STUDIES ON THE EFFECTS OF JUVENILE HORMONE ANALOGUES ON THE DEVELOPMENT OF *Spodoptera mauritia* (BOISDUVAL) (LEPIDOPTERA NOCTUIDAE)

BY

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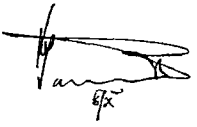

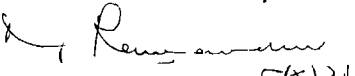

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C E R T I F I C A T E

Certified that this thesis is a record of research work done independently by Sri.V.S Krishnadas under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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

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C O N T E N T S

INTRODUCTION

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The success of modern agriculture depends mostly on efficient use of pest control agents. Broad-spectrum insecticides, the most popular and currently used pest control agents, are not without side effects on the environment and its biota. Hence large-scale research to find out alternative methods was initiated in different parts of the world. A novel approach which has emerged from the endeavours is the use of analogues and antagonists of insect growth regulators such as juvenile hormones and ecdysones to control pest populations. If this approach proves feasible, the insect hormonal analogues can replace insecticides in insect control operations. Hopefully these newer tools could become integral part of integrated control schedules that we are establishing to aid us in our battle against insect pests.

The pioneering studies of Wigglesworth in the mid-1930's showed that moulting and metamorphosis of insects were regulated by hormones. The possibility that juvenile hormones and their synthetic analogues had

practical use in insect management was first recognised by Williams in 1956. Following his discovery that the abdomen of the adult male Cecropia moth was a rich source of juvenile hormone, he proposed juvenile hormone analogues (JH) as powerful "third generation" insect control agents. In subsequent years, a remarkable proliferation of scientific discoveries occurred in this field.

The present study is undertaken to find out the morphogenetic effects of two juvenile hormone analogues on Spodoptera mauritia, one of the important pests of paddy in Kerala.

REVIEW OF LITERATURE

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The important research works on juvenile hormones (JH) and its analogues (JHA) are reviewed in the following heads.

I. History of juvenile hormone and its analogues.

II. Morphogenetic effects.

i) Effects on egg (ovicidal action)

ii) Effects on larva

iii) Effects on pupa

iv) Effects on adult female

v) Effects on adult male.

III. Biochemical effects.

IV. Scope of juvenile hormone analogues in insect control

I. History of juvenile hormone and its analogues

Pioneering studies on insect hormones were done by Wigglesworth (1930) who showed that moulting and metamorphosis of Rhodnius prolixus were regulated by hormones. In 1934, he further observed that juvenile hormone either inhibited or prevented metamorphosis at certain critical periods in the insect life cycle. In a series of classical experiments using decapitated bugs, parabioassays and transplanted organs,

he demonstrated that the juvenile hormone was secreted by the corpora allata and the hormone from one species would also affect other species.

Williams (1956) was the first to recognize the possibility of using JH and its analogues for pest control. He also extracted crude JH from the abdominal tips of the male Cecropia moth, Hyalophora cecropia. Following this discovery he proposed JH-active substances as powerful "third generation" insect control agents.

Slama and Williams (1965) reported the extraordinary morphogenetic activity of the "Paper factor" on linden bug, Pyrrhocoris apterus.

Roller et al.(1965) first reported isolation of the hormone and later in the same year, Williams and Law isolated a highly active fraction from "Cecropia oil". Bowers et al.(1966) identified the "Paper factor" as the methyl ester of todomatuic acid or 'juvabione'.

The sensitivity of early embryonic stages of insects to exogenous JH application was first described by Slama and Williams (1966). Bowers and Blickenstaff (1966) were able to terminate diapause in alfalfa weevil by treating the adults

topically with synthetic JH. Connin et al. (1967) also could demonstrate a similar effect of JH on cereal leaf beetle, Oulema melanopa.

Roller and coworkers (1967) scored a major breakthrough by identifying the major juvenile hormone in Hyalophora cecropia as methyl trans, trans, cis-10, 11-epoxy-7-ethyl-3, 11-dimethyl-2, 6-tridecadienoate, with a molecular weight of 294 and empirical formula $C_{18} H_{30} O_3$. Meyer et al (1968 identified a lower homologue (7-methyl) of the above compound. Thereafter a variety of hormones and their analogues were identified and proved to exert on insects varying degrees of morphogenetic, gametogenetic and diapause disrupting activity.

Slama (1969) suggested that plants could be used as source of materials with insect hormone activity. He reviewed the current knowledge on the occurrence, composition and effects on survival and reproduction of insects to the analogues present in the plants.

Bagley and Bauernfeind (1971) conducted exploratory field tests to find out JHA-induced effects in natural population of alfalfa weevil. He emphasised that there were

serious potential problems in the development of substances with JH-activity suitable for application in the field for insect control. In another field test Chamberlain and Hopkins (1971) demonstrated that biting lice (Mallophaga) on Angora goats could be controlled using synthetic JH.

McGovern et al.(1971) reported JH activity of acetals as a fumigant to yellow mealworm.

Wright (1972) tried hormones for the control of livestock arthropods and reported the effectiveness of three JHA for the control of stable flies.

Redfern et al. (1972) reported aziridines as potentiator of JH activity in tests on yellow mealworm and large milkweed bug. The aziridines synergised the hormonal activity of the hormone mixture. The effect was maximum at a 2:1 ratio of synergist to hormone mixture.

Harris et al.(1973) suggested that hornfly and stable fly could be controlled by inhibiting their development. Development of the flies was inhibited in the faeces of cattle treated orally with JHA and further more, no signs of chemical toxicity were observed in the cattle.

Strong and Dickman (1973) made a comparative study on the effectiveness of 15 insect growth regulators against several pests of stored products. Compounds like dienoates, 'altozar' and 'altosid' were found to be promising.

Mulla et al.(1974)tried insect growth regulators for the control of aquatic midges. They reported that 'altosid' was the most effective compound tested against both resistant and susceptible Chironomus sp. to organophosphorus insecticides.

Some speculations have been made recently that JH was directly involved in honeybee cast differentiation (Wirtz and Beetsma, 1972; Wirtz, 1973) These were mostly based on the claim that treatment of the larvae with a synthetic JH (Biojune-100) led to the development of more queen-like adults under in vivo conditions. However, more recently Hrdy (1973) has reported that feeding of honeybee colonies with JH analogues led to the development of black brood. Direct feeding and spraying of the foraging plots harmfully influenced brood in its early developmental stages and workers removed such a brood. Rembold et al.(1974) undertook a work to study the effect of JH on caste differentiation in honeybee larvae both under in vivo and in vitro conditions. Their results suggested a disturbed development in JH-treated

larvae and did not support a direct role for JH in honeybee caste differentiation.

II. Morphogenetic effects

1) Effects on egg

Slama and Williams (1966) showed that JH disrupted insect embryogenesis. They found that application of JH to young embryo interfered with their development and blocked the metamrophosis of the embryo to the larva. They also demonstrated that application of "juvabione" either to the female bug, Pyrrhocoris apterus, or to the freshly laid eggs, prevented hatching. The same effect has also been found by the application of JH and its analogues to the female or egg of wild American silk moth, Hyalophora sp. and Chinese oak silk moth, Antheraea pernyi (Riddiford and Williams, 1967).

Williams and Lawrence (1970) found that treatment of some insect eggs with synthetic analogue of JH permitted hatching and the resultant larvae to grow normally but inhibited metamorphosis, so that they developed into either intermediates or supernumerary instars. The authors described the fact using Oncopeltus fasciatus as test insect,

and suggested that persistence of the applied hormone might lead to an excess at metamorphosis.

Retnakaran and Grisdale (1970) studied the ovicidal effect of the so called hydrochlorination mixture of Law et al , which has JH-activity, on spruce budworm, Choristoneura fumiferana. Topical application of hormone to the eggs killed the embryos, though development to the black-head stage was seen.

Williams et al.(1970) tested JHA's as potential ovicides to different coleopteran eggs Three methylenedioxy phenoxy-terpenoid ethers with high morphogenetic activity on a variety of insects, prevented egg hatch when they were applied to eggs of Mexican been beetle Epilachna varivestis and cigarette beetle Lasioderma serricorne Eggs of E. varivestis were very sensitive to these synthetic hormones during the first half of the egg stage. Hatch was reduced by 98 per cent when one-day old eggs were exposed to the vapours from 1 /ug of the most potent hormone or dipped for 5 seconds in a solution of 10 ppm

White (1971) in his studies exposed the fact that the degree of activity of corpora allata (and presumably the concentration of JH) in female aphid influenced the

development of wing buds in embryo and by this means, controlled aphid polymorphism.

Riddiford (1971) briefly reviewed the potential role of JH as an ovicide. When cecropia eggs were treated with JH immediately after oviposition, hatching was blocked and the unhatched embryos had not completed blastokinesis. Most of the individuals hatched when JH was applied after blastokinesis, larval life proceded normally but metamorphosis prevented.

ii) Effects on larva

Masner (1969) reported the effect of substances with JH-activity on morphogenesis and on the function of gonads in Pyrrhocoris apterus. Topical application of JH to the fifth instar nymphs resulted into a supernumerary instar when applied within two days after fourth moulting. Treatment to fifth instar nymphs which were more than two days after the moult, frequently resulted in adultoids.

Mehrotra et al. (1969) described the effect of JH-like compounds on desert locust Schistocerca gregaria Injection of farnesyl diethylamine and farnesyl methyl

ether at 0.05-0.4 µl/insect in methanol when tested on fourth and fifth instar nymphs, it was found that both the compounds were toxic, but the former gave about 80 per cent mortality with a high percentage of deformed adults.

Wellington (1969) tested the effect of three hormonal mimics on mortality, metamorphosis and reproduction of Western tent caterpillar Malacosoma californicum. Topical treatment of late fourth and early fifth instar larvae resulted in increased mortality due to structural abnormalities and moulting difficulties.

Effect of JH on adult differentiation of Drosophila melanogaster was claimed by Ashburner (1970). Topical application of principal JH of Cecropia moth to third instar larvae of Drosophila prevented adult emergence or limited the development of specialized sclerites of the external genitalia and caused abnormalities in the bristles and hairs of abdomen.

Retnakaran and Grisdale (1970) tested the hydrochlorination reaction mixture on the sixth instar larvae of Choristoneura fumiferana and observed its juvenilizing effect.

Babu and Slama (1971) investigated the effectiveness of a JHA's against the red cotton bug. They found that a

concentration of 1.5 /ug per 5 ml per plant was sufficient to get profound JH activity, manifested by the production of supernumerary nymphs or half nymphal adultoids.

Bransby-Williams (1971) reported that topical application of ethyl farnesoate dihydrochloride at a dose of 0.08 /ug to newly moulted fifth instar cotton stainer nymphs, produced 50 per cent inhibition of adult characters Juvenilizing effect of farnesol to specimens of Aphis craccivora was reported by Tashev and Danganova (1971) Same effect in apterous forms of been aphid was observed by Hangartner et al.(1971)

Richmond (1972) tested several JHA's on sixth instar larvae of western spruce budworm, Choristoneura fumiferana High dosages resulted in increasing degree of morphogenetic juvenilization Some of the more potent compounds caused supernumerary moults also. Hormonal induction of supernumerary instars in spruce budworms was also reported by Retnakaran (1973). Application of ZR-515, a potent JH, to the early sixth instar resulted in the formation of supernumerary larval instar. Treatment of late larvae, when many structures committed to differentiate towards pupa, resulted in a larval-pupal mosaic.

Kuhr and Cleere (1973) screened certain synthetic JHA's as toxicants against the aphids, Acyrthosiphum pisum and Amphorophora agathonica. First and second instar nymphs and adults of pea aphid and raspberry aphid when exposed to plant material, dipped in 0.1% hormone indicated that certain compounds were very toxic They further observed that exposure to lower levels of hormone did not always produce direct toxic results, but yielded a wide range of morphological abnormalities and considerably altered reproduction. Singh (1974) tested the effects of ZR-515, a JHA, on development of mustard aphid, Lipaphis erysimi. Using different concentrations of hormone sprayed on plants, he was able to obtain upto 60% mortality in released first instar aphids and 35 per cent deformity in development in the surviving individuals.

Cawich et al.(1974) reported that topical application of JH mimics on larvae of pink bollworm, Pectinophora gossypiella caused retention of larval characters in the resulting pupa at lower doses and additional

prepupal and larval stages at higher dosages.

Reissig and Kamm (1974) observed prevention of development of Draeculacephala crassicornis, by the application of JHA. In laboratory and field tests they found that foliage sprays of JHA were effective in preventing emergence of nymphs confined on treated foliage.

Neal and Hower (1974) exposed the fact that when synthetic JHA was applied on fourth instar Hypera postica, it significantly increased the mean weight of normal adults and subsequently increased the mortality of adultoid weevils.

iii) Effects on pupa

Bluemenfield and Schneiderman (1968) observed that injection of JH into the pupae of the silk moth, Antheraea polyphemus caused the pupa to undergo a prepupal-pupal moult instead of a pupal-adult moult. Juvenilizing effects in Western tent caterpillar was also reported by Wellington (1970). Larvae and new pupae when received JH, experienced moulting difficulties and structural abnormalities. Many of the abnormal individuals produced by red cidar extract treatment, died early in the pupal stage.

Mansingh et al.(1970) tested juvenile hormone activity of wood and bark extract of some forest trees, (balsam fir, red cidar etc) on pupae of Galleria mellonella. JH was applied on a wound puncture made in the dorsal surface of thorax. All extracts showed considerably juvenilizing effect on treated pupae.

Wright (1970) reported pupal-adult intermediates in stablefly, Stomoxys calcitrans. Zero-hour old pupae were the most sensitive to JH while the same concentration when applied to 96 hours old or more old pupae had no effect at all.

Sonnet et al.(1971)found the JH activity of citronellyl carbamates and related esters to pupae of yellow mealworm, Tenebrio molitor. Critchley and Campion (1971) reported that treatment of T.molitor pupae with JH or JHA resulted in an arrest of pupal development and produced intermediary forms between pupa and adult. The juvenilizing effect was greatly influenced by the time of application.

Bhatnagar Thomas (1972) showed that young pupae of Trogoderma granarium were highly susceptible to methyl farnesoate dihydrochloride. Metamorphosis was completely inhibited at 300 ppm where as in the case of older pupae more than 50 per cent metamorphosed into normal adults even at 600 ppm.

Wright and Spates (1972) tested JH activity of 62 compounds against pupae of Stomoxys calcitrans for morphogenetic activity and found that a series of six related JHA's had got morphogenetic activity at rates as low as 0.001 µg per pupa.

Redfern et al.(1972) reported synergistic action of aziridines with JH and it was demonstrated on pupae of yellow mealworm and large milkweed bug.

Metwally et al.(1972) administered JH mimics to pupae of Trogoderma granarium in pikogram amounts and observed severe defects in the ovaries and reduction in fecundity of emerging adults.

Outram (1972) reported the effects of synthetic JH on adult emergence and reproduction, in spruce budworm. Many treated pupae contained adults that did not emerge, though a few were alive.

iv) Effects on adult female

Slama and Williams (1966) were the first to show that application of JHA, 'juvabione'to the female Pyrrhocoris apterus would prevent hatching of eggs. The same effect was

reported by Riddiford (1967) in female wild silk moth, Hyalophora sp. and subsequently on many other insect species by a number of workers.

White and Lamb (1968) tested the effect of synthetic JH on adult aphids and their progenies. 0.7-2 µg per adult aphid reduced the survival of both adults and their young ones. Besides reduced fecundity and increased percentage of apterous forms among the nymphs were also resulted.

Masner and co-workers (1968) first observed that sterility was induced to the adult female Pyrrhocoris by the application of juvenile hormone analogues. Morgan and La. Breque (1971) have also found that JH-like substances affected ferility in houseflies. Sterility was occured when adults were treated with JH-like substances. Sterility in female cotton stainer treated with dichlorofarnesenic acid was reported by Homberger et al.(1971). Females treated with JH 10^{-5} g per insect (6-86 hours after emergence) and mated with normal adult males failed to lay viable eggs. Ninetynine per cent of the laid eggs failed to hatch and further it reduced the adult longevity by about 25 per cent and number of eggs laid by the female to 16-30 per cent. Williams (1971)

also reported JHA induced sterility in cotton stainer, Dysdercus cardinalis. Topical application of 100 /ug ethyl farnesoate dihydrochloride to individual adults resulted in complete infertility in females.

Critchley and Campion (1971) found out that topical application of JHA to the adult female Tenebrio molitor at a dose of 5 /ug induced complete sterility. Further they noted the recovery of the fertility by the time fourth and fifth batches of eggs were laid.

Riddiford (1972) made a detailed investigation on the effects of JH on different female insects. His observations revealed that effectiveness of a given dose of a specific compound was dependent upon the time of application, relative to the time of oviposition.

v) Effects on adult male

Masner et al. (1968) exposed the fact that JH can induce sexually spread sterility in insects. Masner (1970) observed that JH topically applied to males when allowed to mate with normal females of Pyrrhocoris sp. the hormones transmitted from treated males to mated females were in doses high enough to cause permanent sterility in females.

The effect was reinforced by repeated mating of females with treated males.

Homberger et al.(1971) found that when treated cotton stainer adult males were allowed to mate with normal females, fertility in females was affected. 95 per cent of the eggs laid by such females, failed to hatch. In normal males that had paired with treated females paired again with normal females induced 60% sterility in the mated female. Partial sterility in JHA treated Dysdercus cardinalis males was reported by Williams (1971). Critchley and Campion (1971) reported JH induced male sterility in Dysdercus fasciatus. Topical application of methyl farnesoate hydrochloride at a dose of 50/µg induced male sterility without any adverse effect on longevity or mating competitiveness.

III. Biochemical effects

Bluemenfield and Schneiderman (1968) observed the effect of JH on synthesis and accumulation of blood protein in silk moth, Antheraea polyphemus. When JH was injected to the female pupa, the synthesis and release of protein was not affected but the oocytes did not accumulate yolk protein,

thus resulting in the concentration of yolk protein in the blood.

Riddiford (1971) suggested the possible mechanism of juvenile hormone on embryogenesis. Application of JH caused blocking of embryonic development in the blastoderm stage, just prior to the germ band formation. The transition from blastoderm to germ band, marks the switch-over from informations taken from the maternal genome to that taken from the zygotic genome and it is this transition that can be blocked by the application of JH to the female during oogenesis.

Siew and Gilbert (1971) analysed the effect of moulting hormone and JH on nuclear RNA synthesis of the corpora allata and prothoracic gland in saturnid pupae. They found that moulting hormone stimulates nuclear RNA synthesis followed by activation of corpora allata three hours later. JH acts directly upon corpora allata and hence application of JH to insects that have just initiated adult development results in drastic decrease in nuclear RNA synthesis.

Slade and Zibitt (1971) made a detailed investigation on the metabolism of cecropia juvenile hormone in

insects and mammals Using two C_{14} labelled hormones *in vivo* and *in vitro* studies with *Manduca sexta* (tobacco hornworm) they have shown that the mechanism of inactivation involved hydrolysis of the ester group followed by hydration of the epoxide.

Schneiderman (1971 a) suggested that in insects ecdysone may act on cells in various phases of cell cycle whereas JH affects most cells prior to or during the replication of DNA. Ilan *et al* (1971) reported the regulation of messenger RNA translation mediated by juvenile hormone. The control of gene expression exercised by JH might be at the translational level which involved the appearance of a new transfer RNA and its activating enzyme.

Gill *et al*. (1971) conducted some preliminary chromatographic studies on the metabolites and photo-decomposition products of juvenoid (JHA) 1-(4-ethylphenoxy) 6, 7-epoxy-3, 7-eimethyl-2-octene. The degradation chemistry studies involved incubation of juvenoid with rat liver enzymes, administration to rats and locusts to obtain excreted metabolities and exposure to sunlight.

IV. Scope of JHA's in insect control

Schneiderman (1971) briefly reviewed the potential insecticidal action of JH and its analogues. According him application of JH to larvae or pupae at a time when some cells have ceased DNA replication and therefore lost their sensitivity to JH but others have not, leads to the production of intermediates. This fact along with JH-induced sterility and its ovicidal action brightens the scope of JHA's as insect control agents. Ellis (1970) considered the new scope for hormone mimics as pesticides. Schneiderman (1971 b) suggested strategy of controlling insect pests with growth regulators. Effective doses of JHA from the point of view of insect control was studied by Varjas (1971).

Wilde (1972) dealt with the present status of hormonal insect control. According to him interference with hormonal balance in insects, though never likely to offer a complete solution to the problem of insect control, is of considerable value as an adjunct to other control measures.

MATERIALS AND METHODS

MATERIALS AND METHODS

Juvenile hormone analogues

'Altosid'- a synthetic juvenile hormone analogue- was a generous gift received from M/s Zoecon Corporation, U S A. The other JHA used in the present studies, farnesyl methyl ether (FME) was a product of M/s Hoffman-La Roche, Switzerland. The hormones were dissolved in acetone or acetone-water and were stored in a refrigerator at 4°C till used.

Preparation of juvenile hormone concentrations

i) Solutions prepared in acetone

4 mg of JHA in 0.2 ml acetone = 200 µg/µl (A)

0.1 ml (A)+ 0.1 ml acetone = 100 µg/µl (B)

0.1 ml (B)+ 0.1 ml acetone = 50 µg/µl (C)

0.1 ml (C)+ 0.1 ml acetone = 25 µg/µl (D)

0.1 ml (D)+ 0.1 ml acetone = 12.5 µg/µl (E)

ii) Solutions prepared in 1% acetone

1 ml acetone + 99 ml distilled water = 1% acetone water.

0.01 gm (10 µl) JHA+ 10 ml acetone water = 0.1% (A)

5 ml (A) + 5 ml acetone water . .. = 0.05%(B)

5 ml (B) + 5 ml acetone water . .. =0 025%(C)

5 ml (C) + 5 ml acetone water .. .. =0 0125% (D)

Glassware and other articles

The glasswares used in the experiment were glass troughs (26 cm diameter and 10 cm height), glass chimneys (5 cm. diameter and 15 cm height), specimen tubes (2 5 cm diameter and 7 cm. height), glass vials (1 cm diameter and 5 cm. height) and micropipette The other articles included camel-hair brush, cotton plugs, muslin cloth for covering the glassware and filter paper

Mass rearing of the test insect

One-day old egg masses, freshly emerged final instar larvae, freshly moulted pupae and just-emerged adult moths of Spodoptera mauritia were used for treatments.

The original culture of the test insect was started from the egg masses collected from the field and those laid by the female moths collected from fluorescent lamps of the college hostel Leaves of a grass weed, Ischaemum aristatum, were used as the food material for caterpillars throughout the rearing period The weed was collected from and around the paddy fields of the college farm.

Two-days old egg masses were transferred to fresh grass leaves kept inside glass chimneys, closed at the both

ends with muslin cloth. The grass leaves were kept turgid by wrapping their cut ends with cotton soaked in water. The leaves generally remained turgid and green for 2 to 3 days. The larvae were transferred to fresh leaves after 3 days of emergence and reared on them for a week. Thereafter the larvae in convenient numbers, were transferred to clean glass troughs. Fresh grass leaves were supplied as and when found necessary. Unhealthy and disease-suspected larvae were culled promptly. The glassware used in the experiment was sterilized frequently.

Whenever the caterpillars reached the sixth instar stage, clean sand (3 to 5 cm. thickness) was provided in the trough for pupation. A circular paper was placed above the soil and the grass leaves and the caterpillars were kept on the paper. Cleaning was done by removing the paper. After pupation, the troughs were kept without any disturbance.

After adult emergence, three to four pairs of moths were kept in each chimney closed both sides with muslin cloth. Adult moths were fed with honey or sucrose solution soaked

in cotton wool. Cotton wool pads were replaced daily in order to prevent fungal growth on them. Female moths laid eggs on the muslin cloth or on the paper folds provided. The eggs were collected using a camel-hair brush and were used for subsequent rearing.

Treatment of eggs with JHA's

One-day old eggs were used for the studies. Three series of experiments were conducted to study the effect of JHA's on the hatchability of eggs.

a) Treating eggs with JHA's diluted in pure acetone

For each treatment, 25 eggs were used. The treatments were 12.5, 25.0, 50.0, 100.0 and 200.0 μg/μl of the hormonal analogues. A batch of 25 eggs was used as control (with no treatment) and another control lot of eggs was treated only with pure acetone. The eggs were placed on clean petridishes and the chemicals were applied. They were then kept under fan for evaporation of the solvent. The eggs were then transferred to clean specimen tubes for easy observation of larval emergence.

b) Treating eggs for observing head capsule development

All the treatments were same as in the previous experiment. Only 10 eggs were used for each treatment.

The development of head capsule was observed under a binocular microscope, three days after the treatment.

c) Treating eggs with JHA's diluted with 1% acetone water

The high egg mortality caused by the solvent in the earlier experiments had prompted to use 1% acetone solution in water for this experiment. The concentrations of the JHA's were also reduced to get a gradation of effects in treated eggs. The concentrations of JHA's used in this experiment were 0.0125, 0.025, 0.05 and 0.1%. Two lots of eggs, one treated with acetone water and another with no treatment, were kept as controls. The mortality of eggs was recorded after the experimental period.

Larval treatment with JHA's

Less than one-day old last instar larvae were used in these studies. Fixed number of larvae were topically treated on the abdominal terga with technical as well as solutions of JHA's. The treatments using 'Altosid' were 1 µl, 3 µl and 5 µl of technical material and 5 µg per µl and 10 µg per µl of solutions in pure acetone. As the preliminary studies have indicated that FME was less active

in producing morphogenetic changes on larvae, only technical material of this compound was used. The treated larvae were then transferred to glass chimney placed over petridish. Soil was provided on the petridish for the larvae to pupate. The top of the chimney was closed with muslin cloth Every day the larvae were provided with fresh grass leaves for feeding. The effects observed were change in colour, consistency of the excreta, morphological changes on the prolegs and thoracic legs, larval mortality, moulting into supernumerary instars, larval duration and any other abnormalities.

Treatment of pupae with JHA's

Freshly moulted pupae (less than 24 hours old) were used for the treatment. The pupae were topically applied on their dorsal abdominal tips with hormones (both technical material and solutions). The concentrations of 'Altosid' in solution were 12.5, 25.0, 50.0, 100.0 and 200 0 /ug per/ul. The technical materials of both the hormonal analogues were used at volumes of 3 /ul and 5 /ul for each test pupa. After treatment, the solvent was allowed to evaporate by placing under a fan The treated pupae were then transferred to glass

chimneys and kept undisturbed for observations. The observations included change in colour, abnormalities during adult emergence, pupal period and any other morphogenetic abnormalities.

Treatment of adult female moths with JHA's

Freshly emerged female moths (less than one day old) were used in this experiment. The moths were inactivated by keeping in the freezer chest of the refrigerator for 10 minutes. After inactivation the female moths were topically applied on their dorsal abdomen with solution of JHA's in pure acetone. The concentration of the solution for both the hormonal compounds was 10 λg per λl. For each treatment, two pairs of moths were used A control group without treatment and another treated only with pure acetone were kept. Treated pairs were then released into clean glass chimneys. Honey solution was provided as food. The adult longevity, time for laying the first batch of eggs after treatment, number of eggs laid per female moth, number of larvae emerged and the latent mortality of caterpillars caused by the hormonal effect were observed.

RESULTS

RESULTS

I. Treatment of eggs with juvenile hormone analogues

a) diluted in pure acetone

Data presented in Table 1, give the percentages of eggs died due to the application of 'Altosid' diluted in pure acetone. It is clear from the table that the solvent acetone itself acted as a powerful ovicide. The egg

Table 1

Mortality of eggs of Spodoptera mauritia caused by the treatment of 'Altosid' diluted in pure acetone

Treatment	No.of eggs treated	Eggs hatched	Eggs died	Mortality of egg (%)
Control(no treatment)	25	17	8	32
Control (acetone)	25	7	18	72
12.5 λug/λul.	25	0	25	100
25 λug/λul	25	0	25	100
50 λug/λul	25	0	25	100
100 λug/λul	25	0	25	100
200 λug/λul	25	0	25	100

mortality caused by acetone application was as high as 72 per cent. All the concentrations of JHA's used in this experiment produced complete lethality in treated eggs The natural mortality of eggs in control was 32 per cent.

The results of the experiment with FME are presented in Table 2.

Table 2

Mortality of eggs of Spodoptera mauritia caused by the treatment of farnesyl methyl ether diluted in pure acetone.

Treatment	No.of eggs treated	Eggs hatched	Eggs died	Mortality of eggs (%)
Control (no treatment)	25	18	7	28
Acetone	25	9	16	64
12.5 λug/λul	25	3	22	88
25 λug/λul	25	1	24	96
50 λug/λul	25	0	25	100
100 λug/λul	25	0	25	100
200 λug/λul	25	0	25	100

It is evident from the data that FME was less lethal than 'Altosid' to the eggs of S. mauritia. The lower concentrations of FME ie., 12.5 and 25.0 µg per µl, caused only 88 and 96 per cent mortality of the eggs while the same concentrations of 'Altosid' produced cent per cent mortality. The rest of the three higher concentrations of FME caused total lethality to the eggs. The ovicidal effect of acetone was confirmed in this experiment as the egg mortality caused by the solvent was 64 per cent.

b) on the development of head capsule in embryos

The purpose of conducting this experiment was to locate the approximate stage of embryo at which the exogenous

Table 3
Number of eggs showing development of head capsule after the treatment with 'Altosid'.

Treatment	No. of eggs treated	Number of eggs showed head capsule development after 3 days	% embryos dead before head capsule formation
Control	10	10	0
Acetone	10	6	40
12.5 µg/ µl	10	5	50
25 µg/ µl	10	5	50
50 µg/ µl	10	2	80
100 µg/ µl	10	2	80
200 µg/ µl	10	0	100

juvenile hormones became lethal. The results obtained on the application of'Altosid' are presented in Table 3.

It is clear from the table that the number of embryos with blocked head capsule development increases progressively with the increase in concentration of the JHA. The control treatment with acetone also inhibited the embryonic development before the stage of head-capsule formation in four out of ten treated eggs.

The number of eggs showing development of head capsule 3 days after the application of FME is tabulated in Table 4.

Table 4
Number of eggs showing head capsule formation after treating with various doses of FME

Treatment	No.of eggs treated	No.of eggs showing head head capsule development	% embryos dead before head capsule formation
Control	10	10	0
Acetone	10	8	20
12.5 µg/ µl	10	6	40
25 µg/ µl	10	5	50
50 µg/ µl	10	5	50
100 µg/ µl	10	3	70
200 µg/ µl	10	1	90

As in the case of 'Altosid', a progressive gradation in the inhibition of embryonic development was discernible with FME, with an increase in the concentration of the juvenile hormone analogue. The percentage of embryos which died by the application of FME ranged from 40 to 90 There was no embryonic mortality in untreated control while in 20 per cent of the acetone-treated embryos the head capsule formation was impaired.

c) diluted with acetone water

The high mortality of eggs caused by acetone and the JHA's in the earlier experiments necessitated diluting down

Table 5

Mortality of eggs of Spodoptera mauritia caused by 'Altosid' diluted in1% acetone

Treatment	No.of eggs treated	No.of eggs hatched	No.of eggs died	Mortality of eggs (%)
Control (no treatment)	25	18	7	28
1% acetone	25	17	8	32
0.0125 per cent	25	15	10	40
0 025 per cent	25	11	14	56
0.05 per cent	25	10	15	60
0.1 per cent	25	9	16	64

the concentrations of the solvent as well as the juvenile hormone analogues. The solvent concentration used in this experiment was 1% acetone in distilled water. The ovicidal action of 'Altosid' expressed in terms of percentage of egg mortality is presented in Table 5. As the concentration of the hormone analogue increased, there was a progressive increase in the percentage mortality of eggs. The values of the effect ranged between 40 and 60 per cent. There was not much difference in the control (no treatment) and in the application of 1% acetone solution as the egg mortality percentages were 28 and 32 respectively.

Table 6
Mortality of eggs of Spodoptera mauritia produced by the application of various concentrations of FME diluted in 1% acetone.

Treatment	No of eggs treated	No.of eggs hatched	No of eggs died	Mortality of eggs (%)
Control(no treatment)	25	19	6	24
1% acetone	25	17	8	32
0.0125 per cent	25	16	9	36
0.025 per cent	25	13	12	48
0 05 per cent	25	14	11	44
0.1 per cent	25	12	13	52

The results on the ovicidal action of FME diluted in 1% acetone solution are given in Table 6. A perusal of the data clearly indicates that the percentages of egg mortality steadily increased with rise in the dosages of the hormone. The highest mortality (52 per cent) was recorded with 0.1 per cent FME. The difference in the egg mortalities in control (no treatment) and 1% acetone solution was only marginal.

II. Treatment of final instar larvae of Spodoptera mauritia with juvenile hormone analogues

a) With 'Altosid' (technical material)

The average duration of larval period, number of larvae moulted to supernumerary instar, duration of supernumerary stadium and remarks on the fate of supernumerary instar larvae are given in Table 7. One out of two larvae treated with each concentration of the hormonal analogue, moulted into a supernumerary instar There was not much difference in the average larval duration between those of control and the treated, except in those treated with 3 /ul of the juvenile hormone analogue. The larval period of caterpillars treated with 3/ul were 9 days in contrast to 7 in control

Table 7

Duration of larval period and details of supernumerary, instars of larvae treated with 'Altosid' technical.

Treatment	No.of larvae treated	Average duration of larval period (days)	No.of larvae moulted to supernumerary instar	Duration of supernumerary stadium (days)	Remarks on supernumerary instar
Control	2	7.0	Nil	..	.
1 µl	2	7.0	1	7	Died as larval-pupal mosaic
3 µl	2	9.0	1	6	Died as larva, shrunken body
5 µl	2	7.5	1	7	Died as pupa

The consistency of excreta of treated larva appeared to be drier than that of the control larvae. Determination of water content in the samples of excreta revealed that excreta of treated larvae in the different doses taken together contained 70.5 per cent water while that of control was having a higher water content of 73.1 per cent Pinkish swellings were observed on the thoracic and abdominal legs of the treated larvae No other abnormalities on treated larvae were recorded.

The supernumerary instar larvae were considerably larger than the normal sixth instar larvae, and although they fed on the grass leaves they were less active than the sixths The integument of the supernumerary larvae appeared abnormal and could be best described as leathery. None of the supernumerary instar larvae developed into adults.

All the larvae in the control had a normal adult emergence. The average pupal period in control was 10 days. The caterpillars in treatment, which did not moult into a supernumerary instar, passed on to the pupal stage normally. But the pupal period (14 days) was significantly longer than that in control. The caterpillar which received 5 µl of the hormone analogue, died during the larval stage itself The adult moths which emerged from the treated larvae remained sluggish throughout the imaginal period.

b) With 'Altosid' diluted in acetone

The fate of the larvae treated with 'Altosid' solutions in acetone is presented in Table 8. The hormonal treatment derranged the normal moulting and metamorphosis. There was a progressive prolongation of the larval period with increase in the hormonal concentration The larvae

Table 8
Duration of larval period and details of supernumerary instars of larvae treated with 'Altosid' diluted in acetone

Treatment	No.of larvae treated	Average duration of larval period (days)	No.of larvae moulted into supernumerary instar	Stadium of supernumerary instar	Remarks on supernumerary instar
Control	2	6.0	Nil	.	.
Acetone	2	7.0	Nil	.	.
5 /ug/ /ul	2	7 8	Nil	.	.
10 /ug/ /ul	2	9.0	2	7 days	Moribund followed by death

which received a dose of 10 /ug per /ul moulted into a supernumerary seventh instar . All the rest of the caterpillars which received treatment failed to pupate normally and died due to unsuccessful moulting During the last days, the larvae ceased feeding and their bodies began to shrink. The cadaver was extremely shrunken The colour of the treated larvae was darkc on the dorsal side and pinkish on the ventral region. The consistency of excreta appeared to be drier than that in the control Slight swelling of thoracic and abdominal legs of the treated larvae was observed

c) With farnesyl methyl ether

Results based on the effects of FME on the last instar larvae of S. mauritia are presented in Table 9 The average larval period of the treated larvae ranged between 6.2 and 8.0 days Only one caterpillar which received a hormonal dose of 5 µl moulted into a supernumerary instar. After 15 days, the supernumerary larva died without undergoing pupation. One out of the three larvae each of which was treated with 1 µl of FME changed into larval-pupal

Table 9

Duration of larval period and details of supernumerary instars of larvae treated with FME.

Treatment	No of larvae treated	Average duration of larval period (days)	Number of larvae moulted to supernumerary instar	Duration of supernumerary stadium	Remarks on supernumerary instar
Control	3	6.2	Nil		..
1 µl FME	3	7.0	Nil	..	
3 µl FME	3	6.2	Nil	.	.
5 µl FME	3	8.0	1	15 days	Died as larvae Highly Shrunken cadaver

mosaic and died. One larva of the 3 µl treatment lot and all the three larvae of 5 µl treatment suffered mortality during their larval stage itself All the rest developed into normal adults.

As observed in the earlier experiments, the colour of the treated larvae was dark on their dorsum and pinkish on their ventral side. The consistency of excreta of the treated larvae appeared to be drier than that of the control Swellings on the thoracic and abdominal legs of the treated larvae were observed in this experiment also.

III. Treatment of pupae of Spodoptera mauritia with juvenile hormone analogues

a) With 'Altosid' diluted in acetone

The data on the effect of 'Altosid' diluted in acetone on the pupae of S.mauritia are presented in Table 10. It could be seen that the hormonal application in all the concentrations tried was lethal to freshly moulted pupae. Stages of development at the time of death were assessed by dissecting out the dead pupae. A perusal of the data indicated that there was no regularity in the stages of mortality, with the hormonal doses. No

Table 10

Observations on the effect of 'Altosid' diluted in acetone on the pupae of *Spodoptera mauritia*

Treatment	No. of pupae treated	Observations
Control	2	Both emerged normally
Acetone	2	One died in early pupal stage
12.5 µg/µl	2	One died early and one late
25.0 µg/µl	2	Both died in the advanced pupal development
50.0 µg/µl	2	One died early and one late
100.00 µg/µl	2	Both died late
200.00 µg/µl	2	Both died late

pupal-pupal moult or any other abnormality was observed. The colour of the treated pupae was more dark brown than the control ones. All the pupae in the control had a normal adult emergence.

b) With technical 'Altosid'

Technical hormonal analogue with lower dosages was used in this experiment. The quantities used were 3 µl and 5 µl The results are presented in Table 11. Even the low dosages of 3 and 5 µl were found to be lethal

Table 11

Observations on the effect of 'Altosid' technical on the pupae of Spodoptera mauritia

Treatment	No.of pupae treated	Observations
Control	3	All emerged normally
3 µl	3	All died in late stage
5 µl	3	All died in late stage

to the pupae of S.mauritia. All the treated pupae died in their late stage of pupal development. As in the earlier experiment, all the control pupae developed to imaginal stage normally.

3) With FME technical

Data based on the effects of FME on the pupae of S. mauritia are presented in Table 12 Three pupae were used in each treatment. All the control pupae, which received

Table 12

Observations on the effect of FME technical on the pupae of Spodoptera mauritia

Treatment	No.of pupae treated	Observations
Control	3	All emerged normally
3 µl FME	3	All emerged normally
5 µl FME	3	One died in the early stage of development and two emerged as normal adults

no treatment, emerged normally. The pupae which were treated with 3 /ul of FME also had a normal adult emergence. Among the three pupae which received a dose of 5 /ul of the hormonal analogue, one died in the early pupal development and the rest passed on to adulthood normally It is clear from the observations that FME was less active than 'Altosid' on the pupae of S.mauritia.

IV. Treatment of adult female moths of Spodoptera mauritia with juvenile hormone analogues

a) with 'Altosid'

The results based on the effects of 'Altosid' on adult female moths are presented in Table 13. The biological parameters recorded in the table are average adult longevity, days for laying the first batch of eggs, eggs laid per pair, percentage reduction in fecundity, number of first instar larvae and percentage mortality of eggs. The latent larval mortality consequent on the treatment of hormonal analogue of female moths recorded in this experiment is tabulated and given separately. The average longevity of moths increased significantly with hormonal treatment but there was a slight reduction in the

Table 13

Adult longevity, fecundity and hatchability of eggs of Spodoptera mauritia moths treated with 'Altosid'.

Treatment	Average adult longevity (days)	Days for laying the first batch of eggs	Eggs laid per pair	% reduction in fecundity with respect to control (acetone)	No.of first instar larvae	% mortality of eggs
Control(no treatment)	7.0	3.0	962	6.3	850	11.6
Control (acetone)	8 5	3.0	1027	..	900	12.3
'Altosid' 10 µg/ µl	9.0	2.5	653	36.4	555	15.0

duration for laying the first batch of eggs in treated moths. A reduction in the fecundity (36.4%) of treated female moths with respect to control was recorded. There was not much difference in the hatchability of eggs as the percentages of mortality of eggs in control (no treatment), acetone-treated control and hormone treated were 11.6, 12 3 and 15.0 respectively.

The percentages of latent larval mortality due to the treatment of 'Altosid' on their mother moths are given in

Table 14

Percentage latent mortality of larvae of Spodoptera mauritia consequent on the treatment of 'Altosid' on adult female moths

Treatment	I instar larvae	II instar larvae	III instar larvae	IV instar larvae	V instar larvae	VI instar larvae	Total larval mortality
Control (no Treatment)	9 0	0.0	19.0	7 4	8.0	5.4	48 8
Control (acetone)	10.6	17.0	20.5	16.6	1 8	7 4	73.9
'Altosid' (10 µg/ µl)	33.5	15 0	21.2	7.5	8.0	5.2	90.4

Table 14 A perusal of the data would indicate that the highest larval mortality in both the controls was recorded in the third instar. But the progeny of the hormonally treated moths died in large scale during the first instar period. The percentage mortality recorded was 33.5 A sizable number (21.2%) of larvae in this category died in the third instar also. The total larval mortalities on control (no treatment), acetone-treated control and hormone treatment amounted to 48.8, 73.9 and 90.4% respectively. The total larval mortality in treatment was significantly higher

than those in controls

b) with farnesyl methyl ether

Average adult longevity, days for laying the first batch of eggs, number of eggs laid per pair, percentage reduction in fecundity, percentage of mortality of eggs are tabulated in Table 15. It would be clear from the table that there was no significant difference among the average adult longevities of control and treated moths.

Table 15
Adult longevity, fecundity and hatchability of eggs of Spodoptera mauritia moths treated with FME

Treatment	Average adult longevity (days)	Days for laying the first batch of eggs	Eggs laid per pair	% reduction in fecundity with respect to control (acetone)	No.of first instar larvae	% mortality of eggs
Control (no Treatment)	6.7	3.5	1452	18.8	1312	9.6
Control (acetone)	7.0	3.5	1787	.	1652	9.3
FME (10 µg/µl)	7.0	4.0	1151	35.6	888	22.8

Similar was the case with the periods for laying the first batch of eggs also. But there was significant reduction

in the fecundity of treated moths with respect to control (acetone) The percentage reduction in the number of eggs laid by the treated moths was 35.6. A reduction in the hatchability of eggs of treated moths was also noted. 22 8 per cent of eggs laid by the treated female moths did not emerge.

The percentages of larval mortality caused by the latent effect of FME treated on female moths are presented in Table 16. The maximum number of larvae in all the three

Table 16

Percentage latent mortality of larvae of Spodoptera mauritia consequent on the treatment of FME on female moths.

Treatment	I instar larvae	II instar larvae	III instar larvae	IV instar larvae	V instar larvae	VI instar larvae	Total larval mortality
Control (no treatment)	30.4	1.5	4.3	8.3	7.6	2.9	55 0
Control (acetone)	45 3	2.7	6.0	2.1	3.5	3.2	62 8
FME (10 µg/µl)	51.7	8.8	2.5	4.7	7.4	2.5	77.6

cases died during the first larval instar. The mortality values recorded in the first instar were 30.4, 45.3 and 51.7 per cent respectively in control (no treatment), control (acetone) and 10 µg/µl FME treatment. The total larval mortality in hormonal treatment was 77.6 per cent while those in control (no treatment) and control (acetone) were 55.0 and 62.8 per cent respectively.

DISCUSSION

DISCUSSION

It is reasonable to believe that juvenile hormone analogues (JHA's) are capable of blocking embryonic development in freshly laid eggs. When eggs of Spodoptera were treated with 'Altosid' diluted in pure acetone, hatching was prevented completely Since pure acetone caused considerable mortality, 1 per cent acetone in water was used as solvent for further investigations Data observed in such experiments indicated a correlation between egg mortality and concentration of the JHA's used. Mortality in all the treatments confirms the capability of the hormone to penetrate into the eggs through the chorion A careful examination of the head capsule development showed varying number of black headed stages in different treatments The highest dosage (200 /ug/ /ul) completely prevented head capsule development while the lowest dosage (12 5 /ug/ /ul) allowed head capsule development in 50 per cent of the eggs treated. JHA application of day-old eggs blocks embryonic development at the embryonic-larval transition stage The progressive increase in hatch as the concentration decreased

may be due to the lack of hormone analogue in sufficient quantities for disrupting embryonic development.

Retnakaran and Crisdale (1970) and Williams et al (1970) found that topical application of JHA's on insect eggs killed the embryos, though development upto the black headed stage was seen The present observations also corroborate their findings.

Embryonic development can be thought of as a progressive utilization of genetic information. In the insect embryo the two major critical steps are the switching on the zygotic genome at blastoderm formation, then of the larval genome at blastokinesis (Riddiford,1971). Similar switching on and shutting-off of the genes in the post-embryonic development of insects were also claimed by Williams and Kafator (1971). The presence of exogenous JHA in the physiological system of insects during these vital stages of development become critical to normal development.

Sensitivity of an insect to exogeneous JHA's has been related to the endogeneous titer of the JH It is generally

believed that young larvae with high concentration of internal JH content are resistant to exogenous application. But last instar larvae have only a low titer of JH compared to young larvae (Slama, 1971) and hence freshly moulted last instar larvae were used in these experiments. The effects of JHA's on last instar larvae of Spodoptera mauritia were generally prolongation of larval instar and delay in pupation, moulting into another supernumerary instar, larval-pupal mosaic, moulting difficulties etc. Many of the treated larvae suffered moulting difficulties and mostly died without completing the metamorphosis. Since the intermediates are not healthy enough for survival in nature they naturally perish soon after the moulting or later in the development.

Metamorphosis of holometabolous larvae to pupal stage takes place when the corpora allata are switched off and a lower titer of JH results in the body system. Any excess JH, through topical application or through any other method, is disruptive. Untimely presence of the JHA is likely to stimulate the prothoracic gland to secrete moulting hormone somewhat prematurely, which can lead to disturbances in developmental and transitional programs, resulting more larval instars or abnormal individuals.

Difference in types of response of the individual insects to the JHA application (individuals of the same treatment) may be due to the difference in penetration or absorption of the chemical into the body system.

Prolongation of larval life and delay in pupation may be due to the inability of the larvae to activate the dormant new set of genes in the presence of high titer of JH The kind of synthetic activities needed for a pupal moult is different from that of a larval-larval moult. Moulting of matured final instar larvae into another larval stage instead of a pupal moult in these experiments suggests the possibility of the above argument.

The relative effectiveness of 'altosid' and FME in the tests showed considerable difference. In most of the tests 'altosid' seems to be more effective than FME except in ovicidal action. No direct toxicity is observed in 'altosid' but FME in higher treatments was found to be lethal to the larvae. Complete mortality in the 5 µl treatment lot indicates the possibility of direct toxicity of FME at higher levels.

The difference in colour was noted between control and treated larvae. Dark pigmentation on the dorsal side

and pinkish tinge on the ventral side may be due to the physiological disturbances in side the body. Similar colour changes and swelling of the legs are present in diseased larvae also They are due to the altered physiology caused by the attacking pathogens. Thus there is every possibility to believe that internal disturbances by JHA at abnormal time may be the reason for changed pigmentation patterns.

Comparative dryness in the faecal pellets and shrunken body were observed in treatments . The body wall is disturbed by the initiation of moulting. Wax layer on the cuticle is responsible for water conservation and if this system is not properly maintained, loss of water would be the result. The excessive absorption of moisture from the food materials in the gut to meet the water requirement of the body may be the possible reason for the comparatively drier faecal pellets. During later stages of the larvae, feeding activity almost ceased probably due to break down of the body tissues and water loss and consequently the body shrinks enormously before death.

Induction of supernumerary instars with the juvenile hormone analogues was successful only when the sixth instars

were treated on the first day of the stadium. It is hypothesized that the differentiation of the various larval structures occurs at different rates and that some remain indeterminate for a longer period than the others. At the early stage of a stadium, differentiation is relatively non-committed. Therefore hormonal treatment at this stage results in the induction of a supernumerary instar. At a later stage in the stadium some structures become committed in their differentiation towards the pupal stage and the developmental programme cannot be reversed Treatment in this stage with exogenous JHA results in maintaining the non-committed structures in the juvenile condition while allowing the committed parts to differentiate towards the pupal stage, the net result is the formation of a larval-pupal mosaic.

From the observations it seems reasonable to assume that the pupae of Spodoptera mauritia are more susceptible to 'altosid' than FME. 'Altosid' either as solution or as technical material caused heavy pupal mortality. To differentiate the pupa into the imaginal stage the synthetic activity has to be reoriented by activating new sets of

dormant genes and switching off of the currently operating genes. The mortality may be due to the interference of the JHA's in the neuclic acid replication needed for adult cuticle synthesis. Pupae were not much affected by FME as most of the treated pupae successfully metamorphosed into normal adults Lack of significant juvenilizing activity in pupae may be due to the unsuccessful penetration of pupal cuticle or may be due to the selective detoxification of the hormone within the body.

Wellington (1970) found that the mortality of new pupae early in the developmental stage was due to exogenous JHA application. Complete inhibition of metamorphosis in young pupae of Trogoderma was reported by Bhatnagar-Thomas (1972). The present observations of the experiments also corroborate the above findings.

Effect of JHA's on freshly emerged female moths were studied at 10 µg/µl dosage Topical application of JHA to female Spodoptera moths resulted in reduced fecundity, decfeased hatchability and disrupted post embryonic development in the emerged larvae Application of JHA to the female during the terminal phases of oogenesis, blocks the eggs in embryonic development. Masner (1967) pointed out

that differentiation of follicular epithelium requires a period of absence of juvenile hormone. So the reduced fecundity may be due to the failure of follicular epithelium differentiation in treated adults Already differentiated follicular cell will develop into mature eggs but JHA prevents further exbryonic developments Probably that may be the reason for high percentage of sterile eggs laid by the females. Normal matured eggs got fertilized and hatched into first instar larvae. But the heavy mortality suffered in the first instar indicates the possibility of internal abnormalities. Riddiford (1971) suggested that the delayed effects of JHA in postembryonic development are due to its action on the developing corpora allata towards malfunctioning.

The effects of FME in female moths are comparatively less marked than those produced by 'altosid' Relative low efficiency of FME may be due to the inability of the chemical to penetrate successfully through the cuticle or may be due to the ability of the moths to detoxify the assimilated hormone analogue. The differences in the molecular configuration of the two JHA's may also be a possible reason for the discrepency of effects on biological systems. High JH activity due to the presence of certain chemical groups were reported by

Wigglesworth (1969). Strong and Dickman (1973) in their comparative studies of several JHA's reported the promising JH-activity of 'altosid' and 'altosar' over other JHA's tested.

In general juvenile hormone analogues offer their potential use as control agents against insect pests Under controlled conditions JHA's are found to be effective in producing disrupted metamorphosis and reproduction They are proven ovicides when gravid females or the female freshly laid eggs are treated. Even the JHA application is not early enough to prevent hatching, the delayed effects will prevent larvae to develop into normal adults. Hence, prospects for the application of juvenile hormone analogues as part of integrated control-the latest in pest management-are highly encouraging.

SUMMARY

SUMMARY

Morphogenetic effects of two juvenile hormone analogues viz 'altosid' and farnesyl methyl ether (FME) on Spodoptera mauritia were studied under laboratory conditions

Ovicidal action was first tested by using different concentrations of juvenile hormone analogues diluted in pure acetone. All the treatments and control (acetone treatment) prevented embryonic development to varying degrees Since the solvent also caused mortality in eggs, one per cent acetone solution in water was used in later experiments Treatments ranging from 0.0125 to 0.1 per cent 'altosid', resulted in egg mortality ranging between 40 to 60 per cent. The corresponding mortality figures caused by FME were 36 and 52 per cent, the values were comparatively less than those with 'altosid'.

Freshly moulted last larval instar was the most susceptible stage to the action of JHA's All the hormonal concentrations used in the study affected normal metamorphosis and moulting Some of the treated larvae moulted into supernumerary larval instars and larval-pupal mosaic. Prolonged larval duration, failure of normal moulting, altered body pigmentation, reduction in water content of

REFERENCES

REFERENCES

Amos, A G., Williams, P., Du Gueselin, P.B. and Schwarz,M.(1974) Effects of a JH-mimic on the pink bollworm J. econ Ent. 67.173-176

Ashburner, M. (1970) Effect of JH on adult differentiation of Drosophila melanogaster. Nature.(Lond) 277(5254)· 187-189

Babu, T H. and Slama, K. (1971). Effectiveness of a juvenile hormone analogue against red cotton bug, Dysdercus cingulatus. Indian J. Ent.33·198-201.

Bagly, R W and Bauernfeind, J.C (1971) Field experiences with JH mimics. In Insect juvenile hormones (Julius J. Menn and Morton Beroza, eds) Academic Press, New York.

Bhatnagar Thomas, P.L.(1972). Laboratory evaluation of synthetic juvenile hormone analogue for the control of Trogoderma granarium. Indian J.Ent. 34:87-93

Bluemenfield, M. and Schneiderman, H.A. (1968) Effect of JH on the synthesis and accumulation of sex-limited blood protein in polyphemus silk moth. Biol Bull 135 466-475.

Bowers, W.S (1972) Biological activity of insect morphogenetic agents. PEST 49 (En) Stauffer chemical company, Richmond, California 94804, U.S A.

Bowers, W S and Blickenstaff,C (1966). Hormonal termination of diapause in alfalfa weevil Science 154(3757): 1673-1674

Bransby-Williams, W.R (1971). JH-activity of ethyl farnesoate dihydrochloride with cotton stainer., Dysdercus cardinalis. Bull Ent. Res. 61.41-47.

Brieger, G (1971). JH-mimics, structure-activity relationship for Oncopeltus fasciatus, J Insect physiol 11. 2085-2095

Cawich, A., Lawry, A., Crowder, L A and Watson, T F. (1974) Effect of a JH-mimic on the pink bollworm. J.econ. Ent. 67 173-176.

Chamberlain, W.F. and Hopkins, D.E.(1971) Synthetic JH for the control of Bovicola limbata on Angora goats. J. econ. Ent. 64.1198-1199.

Connin, R V., Jantz, O.K. and Bowers, W.S (1967). Termination of diapause in the cereal leaf beetle, Oulema melanopus by hormones. J. econ. Ent. 60. 1752-1753.

Critchley, B R. and Campion, D.G (1971) Effects of synthetic JH and a JHA, methyl farnesoate dichloride, on pupal development of the yellow mealworm, Tenebrio molitor Bull. Ent. Res. 61:293-297.

Dyte, C.D. (1972) Resistance to synthetic JH. Nature (Lond.) 238 48-49

Ellis, P.E., Morgan, E.D. and Woodbridge, A.P. (1970) Is there new hope for hormone mimics as pesticides. Pans 16 434-443.

Fales, J H , Bodenstein, O.F. and Bowers, W S (1970) Seven juvenile hormone analogues as synergists for pyrethrins against house flies. J. econ. Ent. 63 1379-1380

Gill,S S., Hammock, B.D., Yamamoto, I. and Casida, J E (1972) Preliminary chromatographic studies on metabolites and photodecomposition product of a juvenoid pp 177-190 In Insect juvenile hormones.(Menn, J J. and Beroza, M eds) Academic Press, New York.

Hangartner, W., Peyer, B. and Meier, W. (1971) Effect of a JHA on the apterus form of bean aphid, Aphis fabae. Med. Fakalteit. Lond. Gent 36. 866-873.

Harris, R.L., Frazer, E D. and Younger, R L.(1973) Horn flies stable flies and house flies: Development in faeces of bovine treated orally with juvenile hormone analogue. J. econ. Ent. 66. 1099-1102

Hamberger, E.,Benz, G. and Thommen, H. (1971). Investigations with dichlorofarnesenic acid ethyl ester on the cotton stainer. Proceedings of the first Swiss symposium on juvenile hormone: pp.193-195.

Hrdy, J. (1973) Effects of juvenoids on termites and honey bees Proc. 7th Congr. IUSSI, London, 1973: pp. 158-161

Ilan, J , Ilan, J. and Patel, N.G. (1971). Regulation of messenger RNA translation mediated by juvenile hormone pp. 43-68 In Insect juvenile hormones, (Julius J. Menn and Morton Beroza, eds) Academic Press, New York

Kamm, J.A and Swenson, K.G. (1972). Termination of diapause in Draeculacephala crassicornis, with synthetic juvenile hormone. J. econ. Ent.65.364-367.

Kuhr, R J. and Cleere, J.S (1973) Toxic effects of synthetic JH on several aphid species. J. econ. Ent. 66: 1019-1022.

Law, J H., Yuan, C. and Williams, C M. (1966) Synthesis of a material with juvenile hormone activity Proc. nat Acad. Sci. U.S A 55: 576-578.

Mansingh, A., Sahota, T S and Shaw, D.A. (1970) Juvenile hormone activity in the wood and bark extracts of some forest trees. Can. Ent. 102 49-53.

Masner, P. (1969). The effect of substances with JH activity on morphogenesis and function of gonads in Pyrrhocoris apterus. Acta. ent. bohemoslov. 66(2): 81-86

Masner, P., Slama, K. and Landa, V (1968). Sexually spread insect sterility induced by the analogues of JH Nature,(Lond) 219: 395-396

Masner, P., Slama, K., Sdarek, J. and Landa, V (1970). Natural and synthetic materials with insect hormone activity: A method of sexually spread insect sterility. J.econ. Ent. 63. 706-710.

McGovern, T.P. and Redfern, R.E.(1971). Juvenile hormone activity of acetals applied topically and as vapour to the yellow mealworm. J. econ. Ent. 64: 238-241.

Mehrotra, K.N. Rao, P.J. and Phokela, A (1969) Effect of JH like compounds on the desert locust, Schistocerca gregaria Indian J Ent. 31. 183-187.

Metwally, M., Schnal, F. and Landa, V. (1972). Reduction of fecundity and control of khapra beetle by juvenile hormone mimics J. econ. Ent. 65: 1603-1605.

Miller, R.W. and Uebel, E.C (1974). Juvenile hormone mimics as feed additives for control of the face fly and house fly. J. econ. Ent. 64: 69-70.

Morgan, P B. LaBrecque, G.C.(1971). Hormones or hormone-like substances on the development and fertility of house flies. J. econ. Ent. 64(6): 1479-1481.

Mulla, M.S. Norland, L.R , Toshiakai and Karamer, W.L. (1974). Insect growth regulators for control of aquatic midges. J. econ. Ent. 67: 165-170.

Neal, J.W. and Hower, A.A (1974). Small pot tests with synthetic JH on alfalfa weevil in Maryland and Pennsylvania. J. econ. Ent. 67: 93-96.

Outram, I. (1972). Effect of synthetic JH on adult emergence and reproduction of the female spruce budworm, Choristoneura fumiferana. Can. Ent. 104: 271-273

Outram, I. (1973) Synthetic juvenile hormone: Effect on pupae of the spruce budworm. J. econ. Ent. 66: 1029-1031.

Pallos, F.M. and Menn, J.J. (1972). Synthesis and activity of juvenile hormone analogues. pp. 303-316. Insect juvenile hormones (Menn, J.J. and Beroza, M.eds) Academic Press, New York

Pallos, F M. Menn, J.J. Letchworth, P.E. and Miaullis, J.B.(1971) Synthetic mimics of juvenile hormone. Nature,(Lond.) 232. 486-

Ratusky, J , Slam, K. and Sorm, F.(1969). Natural and synthetic materials with insect hormone activity. Juvenile hormone effect of some alkyl-esters derived from aliphatic hydroxy acids. J. Stored prod. Res. 5: 111-117.

Redfern, R.E. McGovern, T.P. and Beroza, M. (1970) Juvenile hormone activity of sesamex and related compounds in tests on the yellow mealworm. J. econ. Ent. 63. 540-545.

Redfern, R.E., McGovern, T.P. Sarmiento, R and Beroza, M.(1971). Juvenile hormone activity of mixed ethers containing a phenyl and a terpenoid moiety, applied topically to large milkweed bug and yellow mealworm. J. econ. Ent. 64. 374-376.

Redfern, R.E Mills, G.D. and Sonnet, P.E.(1972) Aziridines as potentiators of juvenile hormone activity in tests on the yellow mealworm and large milked bug. J econ. Ent. 65 1065-1067.

Reissig, W.H. and Kamm, J.A (1974) Effect of foliage sprays of JHA on development of Draeculacephala crassicornis J. econ. Ent. 67. 181-183.

Rembold, H., Czoppelt, Ch. and Rao, P.J. (1974). Effect of juvenile hormone treatment on caste differentiation in the honey bee Apis mellifera. J. Insect Physiol 20 1193-1202

Retnakaran, A.(1973 a). Ovicidal effects in the white pine weevil, Pissodes strobi, of a synthetic JHA. Can. Ent. 105. 591-594.

Retnakaran, A. (1973 b) Hormonal induction of supernumerary instar in the spruce budworm, Choristoneura fumiferana.Can. Ent. 105 459-461.

Retnakaran, A and Grisdale, D (1970). The ovicidal effect of hydrochlorination reaction mixture on the spruce budworm. Ann. Ent. Soc. Am. 63: 907-909.

Richmond, C.E. (1972). Juvenile hormone analogues tested on larvae of Western spruce budworm. J. econ. Ent. 65 950-953.

Riddford, L.M. (1972) Juvenile hormone and insect embryonic development its potential role as an ovicide pp.95-112. In Insect Juvenile Hormones (Menn, J.J. and Beroza, M. eds) Academic Press, New York.

Roller, H., Bjerke, J.S. and Meshan, W H.(1965) Isolation of hormone from cecropia oil. J. Insect Physiol 11. 1184-1197.

Roller, H.,Bjerke, J.S., Holthaus, L.M , Norgard, D.S. and McShan. W.H. (1969). Isolation and biological properties of juvenile hormone. J. Insect Physiol. 15. 379-389.

Romanuk, M., Slama, K. and Sorm, F.(1967). Constitution of a compound with a pronounced juvenile hormone activity. Proc. natn Acad Sci. U S.A. 57: 349-352.

Schaefer, C.S and Wilder, W.H. (1972). Insect developmental inhibitors: A practical evaluation on mosquito control agents. J. econ.Ent. 65: 1066-1071.

Schneiderman, H A. (1971 a). Control systems regulating insect development PEST 48 (En) School of Biological Sciences California University, Irvine, California U.S A.

Schneiderman, H A (1971 b). The strategy of controlling insect pests with growth regulators Bull. Sc. Ent. of Swiss. 44. 441-449.

Schneiderman, H.A (1972) Insect hormone and insect control pp. 3-29 In Insect Juvenile Hormones (Menn, J.J. and Beroza, M.eds) Academic Press, New York.

Schwarz, M. Wright, J E , Redfern, R E and Mills, G D (1974) Compounds related to JH Activity of aryl terpenoid compounds in four insect species. J. econ. Ent.67: 177-180.

Siew, Y.C and Gilbert, L.I.(1971). Effect of moulting hormone and juvenile hormone on insect endocrine gland activity J Insect Physiol. 17: 2095-2104.

Singh, D.(1974). Effect of ZR-515, a JHA on development of mustard aphid, Lipaphis erysimi. Abstract of the talk delivered on the occasion of short course on insect hormone from 27th November-3rd Dec. 1974, at Hebbal, Bangalore.

Slade, M. and Zibitt, C H.(1971) Metabolism of cecropia juvenile hormone in insects and mammals. pp. 155-175 In Insect Juvenile Hormones (Menn, J.J. and Beroza, M. eds) Academic Press, New York.

Slama, K.(1969). Plants as a source of materials with insect hormone activity. Proc. IInd Intern. Symp. Insect and host plants, held at Wageningn,Netherland

Slama, K.(1971). Insect juvenile hormone analogues. A. Rew. Biocnem, 40: 1079-1102.

Slama, K. and Williams, C M.(1965). Juvenile hormone activity for the bud Pyrrhocoris apterus. Proc. Nat. Acad Sci. U.S.A. 54 411-424.

Slama, K. and Williams, C.M.(1966). Paper factor as an inhibition of the embryonic development of European bug, P. apterus. Nature, (Lond.) 210: 329-330.

Slama, K. Romanuk, M. and Sorm. F.(1969). Natural and synthetic materials with insect hormone activity: Juvenile hormone activity of some derivatives of farnesenic acid. Biol. Bull. 136. 91-95.

Sonnet, P.E., Redfern, R.E. Schwarz, M. Wakabashi, N. and Waters, R.M. (1971). Compounds related to JH Activity of aryl citonellyl carbamates on yellow mealworm and large milkweed bug J. econ. Ent. 64. 1378-1381.

Strong,R G. and Dickman, J. (1973) Comparative effectiveness of fifteen insect growth regulators against sevdral pests of stored produces. J. econ. Ent. 66: 1167-1173.

Tashev, D.G and Damyanova, S.A.(1971) Juvenilizing effect of farnesol on the apterous specimens of Aphis craccivora. Doklady Bolgarskoi Akademi Nank 24. 659-662.

Varjas, L. (1971) The effective doses of juvenile hormone analogues from the point of view of insect control. 219-227 Proceedings of a Symposium held on the occasion of the 90th anniversary of the Hungarian Research Institute for plant protection. Budapest, Hungary.

Walker, W.F. and Bowers. (1970). Synthetic juvenile hormone as potential coleopteran ovicides. J. econ. Ent. 63: 1231-1233.

Wellington,W G. (1969) Effects of three hormonal mimics on mortality, metamorphosis and reproduction of Western tent caterpillar, Malacosoma californicum. Can. Ent. 101 1163-1172.

White, D.F. (1968) Postnatal treatment of the cabbage aphid with a synthetic JH. J. Insect Physiol. 14.901-912.

White, D F. and Lamb, K.P. (1968). Effect of a synthetic JH on adult cabbage aphids and their progenies. J. Insect Physiol. 14: 395-402.

Wigglesworth, V B. (1930). In Insect Hormones. Oliver and Boyd. Edinburgh. pp. 141. (Published in 1970).

Wigglesworth, V B. (1934). Hormone controlling moulting and metamorphosis in Rhodnius sp. Quart. J. Microscop. Sci. 77. 191.

Wigglesworth, V.B. (1969) Chemical structure and juvenile hormone activity Nature, (Lond.) 221: 190-191.

Wilde, J.de.(1972) The present status of hormonal insect control. Department of Entomology, Wageningen, Netherlands.

Williams, C.M.(1956) Juvenile hormone in insects. Nature, (Lond.) 178: 212

Williams, C.M.(1969). Juvenile hormone insecticides Sci. American 217: 13-17

Williams, J.H. and Lawrence, P.A (1970) Deferred action of juvenile hormone Nature, (Lond.) 225 81-83.

Wright, J E (1970). Hormones for control of live-stock arthropods. Development of an assay to select candidate compounds with JH activity in the stable fly. J econ. Ent. 63: 878-883.

Wright, J.E (1972). Hormones for control of live-stock arthropods. Effectiveness of three JHA for control of stable flies. J. econ.Ent. 65: 1361-1364.

Wright, J.E.and Schwarz, M. (1972) Juvenilizing activity of compounds related to JH against pupae of stable fly. J. econ. Ent. 65· 1644-1647

Wright, J.E. and Spates, G E. (1972) Laboratory evaluation of compounds to determine JH activity against the stable fly. J. econ. Ent. 65 1346-1349.

Writz, P. (1973) Differentiation in honey-bee larvae. A Histological study of caste induction of Apis mellifera L.Commun. Agri. Univ. Wageningen 73(5) 1-55

Writz, P. and Beetsma, J. (1972) Induction of caste differentiation in the honeybee Apis mellifera by juvenile hormone. Entomologia exp. appl. 15· 517-520

PLATE

Plate No. 3. The shrunken cadaver of a supernumerary larval form from the treatment with FME 5 µl., along with a control larva.