level of DFB in both the test insects.

5. Longevity and fecundity of adults; viability and hatchability of eggs

Only a very few S. litura and E. virgincula moths, emerged in lower doses. They lived for shorter period in comparison to control moths. None of these emerged moths layed eggs.

III. FIELD EVALUATION OF DFB AND ITS PERSISTENCE

(i) Efficacy of DFB was tested against S. litura and E. virgincula in field, using five concentrations viz., 0.2, 0.1, 0.05, 0.02 and 0.01 per cent. Its persistence was also observed upto 10 days.

(ii) Initially at 0 day after spray 100 per cent pupal inhibition was obtained in S. litura with 0.05 per cent and higher concentrations. In E. virgincula with 0.02 per cent and higher concentrations, hundred per cent pupal inhibition was met with.

(iii) The persistence of the chemical was fairly high. It was effective upto 10 days resulting 55 to 77 per cent pupal inhibition in S. litura. In E. virgincula it gave about 42 to 74 per cent pupal inhibition for the same period.

IV. HISTOPATHOLOGICAL STUDIES (on Spodoptera litura only)

The oral dose of DFB at 5 µg/larva resulted severe lesions and disintigration in the endocuticular zone of the larvae. A space was seen developed between cuticle and epidermis. The epithelial layer also got affected and developed intercellular spaces. The effects were found more pronounced after 72 and 96 hrs of the incorporation of doses. There were many lesions and increased gaps between epidermis and the cuticle which contained coagulated material. At the latter stage the cuticle was also found completely obliterated disconnecting the cuticular layer with the epidermis.

V. BIOCHEMICAL INVESTIGATIONS : (on Spodoptera litura only)

Changes in haemolymph and cuticular protein patterns of S. litura larvae were studied at 24, 48 and 72 hrs after administering DFB doses of 5.0 and 1.0 µg/larva by feeding method. The separation of proteins was done by disc gel electrophoresis.

Results obtained revealed definite changes in the protein pattern in both, the haemolymph and the cuticle. Appearance of new and disappearance of old protein bands were observed due to the incorporation of diflubenzuron.

The cuticular protein pattern in the control was more or less similar to that obtained by Scanning the haemolymph. After treating with DFB the protein bands decreased in both 1.0 /ug and 5.0 /ug dose levels.

In cuticular proteins also there was appearance and disappearance of protein bands due to the incorporation of DFB, indicating thereby that DFB had triggered a process of reorganization of proteins.

*****

## REFERENCES


Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. econ. Ent. 18(4):265-67.

Ables, J.R., Jones, S.L. and Bee M.J. 1977. Effect of diflubenzuron on beneficial arthropods associated with cotton SH. Ent. 2. 66 : 72.

Ables, J.R., Robert, P. West and Merleshepard. 1975. Response of the housefly and its parasitoids, Dimilin TH 6040. J. econ. Entomol. 68:5.

Abo El-Ghar, M.R.A., El Keie, I.A., Mitri, S.H. and Radwan, H.S. 1980. Field evaluation of certain insecticides for ovicidal activity of the cotton leaf, Spodoptera littoralis (Boisd). Z. angew. Entomol. 89:100-104.

Abo-Elghar, M.R.; Radwan, H.S.A.; Ammar, I.M.A. 1976. The ovicidal effect of a chitin biosynthesis disruptor on Spodoptera littoralis egg-masses. The 3rd pest control Conf. Ain Shams Univ. Egypt.

Abo-Elghar, M.R.; Radwan H.S.A.; EL-Bermawy, Z.A. 1978. Development of a lab procedure for computing the toxicity of certain soil gramular pesticides against the cotton leafworm. Proceeding 4th Conf. Pest control, NRC, Cairo Egypt. 437-445.

Abo-Elghar, M.R.; Radwan, H.S.A. and Shoaban, M.A. 1977. Further field studies on the control of some major cotton pests with soil insecticides. Proc. 2nd Ara-Pest. Conf. Tanta University. 986-91.

Alfred, R. Hopkins; Raymond F. Moore and William James. 1982. Efficacy of diflubenzuron (DFB) diluted in three volumes of oils on bollweevil progeny. J. econ. Entomol. 75:385-86.

Anderson, D.W. and Elliott, R.H. 1982. Factors influencing the activity of DFB against the codling moth, Laspeyresia pomonella (Lepidoptera : Olethreutidae). Can Ent. 114(3):259-68.

Anonymous. 1974. TH 6040 Insect Growth Regulator. Thompson Hayward Tech. Inf.:25

Argauer, R.J. and Cantelo, W.W. 1980. Stability of three ureide insect chitin synthesis inhibitors in mushroom compost determined by chemical & Bioassey techniques. J. econ. ent. 73(5):671-73.

Ascher, K.R.S. and Nemny, N.E. 1976a. Toxicity of the chitin synthesis inhibitors, diflubenzuron and its dichloro-analogue to Spodoptera littoralis larvae. Pestic. Sci. 7, 1-9.

Ascher, K.R.S. and Nemny N.E. 1976b. Contact toxicity of diflubenzuron against Spodoptera littoralis larvae. Pestic. Sci. 7:447-52.

Ascher, K.R.S. and Nemny, N.E. 1980. Pestic. Sci. 11,90.

Audemard, H.; Guennelon, G. and Baudouin, J.M. 1975. Essais Preliminaires Sur Le Carpocapse (Laspeyresia pomonella L.) D'un Insecticide Presentant UnNoyreau Mode D'action; Le Diflubenzuron. Phytiarie Photopharmacie 24:179-92.

Baeumler, W. and Salama H.S. 1976. Some bio-chemical changes induced by dimilin in the Gypsy moth, Porthetria dispar. Z. Angew Entomol. 81(3):304-10.

Beaver, J.B.; Schroeder, W.J. and Selhime, A.G. 1976. Effect of diflubenzuron on larvae of Diaprepes abbreviatus. Fla. Ent. 59, 434.

Bijloo J.D. 1975. An original insecticide : diflubenzuron, Physico. chemical characteristics - Biological properties - Mode of action. Phytiatric Phytopharmacie. 24(3):147-158.

Busschbach E.J. Van. 1975. General outline of the possibilities of the application of Dimilin. Phytiatric-Phytopharmacie. 24(3):159-77.

Brusschwein, J. and Granett, J. 1977. Laboratory tests of two moulting disruptants. EL-494 and Dimilin, against the spruce budworm (Lepidoptera : Tortricidae) : Mortality and Reproductive effects. Marine Life Science and Agric. Expt. Station. Misc. Rept. No. 192.

Calkins C.O. Hill A.J. & Huettel M.D. 1977. Effect of diflubenzuron on plum curcutionid population in Laboratory & field tests. J. econ. Entomol. 70(4):463-66.

Chapman, R.F. 1969. The insects : Strcture and function. Publ. The English Univ. Press Ltd. London.

Ching-Muh, W. and Pitton, R.L. 1968. The separation and characterisation of the haemolymph proteins of several insects. J. Insect Physiol. 14:1069-75.

Choklingam S., Krishnan M. & Velpandi P. 1984. Efficacy of thiourea compound on the food utilization in lepidopteran pests. Abstracts III Oriental Entomology Symposium. February 21-24,: 76.

Clarke, L.; G.H.R. Temple and J.F.V. Vincent. 1977. The effect of a chitin inhibitor - dimilin on the production of peritrophic membrance in the locust, Locusta migratoria. J. Insect Physiol. 23:241-46.

Das N.M., Saradamma K., Ambika Devi D. and Dale D. 1984. Evaluation of diflubenzuron against some important crop pests. III Oriental Entomology Symposium. Feb. 21-24, 1984. :129.

Deul, D.H., B.F. deJong and Kortenbach 1978. Inhibition of chitin synthesis by two 1-(2-6 disubstituted benzoyl)-3-Phenylurea insecticides. J. Pestic. Biochem. Physiol. 8: 98-105.

Donaubauer, E. 1979. Dimilin (PH 6040) a 'Biotechnical insecticide. Allgemeine Forstzeitung 87:108-9.

Earle, N.W., Simmons, L.A., Nilakhe, S.S., Villavaso, E.J., McKibben, G.H. and Sikorowski. P. 1978. Pheromone production and sterility in boll weevils; effects of acute and fractional gammaiirradiation. J. Econ. Ent. 71. 591-95.

EL-Badawy, F.A., Farag, A.R. & Halawa L.A. 1980. Field evaluation of certain insecticides against egg masses of Spodoptera littoralis in Gemmiza Experimental farm. 1st Conf. Pl. Prot. Res. Institute Cairo Egypt. 21.

El-Gayar, F., Watson,W.M. and Soliman,M. 1978. Ovicidal and larvicidal activity of different formulations of Dimilin against Spodoptera littoralis (Boisd) under field condition (in press).

Elings H. and Dieperink J.G. 1977. Practical experience with the experimental insecticide. TH 6040. Meded. Fac. Laydbouwwet. Rijksunir Gent 31:833-46, Rev. Appl. Ent. 65(4):1908.

Elliott, R.H. and Iyer, I. 1982. Toxicity of DFB to nymphs of the migratory grasshopper, Melanoplus sanguinipes. Can. Ent. 114(6):479-84.

EL-Sayed, E.I. 1978. Larvicidal & ovidical effects of Dimilin, PH 66-13 and PH 66-14 on the Egyptian cotton leafworm. Proceedings 4th Conf. pest control, Cairo,Egypt. 381-91.

El Tantawi, M.A.; Belal, M.B. and Abdallah, M.D. 1976. Effects of two anti-moulting compounds on the larvae of Spodoptera littoralis (Boisd.). Schadl. Pflazensch. Umweltsch. 49 : 153-155.

Ferkovich, S.M., Oberlander H. and Leach C.E. 1981. Chitin synthesis in larval and pupal epidermis of the Indian meal moth, Plodia interpunetella and the greater wax moth, Galleria mellonella, J. Insect Physiol. 27(8):509-14.

Finney, D.J. 1971. Probit analysis. S. Chand & Company Ltd., New Delhi. P.333.

Flint, H.M. and Smith, R.L. 1977. Laboratory evaluation of TH 6040 against the pink bollworm (Lepidoptera). J. econ. Entomol. 70(1):51-53.

Fytizas, E. 1976. The effect of TH 6040 on the metamorphosis of Dacus oleae (Diptera : Tripetidae). Z. angew. Entomol 81(4):440-44.

Gijswijt, M.J.; Deul, D.H. and deJong, B.J. 1979. Inhibition of chitin synthesis of Benzoyl-Phenylurea Insecticides, III Similarity in action in Pieris brassicae (L.) with Polyoxin D. Pest. Biochem. and Physiol., 12:87-94.

Granett, H.J. and Dunbar D.M. 1975. TH 6040 Laboratory and field trials for the control of gypsy moths. J. econ. Entomol. 68:99-102.

Granett, J., Bisabri-Ershadi, B. and Hejazi, M.J. 1983. Some parameters of benzoylphenyurea toxicity to beet armyworm. J. Econ. Ent. 76. 403-6.

Greene, J.R. and Dahlman, D.L. 1973. Haemolymph protein pattern in developing tobacco horn worm larvae. J. Insect Physiol. 19:1241-50.

Grosscurt, A.C. 1978. Effects of diflubenzuron on mechanical penetrability, chitin formation, and structure of the elytra of Leptinotarsa decemlineata. J. Insect Physiol. 24:827-831.

Gund Pushpa and Sharma, R.N. 1984. Aetion of Dimilin on the potato tuber moth. Abstract III Oriental Entomology Symposium, Feb. 21-24, 1984:110.

Hackman, R.H. 1953. Chemistry of insect cuticle-1. The water soluble proteins. Biochem. J. 54, 362-67.

Hackman, R.H. 1964. Chemistry of insect cuticle.In The Physiology of Insecta Ed. by Rockstein M.) Academic Press, New York.

Hoying,S.A. and Riedl, H. 1980. Susceptibility of the codling moth to Diflubenzuron. J. econ. ent. 73(4):556-60.

Hunter, E. and Vincent, J. 1974. The effect of a novel insecticide on insect cuticle. Experientia 30.1432-33.

Ishaaya, I. and Ascher, K.R.S. 1977. Effect of diflubenzuron on growth and carbohydrate hydrolyses of Tribolium castaneum.Phytoparasitica. 5:149-58.

Ishaaya, I. and Casida,J.E. 1974. Dietary TH6040 alters composition and enzyme activity of housefly larval cuticle. Pestic. Biochem. Physiol. 4:484-80.

Keever, D.W., Bradley,J.R.J. and Ganyard, M.C. 1977. Effects of diflubenzuron (Dimilin) on selected beneficial orthropods in cotton fields. Environ. Ent. 6:732-36.

Ker,R.F. 1977. Investigation of locust cuticle using the insecticide diflubenzuron. J. Insect Physiol. 23: 39-48.

Ker, R.F. 1978. The effects of diflubenzuron on the growth of insect cuticle. Pesticide Sci. 9, 259-65.

Khalil, P.A. and Watson, W.M. 1979. Residual effectiveness of binary mixtures of Dimilin (IGR) with certain insecticides against Spodoptera littoralis (Boisd.) larvae. 1st Conf. Agri. Res. Center.

Kimura, S. 1973. The control of chitin deposition by ecdysterone in larvae of Bombyx mori. J. Insect Physiol. 19 :2177-81.

Kramer, K.J., Turner, C.D. and Daizo, K. 1985. Chitin metabolism in insects. Capter in "Comprehensive Insect Physiology, Biochemistry and Pharmacology. Ed. Kerkut G.A. and Gilbert, L.I. Vol. 12. Insect control. Publ. Pergamon Press. 79-115.

Lewo,1982. International conference on plant protection in the tropics Kuala Lumpur. Tropical Pest Mgt. 28(3):322-25.

Madrid, F.J. and Stewart R.K. 1981. Impact of DFB spray on Gypsy moth, parasitoids in the field. J. econ. Entomol. 74(1).

Maurer, R.H. 1968. Disk-Elektrophorese, Therie and Praxis der diskontimuierlichen Poly-acrylamedgel-Elektrophorese Walter de Gruyter, Berlin.

McCoy,C.W. 1978. Activity of Dimilin on the developmental stages of Phyllocoptruta oleivora and its performance in the field.J. econ. Entomol. 71(1):122-24.

McLaughlin, R.E. 1976. Response of the boll weevil to TH 6040 administered by feeding. Jr. econ. Entomol. 69(3),317-318.

Miller, R.W., Corley C. and Hill, K.R. 1975. Feeding TH 6040 to chickens. Effect on larval houseflies on manure and determination of residues. J. econ. Ent. 68(5):181.

Miltin, N. Wiygul. G. and Haynes, J.W. 1977. Inhibition of DNA synthesis in boll weevils (Anthonomus grandis Boheman) sterilized by Dimilin. Pestic. Biochem. Physiol. 7 : 559-63.

Mitsui, T., Nobusawa, C. and Fukami, J. 1981. Inhibition of chitin synthesis by diflubenzuron in Mamestra brassicae L. J. Pestic. Sci. 6. 155-61.

Moore Jr. R.F. and Taft, H.M. 1975. Boll weevils : Chemosterilization of both sexes with Busulfan plus. Thompson-Hayward TH 6040. J. econ. Entomol : 68-96.

Mulder, R. and Gijswijt, M.J. 1973. The laboratory evaluation of two promising new insecticides which interfere with chitin deposition. Pestic. Sci. 4, 737-45.

Natarajan, N., Kumaraswami, T. and Balasubramanian, M. 1984a. Efficacy of moult inhibitor BAY SIR 8514 on cotton pests under field conditions. . Abstract. Nat. Seminar, TNAU Coimbatore, June 21-23. 1984. 41-42.

Natrajan, N., Kumaraswami, T. and Balasubramanian, M. 1984b. Field efficacy of BAY SIR 8514 on groundnut pests. Abstract. Nat. Seminar, TNAU Coimbatore, June 21-23, 1984. 42.

Natesan R. and Balasubramanian, M. 1979. Effect of Diflubenzuron against tobacco caterpillar, Spodoptera litura F. on Tobacco Nicotiana tabacum L. Madras agric. J. 66(12):799-801.

Neal, J.W. Jr. 1974. Alfalfa weevil control with the unique growth disruptor TH 6040 in small test plots. J. Econ. Entomol. 67:300-1.

O'Niell, M.P., Holman, G.M. and Wright, J.E. 1977. $\beta$ - ecdysone levels in pharate pupae at the stable fly, Stomoxys calcitrans and interaction with the chitin inhibitor diflubenzuron. J. Insect Physiol. 23:1243-44.

Pant, R. and Agarwal, H.C. 1965. Some quantitative changes observed in Philosamia ricini pupal haemolymph during metamorphosis. Biochem. J. 96:824-28.

Philips, Daniet R. and Loughton B.G., 1982. Cuticle protein in adult Locusta migratoria. J. Insect Physiol. 27(7):475-84.

Philips-Duphar, B.V. 1977. Dimilin, a new insecticide interfering with chitin deposition. Technical Information Crop Protection Division, Holland, 7th edn: 43.

Post, L.C., Dejong, B.J. and Vincent, W.R. 1974. 1-2,6-Disubstituted benzoyl)-3-phenylurea insecticides : inhibitors of chitin synthesis. Pestic. Biochem. Physiol. 4, 473-83.

Post, L.C. and Vincent, W.R. 1973. A new insecticide inhibits chitin synthesis. Naturwiss ehchflen, 60; 431-32.

Rabindra, R.J. and Balasubramanian, M. 1981. The effect of diflubenzuron on the castor semilooper Achaea janata Linn. (Lepidoptera : Noctuidae). Entomon. 6(1):15-18.

Rabindra, R.J. and Balasubramanian, M. 1984. Diflubenzuron and some new insecticides for the control of the groundnut red hairy caterpillar Amsacta albistriga Walker. Abstract. Nat. Seminar, TNAU Coimbatore, June 21-23, 1984. 43.

Radwan, H.S.A., Rizk, G.A.M. Assal, O.M. 1978. The dose-toxicity of certain I.G.R.'s on cotton leafworm larvae in relation to developmental inhibitory effect. Procedings 4th Conf. Pest Control, Cairo, Egypt. 413-23.

Rajasekaran, B. and Kumaraswami, T. 1984. Effect of chitin inhibitors on rice weevil Sitophilus oryzae. Abstract. Nat. Seminar, TNAU Coimbatore, June 21-23, 1984.44.

Rana, B.S. 1983. Studies on the effectiveness of some insecticides against insect pests of bhindi (Abelmoschus esculentus L.) Moench. M.Sc. Ag. Thesis submitted to M.L.S. University, Udaipur, India.

Reed, T. and Bass M.H. 1980. Larval and post larval effect of diflubenzuron on the soybean looper. J. econ. ent. 73(2):332-38.

Retnakaran, A. 1981. Toxicology and efficacy of insect growth regulators aerially applied against the spruce budworm at Hearst (1978), Wawa (1979) and the French River area (1980). Canadian Forestry Service Deptt. of Environ. Rept. FPM-X-45.

Retnakaran, A., Granett. J. and Ennis T. 1985. Insect Growth Regulators. Chapter in "Comprehensive Insect Physiology, Biochemistry and Pharmacology. Ed. Kerkut, G.A. and Gilbert L.I. Vol. 12 Insect Control. Publ. Pergamon Press. 529-601.

Retnakaran, A. 1978. Insect Growth Regulators as control agents for the Eastern spruce budworm. In "Insect Growth Regulators for Eastern spruce Budworm control". Edited by Granett. P.9-30. Maine Life Science and Agriculture Experiment Station. Misc. Rep. No.198.

Retnakaran, A., Smith, L. and Tomkins, B. 1976. Application of Dimilin effectively controls forest tent caterpillar population and afford foliage protection. Bi-monthly. Research notes 32(5):26-27.

Rishi, N.D., and Shah, K.A. 1984. Efficacy of growth regulator against Indian Gypsy moth Lymantria obfuscata Walker (Lepidoptera : Lymantridae). Abstract. III Oriental Entomology Symposium, Feb. 21-24, 1984: 111.

Rizk, G.A.M. and Radwan, H.S.A., 1974. Proc. 2nd Pest Control Conf. Alexandria Univ. Egypt.

Rizk, G.A.M. and Radwan, H.S.A. 1975. Potency and residuality of two antimoulting compounds against cotton leaf worms and bollworms. Z. Angew. Entomol. 79:136-40.

Rupes, V., Zdarek, J. and Pintersova, J. 1977. Reinvestigation of effects of diflubenzuron on the development and reproduction in susceptible and organophosphate resistant strains of housefly (Musca domestica L.) (Dipt., Muscidae). Z. Ang. Ent. 84:328-34.

Saad, A.S.A., Elewa, M.A. and Aly, N.M. 1981. Toxicological studies on Egyptian cotton leafworm Spodoptera littoralis I. Potentiation and antagonism of synthetic pyrethroid, organophosphorus and urea derivative insecticides. Med. Fac. Landouww. Rijksuniv. Gent. 46(2) 559-71.

Sahota, T.S. and Shepherd, R.F. 1975. Laboratory tests of 1-(4-chlorophenyl)-3-(2, 6 difluorobenzoyl)-urea on survival of Western hemlock looper. Canad. For Serr., Bi-monthly. Res. Notes. 31. 39.

Salama, H.S., Z.A. Motagally and Skatulla, U. 1976. On the mode of action of dimilin as a moulting inhibitor in some lepidopterous insects. Z. angew. Entomol. 80(4):396-407.

Santharam , G. Balasubramanlam, M. 1980. Note on the control of Spodoptera litura on tobbaco with a nuclear polyhydrosis virus and diflubenzuron. Indian J. Agric. Sc. 50(9):726-27.

Saradamma, K., Dale, D. Ambika Devi D. and Mohandas N. 1984. Bioefficacy of two insect growth regulators on spotted beetle Hense epilachna vigintioctopuntata on bitter gourd. III Oriental Entomology Symp. Feb. 21-24, 1984. 129-30.

Satya Narayana K. and Sukumar Kumuda 1984. Histopathological and biochemical effects of a chitin synthesis inhibitor on female red cotton bug (D. cingulatus F.) Abstracts. III Oriental Entomology Symposium, Feb. 21-24, 1984. 88.

Saxena, S.C. and Kumar V. 1981a. Effect of Difluron and Penfluron on integumentary chitin, protein and lipid of Chrotogomus trachypterus (Orthoptera : Acrididae). Ind. J. Exp. Biol. 19:669-90.

Saxena, S.C. and Kumar, V. 1981b. Blockage in chitin biosynthetic chain in the grasshopper Chrotogomus trachypterus treated with Diflubenzuron and Penfluron. Ind. J. Exp. Biol. 19:1199-1200.

Srivastava, R.C., Sharma, G.K. and Qureshi, Q.G. 1984. Testing of diflubenzuron (Dimilin) as a controlling agent for insect pests. Ann. Report., 1985. Sukhadia University, Udaipur.

Srivastava, R.P. 1970. Electrophoretic behaviour of cuticular proteins of different developmental stages of Galleria mellonella. J. Insect Physiol. 16:2345-51.

Subrammanyam, B., Rao, P.J. and Tiwari, L.D. 1980. Juvenomimetic effects of Diflubenzuron on Achaea janata Linn. Ind. J. Expt. Bio. 18(3):324-25.

Sundaramurthy, V.T. 1976. Effect of insect growth regulator on growth and differentiation of the tobacco caterpillar Spodoptera litura Fb.(Noctuidae : Lepidoptera). Phytoparasitica 4:19-20.

Sundaramurthy, V.T. 1977. Effect of an inhibitor of chitin deposition on the growth and differentiation of tobacco caterpillar Spodoptera litura Fb. (Noctuidae : Lepidoptera). J. Plant diseases and Protection. 84(10): 597-601.

Sundaramurthy, V.T. and Santhanakrishnan, K. 1979. Morphogenetic effects of diflubenzuron, an inhibitor of chitin deposition on the cocomut black headed caterpiller (Nephantis serinopa Myer). Pesticide Sci. 10:147-50.

Svaboda, J.A., Kapalins, J.N., Robins, W.E., and Thompson, M.J. 1975. Recent development in insect steroid metabolism. A. Rev. Ent. 20:205-20.

Svaboda, J.A. and Robins, W.E. 1967. Conversion of β -sito sterol to cholesterol blocked in an insect by hypocholesterolemic agents. Science, N.Y. 156, 1637-38.

Taft, H.M. and Hopkins 1975. Boll weevils : Field populations controlled by sterilizing emerging overwintered females with a TH 6040 sprayable dust. J. econ. Entmol. 68(4)

Tamaki, G. and Turner, J.E. 1974. The Zebra caterpillar on sugarbeets; control with two phenylurea compounds. J. econ. Entomol. 67:697-99.

Technical Information Bulletin 1983. An Insecticide interfering with chitin deposition - Dimilin. Duphar B.V. Netherland. 8th edition, 35.

Thangavelu, K. 1982. Ovicidal effect of diglubenzuron on ash weevil. Current Science. 51(8):439-40.

Thompson, M.J. and Robins, W.F. 1972. Azasteroids : Potent inhibitors of insect moulting and metamorphosis - Lipids. 7:553-56.

Tsutomu Negishi, Shuya Shimada and Shoji Asana. 1976. Effect of Insect growth regulators on the tobacco cutworm, Spodoptera litura, Reetal Prolapse. J. Insect Physiol. 22:1523-27.

Turnipseed, S.G., Heinrichs, E.A., DaSilva,R.F.P. and Todd, J.W. 1974. Response of soybean insects to foliar applications of a chitin synthesis inhibitor TH 6040. J. Econ. Entomol. 67:760-62.

Villavaso, E.J. 1982. Boll weevil (Coleoptera : Curculionidae). Field competitiveness of DFB fed, irradiated males. J. econ. Entomol. 75:662-64.

Vaan Daalen, J.J., Meltzer, J., Muldur, R. and Wellinga,K. 1972. Naturewissenschaften, 59:312. A selective insecticide with novel mode of action.

Watson, W.M., Khalil, F.A. Guirguis and El-Gayer,F. 1981. Lab and Field Evaluation of different insecticides and their mixture with Dimilin against Spodoptera littoralis (B.) larvae. Proc. 3rd Arab Plant Protection Conf. Tanta Univ. Faculty of Ag. Kafr EL Sheikh.

Wellinga, K., Mulder, R. and Vaan Daalen, J.J. 1973. Synthesis and laboratory evaluation of 1-(2,6-disubstituted benzoyl)-3-phenyl urea, a new class of insecticides-1-1-(2,6-dichlorobenzoyl) 3-phenyl ureas. J. agric. Food Chem. 21:348-54.

Westigard, P.H. 1979. Codling moth : Control of Pears with diflubenzuron and effects on non target pest and beneficial species. J. econ. Ent. 72(4):552-54.

~~Wigglesworth, V.B. 1965. The principles of insect physiology. London. Methuen.~~

Wigglesworth, V.B. 1965. The Principles of insect physiology. Mathuen and Co., London.

Willis, J.H. 1974. Morphogenetic action of insect hormones. A. Rev. Ent. 19:97-115.

Wright, J.E. and R.L. Harris 1976. Ovicidal activity of Thompson-Hayward TH 6040 in the stable fly and horn fly after surface contact by adults. J. econ. Entomol. 69(6):728-30.

Wyatt, G.R. 1961. The bio-chemistry of insect haemolymph. Ann. Rev. Ent. 75-102.

Yu, S.J. and Terriera, L.C. 1975. Activities of hormone metabolizing enzymes in house flies treated with some substituted urea growth regulators. Life Sci. 17: 619-25.

Yu, S.J. and Terriere, L.C. 1977. Ecdysone-metabolism by soluble enzymes from three species of Diptera and its inhibition by the insect growth regulator TH-6040. Pesticide Biochem. Physiol.7:48-55.

*******

APPENDIX I

Actual cumulative mortality of *S. litura* larvae (appendix to table 8)

| Treatments µg/larva | Number of insects treated | Mortality | | | LPI | Pupation | | Pupal mortality | | Adult emergence | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | larval | prepupal | total | | Abnormal | Normal | Among normal | Total | Abnormal | Normal |
| 5.0 | 57 | 28 | 16 | 44 | 13 | 0 | 0 | - | - | 0 | 0 |
| 1.0 | 59 | 21 | 9 | 30 | 22 | 2 | 5 | 5 | 7 | 0 | 0 |
| 0.5 | 57 | 18 | 6 | 24 | 20 | 2 | 11 | 6 | 8 | 4 | 1 |
| 0.1 | 40 | 6 | 1 | 7 | 17 | 3 | 13 | 5 | 8 | 5 | 3 |
| 0.0 (Control) | 60 | 3 | 0 | 3 | 0 | 0 | 57 | 3 | 3 | 0 | 54 |

APPENDIX II

**Actual** cumulative mortality of *E. virginicula* larvae (appendix to table 22)

| Treatments DFB µg/larva | Number of insects treated | Mortality | | | LPI | Pupation | | Pupal mortality | | Adult emergence | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Larval | prepupal | Total | | Abnormal | Normal | Among normal | Total | Abnormal | Normal |
| 5.0 | 55 | 21 | 13 | 34 | 19 | 2 | 0 | - | 2 | 0 | 0 |
| 1.0 | 58 | 16 | 6 | 22 | 25 | 5 | 6 | 4 | 9 | 2 | 0 |
| 0.5 | 59 | 13 | 5 | 18 | 26 | 5 | 11 | 6 | 11 | 5 | 2 |
| 0.1 | 54 | 5 | 3 | 8 | 24 | 4 | 18 | 6 | 10 | 7 | 5 |
| 0.0 (Control) | 60 | 1 | 0 | 1 | 0 | 0 | 59 | 1 | 1 | 0 | 58 |