THE ACTION OF SOME INSECT GROWTH REGULATORS ON BEHAVIOUR, DEVELOPMENT, METAMORPHOSIS AND BIOCHEMISTRY OF THE YELLOW FEVER MOSQUITO *Aedes aegypti* (L) (Diptera : Culicidae)

A Thesis Submitted to the
Shivaji University, Kolhapur
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in Zoology

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DECLARATION

I hereby declare that the thesis entitled "The action of some Insect Growth Regulators on Behaviour, Development, Metamorphosis and Biochemistry of the yellow fever mosquito Aedes aegypti (L) (Diptera : Culicidae)" completed and written by me has not previously formed the basis for the award of any degree or diploma or other similar title of this or any other University or examining body.

Place : PUNE

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CERTIFICATE

This is to certify that the thesis entitled "The action of some Insect Growth Regulators on Behaviour, Development, Metamorphosis and Biochemistry of the yellow fever mosquito Aedes aegypti (L) (Diptera : Culicidae)" which is being submitted herewith for the award of the Doctor of Philosophy in Zoology, of Shivaji University, Kolhapur is the result of the original research work completed by Shri Sudhakar Gopal Deshpande under my knowledge and belief. The work embodied in this thesis has not formed earlier the basis for the award of any degree or similar title of this or any other University or examining body.

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Date : 18 AUG 1994

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(*SUDHAKAR GOPAL DESHPANDE*)

CONTENTS

General Introdu[redacted]

General Introduction

As the most successful and abundant group of animals, insects have been competing with man for food and fibre ever since the rise and emergence of the Homo sapiens. Primeveal man also had to contend with insect borne various maladies and other ectoparasites. It was natural, therefore, that with the beginning of civilisation and organised living activities of reducing insects ravages on crops, stored foods, fabric materials, the humans themselves and their live stock also developed from mere mechanical sloughter to the use of fire, smoke and finally toxic chemicals. Historical records of use of chemicals to control insects start appearing from classical Greek and Roman times. Theophast mentions the fumigant action of burning sulphur and Pliny the elder, advocated the use of arsenic as an insecticide, apart from referring to the use of soda and olive oil for seed treatments of legumes (Cremlyn, R., 1978).

The Chinese have also been recorded (Konishi and Ito 1973) as using moderate amounts of arsenicals as insecticides in the 16th century. Not long afterwards, the first botanical in the form of nicotine came into use for control of insects. By the 19th century, pyrethrum as well as soap had also been used.

The middle of the 19th century saw the beginning of systematic scientific methods in the use of crop protection. Various chemicals in use included arsenical compounds, particularly paris green, which was an impure form of copper arsenite. The USA pioneered its use to check the spread of the devastating Colorado beetle. Paris green came to be used so extensively that it led to the introduction of what was probably the first pesticide legislation in the world. The Dowhy Mildew of Vine was controlled by the famous Bazadeux mixture, and by the turn of the century lime sulphur was being used widely in Europe and America as a fungicide in orchards.

The era of the 1st botanical and miscellaneous synthetics (also called the 1st Generation Pesticides) was terminated by the discovery of DDT during the 2nd World War (West and Campbell 1950). Inspite of its initial high success and promise, it was followed by more and more potent toxicants such as organo-phosphates, carbamates as well as a host of more powerful organochlorines. Whereas the latter caused the most dramatic decline in dreaded human afflictions such as malaria, typhus etc. at the same time were responsible for ushering in major agricultural advances, they were also the ultimate causes of eventual disenhantment with the synthetic insecticides. Apart from development of resistance to insects, which led to multiplication of the more toxic pesticides, widespread distribution as well as persistence of their residues, in nearly all spheres of the environment, plus definite serious hazards to man himself were responsible for the energetic search for alternatives or supplements. This began towards the latter half of the present century. This movement has gained momentum as well as wide sympathy with the pioneering work of Wigglesworth, Williams, Slama, Staal etc. in the discovery and elucidation of control potential of insect (juvenile) hormones and other growth regulators, aptly termed the "Third Generation Pesticides".

Mention must now be made of an interesting new development of the last decade, namely the discovery and promotion of a new class of insect growth regulators (IGRs) based on the principle of chemical mediated inhibition of insect ecdysis. Aptly termed insect ecdysial inhibitors, the most well known among these are the diflurobenzoides, commercialised as the product Dimlin. (Philips Duphar Company, Holland). Highly potent inhibitors of chitin synthesis in the insect integument have been developed, some having excellent field effectiveness (F. Rettich 1978). As chitin has no essential role in the biochemical economy of vertebrates, these compounds are relatively safe to higher organisms.

Parallel with these developments was the emergence of the concept of pest management defined as control or reduction of insect pest population to levels which would not produce economic injury (Geier 1966). In other words, the older concepts of complete eradication or 'overkill' were discarded as unscientific. The ecological unacceptability of complete eradication of any species becomes apparent when total eco-system dynamics are taken into consideration. Thus any natural population has a complex of predators, parasites and prey species. Upsetting this delicate balance by elimination of any one of these components immediately produces highly undesirable consequences. Thus the natural enemies of the eradicated pest population suffer on two counts of prey deprivation and the insecticidal toxicity itself. This may eventually cause a resurgence of the pest population or pave the way for emergence of minor or secondary pest outbreaks which may not be controlled by the specific chemical being used.

Awareness of various complications and aggravations briefly described above has led to greater faith in non-toxic chemicals which may affect the physiology or biology of the pest adversely but without causing its complete eradication or producing unwanted effects on beneficial and non target species.

Chemicals which largely meet some of these requirements are the insects, own hormones, whose various synthetic analogues have proved to be more potent than the original base material. Whereas both 'moulting' hormones (Williams 1952 a,b, Butenandant and Karlson 1954) and anti-juvenile hormones (AJH), sometimes also called the IV generation pesticides (Staal 1961, Bowers et al. 1976) are also known and their synthetic analogues available, the Juvenile Hormone Analogues (JHAs) probably remain the most promising and favoured of the new Third/Fourth Generation Pesticides. The diflurobenzoides too, because of greater field suitability and success, have

become equally or even more popular.

The insect juvenile hormone was initially discovered by Wigglesworth (1935, 1936). Williams (1956) was able to demonstrate the practical potential of juvenile hormone analogues for field use. Staal (1972) must be credited with the first successful efforts for commercialisation of the JHA potential. The Zoecon Research Corporation, Palo Alto, California, USA was able to come out with powerful synthetic juvenile hormone analogues such as hydroprene (Altozar), methoprene (Altosid) and Kinoprene. Of these, hydroprene proved more successful in control of live stock pests while methoprene and especially its controlled release formulation (SR-10) (Dunn et al. 1973) were unquestionably successful in non-hazardous control of swamp mosquitoes in USA.

Several limitations still restrict more widespread employment of these modern JHA's. These include high cost, high specificity of action, limited range of target pests and effectiveness only at particulate sensitive stages, plus comparatively higher instability in field conditions. However, it must be realised that the latter three characteristics are actually highly desirable in scientific terms and in the context of environment. To this extent, such chemicals have actually been called 'biorational' (Menn and Henrick 1981) meaning biologically logical. Because of this, considerable work has been done and continues on various aspects of activity, persistence, behaviour and other implications of known and new JHA's. Chemists all over the world continue to synthesize newer juvenoid molecules, and biologists continue to assess their promise and potential (Hebbalkar and Sharma 1979, Sharma et al. 1980, Patwardhan et al. 1982, Phadnis et al. 1987).

Recently the Zoecon Corporation under the stewardship of G.B. Staal have synthesized even more potent isomers (S-Hydroprene, S-Methoprene) of the well known hydroprene and methoprene respectively. In these, older compounds i.e. inactive stereoisomers are also present. These stereoisomers simply dilute the active principles in the racemic mixture.

In the present study the more active optical isomers namely S-Hydroprene and S-Methoprene, have been used which are more potent and effective than earlier ones. Zoecon Corporation have replaced racemic methoprene and hydroprene in many of its products with the S isomers of high geometric and optical purity. (Henrick et al.1988).

In case of insect ecdysial inhibitors, their use has been fairly successful in certain selective situations particular mention may be made of the effective deployment of dimlin (Zabel and Ostojic 1973) for control of leaf rollers. This compound is not a plant systemic one but is mainly a stomach poison (Datebout 1985). The major limitation of dimlin as a pest control agent seems to lie in its greater effectiveness by administration through the oral route even though there are sporadic reports of equal cuticular efficacy of this product.

The present thesis incorporates results of an extensive investigation into biological activity, persistence and potential of these two different classes of modern pest control agents. (The JHA's and Dimlin).

The thesis has been divided into three sections for convenience of presentation. Section I deals with the effects of these new super active molecules on the development, metamorphosis and certain aspects of behaviour of the chosen test species, the yellow fever mosquito Aedes aegypti (L.). The second section deals with certain biochemical

changes induced in the test organism by the new compounds. The third section deals with general persistence of these IGR's. In all these studies the common design consists of assessment of effects of the test compounds when the insects were exposed continuously or for short durations at different temperature levels. Again, these variables have been used for all stages of test organisms ranging from II instar larva to pupa. It is worth mentioning here that the latter stage i.e. pupa has been very sparingly used in such investigations by other workers as per the literature survey and the present study has been very fruitful in producing highly significant results.

Based on these, it has been possible through this exhaustive undertaking, to enunciate and elucidate potential of the new, more potent optical isomers of hydroprene and methoprene, as also to generate new data on the use and properties of the rapidly emerging IGR dimlin. The findings projected in this thesis should be valuable from the view point of field applications also. It is expected that results and conclusions presented here will provide basic new as well as additional information for the sagacious employment of these newer Third Generation Pesticides.

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CHAPTER ONE

General Review of Literature on IGR's

REVIEW OF LITERATURE ON IGR'S

The role of internal secretions or hormones in animal physiology, growth and development has been well recognised and established since long. In invertebrates, especially the insects, presumably glandular or secretory organs, most prominent being the corpora allata, had been noted by morphologists as early as 1762 (Pflugfelder, O 1958). However, definite histological and other evidence suggesting their endocrine nature began accumulating only towards the beginning of 20th century (Novak, V.J.A. 1966). Wigglesworth (1935, 1936) was the first to investigate possible hormonal regulation of insect growth, development and metamorphosis in a series of classical experiments mainly on the blood sucking bug (Rhodnius Prolixus). It was then only a matter of time before the hormone itself was isolated from insect tissues and eventually characterised chemically (Findlay, J. 1971, Roller, H. et al. 1967). The hormone suggested by Wigglesworth, and later confirmed by several others from various chemical and biological studies, was shown to be originating from the corpora allatta. In the early or juvenile stages of the insect it was shown to be responsible for maintenance of juvenile condition and hence it was appropriately called Juvenile Hormone (JH). The latter was apparently acting on the cells of juvenile insects to prevent metamorphosis, which would take place only when the titre of JH in the insect went down, with a concomittant rise in concentration of another hormone called the moulting hormone (MH). Subsequently chemical structures of both these remarkable regulatory chemicals were elucidated. The JH was shown to be a terpenoid moiety while MH was identified as ecdysterone.

Concurrent with the above academic studies, Williams (1956) was able to obtain substantial JH activity in lipid extracts of abdomens of cecropia silk moth. More interestingly, he was able to detect potent JH activity in extracts of certain American newspapers. This was later confirmed for certain Indian newspapers also by Saxena and

Williams (1966). Later it was possible to show that JH activity in newspaper print was originating from the wood pulp, and the chemical principle was identified as juvabione, found especially in balsm firs in the Western hemisphere and the Deodar tree in India.

Williams (1956) reported the preparation of an active extract of a differentiation controlling hormone from the adult male cecropia moth. History of various studies on juvenile hormones has been reviewed in detail by Bowers (1971) Wigglesworth (1964), Berkoff (1969), Gilbert (1964) etc. Here only salient highlights will be briefly noted. Williams (1956) referred to this class of compounds as third generation pesticides. Schmialek (1961) isolated from the faeces of the yellow meal worm Tenebrio molitor two compounds with JH activity, farnesol and farnesal. To date five natural JHs have been identified. The first JH to be isolated and identified was announced by Roller and co-workers in (1967). A second hormone JH-II was isolated from the same species, the cecropia moth Hyalophora cecropia by Meyer et al. (1968). Williams (1967) was the first to suggest that JHs could be used as insect specific control agents to which the pest species may be unable to develope resistance.

The third hormone, JH III was isolated and identified from the tobacco horn-worm Monduca sexta (Judy 1973). The fourth hormone JHO and fifth hormone 4 me JHI (iso-JH O". 5) were isolated from developing embryos of the same species (Bergot 1980, 1981). Schooly and co-workers at the Zoecon Research Institute in collaboration with scientists in several countries have studied the qualitative and quantitative determination of natural JHs. They have identified the actual JHs present at physiological levels in samples of either whole bodies or haemolymph in several insect species (Schooly et al. 1984). Insect metamorphosis is under hormonal control. The two principal hormones controlled by the brain are juvenile hormone and ecdysone. It is thought that JH secretion of the corpus allatum is controlled by allatotropin and allatohibin from

the brain (Williams C. 1976). Ecdysone secretion by the prothoracic gland is controlled by the prothoracictropic hormone from the brain. (Gilbert et al. 1981). Upsetting the titre of juvenile hormone at certain periods during the life history will adversely affect metamorphosis. One of the main reasons for juvenile hormone being attractive as a control agent is its terpenoid nature, which enables it to penetrate the cuticle with great ease and exert its effects on the target tissue, the epidermis. More than 500 analogues with different substitutions and varying degrees of insecticidal activity and specificity have been synthesized (Romonuk M. 1981, Slama K. et al. 1974).

Chemistry :-

Structure-activity relationships of JH analogues are extremely complex. Details of synthesis, relative potencies and studies on structure activity relationships have been extensively reviewed (Henrick et al. 1976, Jarolim 1981, Romonuk and Wimmer, 1981, Slama et al. 1974, Sobotka and Zabra, 1981). Bowers (1969) synthesized certain aromatic terpenoid ethers that were potent mimics of the natural hormone. Methoprene, Triprene, Hydroprene and Kinoprene are dodecadienoates developed by Zoecon Research Laboratories of Palo Alto, California under the stewardship primarily of G.B. Staal. Two of these, Methoprene and Kinoprene have been registered in the United States (Staal, 1982). The rest of the compounds have aromatic moieties with or without an epoxide.

Work is still continuing the world over on synthesis of new JHAs and/or isolation of active compounds/principles from plants. In India, the most notable contributions in this area are those from the National Chemical Laboratory (N.C.L.), Pune (Sharma et al. 1980, Patwardhan et al. 1982).

Effects :-

The effects of juvenile hormone analogues in most instances cannot be differentiated from the effects of the natural hormone itself.

The biochemical effects of juvenile hormone analogues are complex and vary from one analogue to another (Kramer and Staal, 1981). Juvenile hormone has two distinct biochemical effects : One during the larval stage it suppresses metamorphic change during moulting and in the adult it induces vitellogenin synthesis during ovarian development. Both functions are associated with the transcription of m-RNA (Coudron et al. 1981). Tobe and Stay (1979) showed that hydroprene stimulated juvenile hormone synthesis at low doses and inhibited synthesis at higher doses. Juvenile hormone analogues act on the juvenile hormone receptors responsible for feed back control of the hormone titre. Hydroprene and methoprene stimulate the esterase activity for the natural hormone (Kramer et al. 1978, Kramer. 1978). In the mosquito Aedes aegypti it was shown that the pupal esterase activity was suppressed by methoprene (Downer et al. 1975). Effects of methoprene on DNA, RNA and protein synthesis indicate that it has no effect on thymidine incorporation in DNA but decreases uridine incorporation in RNA (Scheller K. et al. 1978). Juvenile hormone action depends on the developmental stage and varies from species to species. Effects at the biochemical level appear to be scattered. Recently it has been shown that the enzyme hydroxymethyl glutazyl CoA reductase (HMG-CoA reductase) is the rate limiting enzyme in juvenile hormone synthesis (Monger et al. 1982).

Juvenile hormone analogues affect the physiology of morpho-genesis, reproduction and embryogenesis. The initial effect is seen during larval pupal transformation. Due to the action of juvenile hormone analogues, various degrees of incomplete meta-

morphosis are observed. Juenile hormone analogues also affect the endocrine physiology of the insect which ultimately results in abnormal morphogenesis. Methoprene inhibits release of prothoracitropic hormone from the brain which inhibits the prothoracic gland activity early in the last instar but stimulates the gland prior to pupation (Hiruma et al. 1978, a and b). Hydroprene stimulates juvenile hormone synthesis by the corpora allata (Tobe and Stay 1979). The role of juvenile hormone of insects is relatively well studied (Chen and Wyatl 1981, Koeppe 1981). Juvenile hormone analogues block embryonic development (Saxena and Sharma 1972). Since this blockage occur at blastokinesis it can be deemed as ovicidal in nature (Retnakaran 1980, Riddiford and Williams 1967). Juvenile hormone also depresses respiratory rate (Retnakaran 1975) and mobilizes and deplete the reserve food (Downer et al. 1976, Retnakaran 1974).

Organismal effects were observed at different stages such as egg, larva, pupa and adult. Embryogenesis is disrupted if juvenile hormone analogues are applied to the eggs. (Saxena and Sharma, 1972). Various types of delayed effects during postembroyonic life have been reported. (Riddiford 1971). Treatment of the last larval instar with a JHA results in abnormal pupation. This effect has been called the morphogenetic effect (Novak 1966). Effect of exogenous application of juvenile hormone to the last instar depends on the age as well as period of exposure. Early instar larvae treated with juvenile hormone analogues usually give rise to supernumerary instars. If treated late, they usually end up as larval pupal mosaics. Treatment of pupae with juvenile hormone analogues results in a further pupal molt either complete or incomplete depending on the age of the pupa and potency of the compound used.

The control potential of JHAs can be adequately assessed by investigating its effects on pest population. It is important here to note that insects are susceptible to

JHAs only at certain stages of their life cycle which are therefore known as the JH sensitive stages. Before studying any insect population it is therefore necessary to establish the specific sensitive stage of a given taxonomic group. Many important agricultural pests are lepidopterans. In this order, among effects observed after JH treatment of the susceptible stages are embryonic inhibition, diapause and morphogenetic disruption. For the stored grain, pests mainly coleopterans, the JHAs are generally mixed in stored commodities. The effect here is manifested in the larvae in the form of morphogenetic deformities. In some cases eg. in Tribolium castaneum (Amos et al. 1978) there may be a combination of morphogenetic effects on larvae and ovicidal effects on the eggs. Among insects of public health importance such as mosquitoes JHAs are most effective on last instar larvae, which makes them deformed as a result. (Phadnis et al. 1988). Special formulations of JHAs e.g. Controlled Release SR 10 of methoprene have been developed for better efficacy. Recently some JHAs have been shown to exercise embroyonic inhibition (ovicidal) delayed larval toxicity as well as morphogenetic disruption of larva, pupa-adult transformation resulting in overall dramatic decline in total adult emergence. Cockroaches and ants have also been controlled by JHAs used in suitable baits. These treatments have been found to be especially useful and appropriate in e.g. hospital premises. Termites have also been controlled by JHAs by treating wood and inserting it into the termite colonies. This results in gross disruption of normal larval development in the termiteria and consequent mayhem and destruction of the colony. Effects of JHAs have also been extensively studied on various non-target as well as beneficial species. JHAs, especially methoprene have been successfully used in silk production, in silk worm Bombyx mori (Akai et al. 1971, Akai 1979, Murakoshi et al. 1972).

Studies on JHAs have largely been restricted to the major insect orders of Diptera and Lepidoptera on account of their overriding economic importance. The

order Hemiptera has also figured fairly prominently in JH studies mainly because many hemipterans were found to be extra sensitive to JH action. In this resume details of various investigations on JHA effects on specific orders of insects have not been reviewed as has been done in for other test compounds used in the present work, viz. the IGR Dimilin.

Pharmacodynamics :-

JHAs gain entry into the tissue of insects more readily by ingestion. However, on account of their terpenoid nature they are also able to penetrate the insect cuticle and are therefore effective by contact also (Staal G. 1972). JHAs are usually formulated as a solution in oil or as emulsion in water. Metabolism/fate of a few JHAs analogues has been studied in insects, mammals and the environment and results have been succinctly summarised by Hammock and Quistad (1981). The metabolism of methoprene has been studied in various insects and well documented by some authors. The metabolism of hydroprene has been studied in house fly Musca domestica and Dysdercus Koenigii (Tungikar et al. 1978). Surprisingly resistance has also been established against these compounds and has been attributed to slower intake, faster elimination and increased detoxification resulting in overall reduced accumulation of the bioactive molecules (Brown et al. 1978, Brown and Brown 1980).

Recent work done on Juvenile Hormone analogues :-

JHAs have been implicated in the control of diuresis after a blood meal in Aedes aegypti by Wheelock et al. (1988). Effects on ovarian development, embryogenesis, larval, pupal and adult deformities as well as mortality as a consequence of JHAs treatments in mosquitoes/houseflies has been reported variously by Klowden and Chambers

(1981), Pawar et al. (1989) Sinha, et al. (1992). Regulation of reproduction by JHA mimic pyriferon has been reported in Rhodnius prolixus by Longley et al. (1990). Idriss (1990) has studied the action of JH and ecdysone in the metamorphic endocrine centre. Several authorities have studied the role of the JHA, esterase in Diptera. (Rauschenbach et al. 1991). Organophosphorus inhibitors of JHA esterase have been studied by Linderman et al. (1991). Chinzes et al. (1991) have studied the vitellogenesis synthesis and overian development in JHA treated Ornithodoros moubata. Bogus and Scheller (1991) have studied the action of hydroprene on the JH synthesizing system of Galleria mellonella larva.

Continuing work on synthesis of new JHAs as well as identification of plant products exhibiting JH effects have been cited earlier in the Introduction.

BENZOYLPHENYL UREAS

In the year 1970 the Philips Duphar Company of Holland discovered the insecticidal activity of benzoylphenyl urea analogue. This analogue was demonstrated to be effective against insects and was designated as DU 19.111. The latter is basically a combination of the herbicide dichlobenil with the urea herbicide diuron. The chemistry and effects of this analogue are unique and differ from conventional synthetic organic insecticides. Hence this is now considered as a new class of insecticide. Since the discovery of the base molecule, several variations (different chemical configurations) of benzoyl ureas have been prepared. Many of these are now available for field use also (Retnakaran et al. 1985).

Chemistry :-

Benzoylphenyl ureas consist of two substituted ring structures connected by urea bridge. The substituents are generally halogens (chlorine and fluorine). These compounds are highly insoluble in water and many organic solvents. They are, however, soluble in acetone, dioxane, dimethylformamide (DMF) and dimethylsulfoxide (DMSO) and are commonly used as solvents for these chemicals. Vapour pressure of these compounds are low. They tend to be stable in non-biological environmental conditions although they are degraded in basic solutions. (Maas et al. 1981, Verloop and Ferrell 1977).

Effects :-

Effect of benzoylphenyl urea on organisms can be studied at many levels of complexity. The main level is the biochemical wherein the chemical interacts with specific definable sites within the organisms. This level indicates the mode of action of the toxicant. These basic biochemical effects induce physiological disturbances leading to functional disturbances. The overall consequence of all these activities is mortality which is reflected at the population level also.

The nature of chitin polymers as well as their chemical structures are well known (Candy and Kilby 1962, Chippendale 1978). Muzzarelli (1976) and Neville (1975) have reviewed information currently available on chitin chemistry.

Biochemical effects :-

Biochemical effects of benzoylphenyl urea analogues on insects have been extensively studied especially in context of the moulting process.

Various authors (Post and Vincent 1973 Post et al. 1974) have studied the fate of injected labelled glucose in cabbage butterfly larvae and found that less labelled chitin was produced in benzoylphenyl urea treated than in non-treated larvae. This result was confirmed by Deul et al. (1978). Inhibition of chitin formation was also seen with other benzoylphenyl ureas in gypsy moths and stable flies with in vitro tissue systems (Abdel-Mohem et al. 1980, Mayer et al. 1981). Inhibition of chitin synthesis is the primary result of insecticidal action. This conclusion is supported by Von Eck (1979) from in vitro cuticle studies using house fly larvae. These findings indicate the enzyme chitin synthetase as the actual biochemical moiety which interacts with the

toxicant. Several workers have isolated chitin synthetase and tested in vitro against diflubenzuron. The first purified cell free system from insect tissues was established by Mayer et al. (1981). Cohen and Casida (1980 a,b) isolated a chitin synthetase cell free system from the gut of Triboleum castaneum. Lack of diflubenzuron activity directly on chitin synthetase was demonstrated by Leighton et al. (1981). Leighton et al (1981) found that dimlin inhibited chymotrypsin. Other biochemical anomalies such as increased chitinase etc. have also been reported (Ishaaya T. and Casida, 1974) in houseflies. The effect of diflubenzuron on chitinase was contested by Deul et al. (1978) who found no effect on chitinase in cabbage butterfly larvae. Yu and Terriere (1975, 1977) showed that activity of ß-ecdysone metabolizing enzymes was increased after diflubenzuron treatment. Ishaaya and Asher (1977) reported a significant depression in the activities of trehalose, amylase and invertase in Triboleum castaneum treated with diflubenzuron. Increased phenoloxidase activity due to diflubenzuron treatment was observed by Ishaaya and Casida (1974). Increased activity of this enzyme would possibly accentuate the darkening and hardening of exocuticle. Deul et al. (1978) explained the darkening of the cuticle of insects dying of diflubenzuron ingestion as due to increased phenoloxidase activity. Mitlin et al. (1977) showed that DNA synthesis in diflubenzuron-sterilized boll weevils was inhibited. RNA and protein synthesis in males was not affected, but lipoprotein synthesis was decreased. These results supported the hypothesis that the juvenilizing symptoms seen in diflubenzuron treated larvae and the sterility in adults are due to inhibition of DNA synthesis.

Physiological effects :-

Biochemical effects of benzyolphenyl ureas on chitin synthesis directly affect the insects moulting physiology. Additional physiological effects have been found which may indicate a biochemical site independent of chitin synthesis. Disruption of imaginal

disc development of flies has been shown by Meola and Mayer (1980). Various authors have noted JH mimetic effects with diflubenzuron (Retnakaran and Smith 1975).

Organismal effects :-

The spectrum of effects of benzoylphenyl ureas treatment follows a consistent pattern which reflects the primary site of action namely disruption of chitin synthesis. The effects may be categorized as namely - (1) Disruption of ecdysis (2) Failure to feed (3) Factors related to delayed mortality.

Survey on Action of Benzoylphenyl Ureas on Different Insects

(A) Lepidoptera :-

A large number of lepidopterans have been tested with benzoylphenyl urea analogues. The moult disrutpion syndromes in larvae are the most common symptoms observed. If treatments are made during the last instar stage, pupation is either prevented or the larval-pupal moult is initiated but not completed (Mulder and Gijswijit 1973). In caterpillars treated at the last instar stages with diflubenzuron, pupal abnormalities were observed. Lepidopteran larvae were unable to feed and died of starvation after an apparently successful ecdysis with no obvious morphological abnormalities (Zabel and Ostojic 1973, Abid et al. 1978, Brushwein, 1980). Effects of benzoylphenyl ureas on lepidopteran reproduction through treatments of adults have not been commonly studied. Slama et al. (1976) reported no effects on spermatogenesis, mating or oviposition of nun moth Lymontzia monacha. Direct effects after treatment of eggs have been observed in a number of species such as codling moth Laspeyresia pomonella (Hoying and Riedl 1980) the Egyptian cotton worm Spodoptera littoralis (Ascher and

Nemny 1974) and the soyabean looper Pseudoplusia includens (Reed and Bass 1980).

(2) **Coleoptera** :-

Effects on beetles have been characterized as disruption of molt as well as reproduction. Treated larvae lose their locomotor ability, stop feeding, become dessicated, darken and die. Larvae of alfalfa weevils Hypera postica treated with diflubenzuron developed into adults which were not able to escape from the pupal cuticle. Ovicidal effects were also observed when adult females ingested diflubenzuron. Earle and his co-workers (1978) found that diflubenzuron also affected mating behaviour of young male boll weevils. Mating success could be reduced as much as 50% by dipping adults in acetone solutions of diflubenzuron.

(3) **Diptera** :-

Treatment of the aquatic habitat of mosquitoes with benzyolphenyl ureas resulted in larval mortality. Malformation of pupae resulted either in immediate death or delayed mortality due to incomplete emergence of adults from the pupal cuticle (Jacob 1973). Malformed adult appendages have been observed which interfere with take off and flight of the mosquito (Dame et al. 1976, Self et al., 1978). Effects on house flies were described by Rupes et al. (1977). Larvae treated at high concentrations became immobile, stiff and not able to rupture the cuticle at the moult. Treatment at low concentrations allowed moulting from one instar to the next but frequently resulted in elongated pupae. Ovicidal effects have been observed in a number of fly pests of cattle, including horn flies, stable flies, house flies. These effects occured after topical treatments of adults and through feeding. The larvae died within the eggs only (Ivic and Wright 1978, Kunz and Bay 1977, Wright and Spates 1976). At high doses of diflubcn-

zuron the ovicidal effect was permanent, whereas at low doses after a period of time without access to diflubenzuron individual flies oviposited viable eggs (Kunz and Bay 1977, Rupes et al. 1977). No ovicidal effects were seen with treatment of mosquito larvae (Arias and Mulla 1975). However, treatments of adults or eggs did result in ovicidal effects (Miura et al. 1976).

Field use of Benzoylphenyl Urea :-

In the aquatic environment, benzoylphenyl ureas including prominently dimlin, are highly active against a range of aquatic insects such as mosquitoes, midges and chronomides.

Diflubenzuron use has been proposed for controlling flies which breed in livestock manure. Pickens and Miller (1975) studied the use of diflubenzuron as a feed additive for cattle to control face flies. Ables et al. (1975) considered the use of diflubenzuron to control house flies which breed in poultry manure. Various crops, especially in cotton have a wide variety of insect pests associated with it as well as beneficial insects. Integrated control of cotton pests is becoming important and hence diflubenzuron effects on the natural enemies are important. Diflubenzuron has been shown to be responsible for the decrease in number of predators namely geocordis and nabids in soyabean fields. Diflubenzuron has been tested against a number of forest insects with their parasite complex. Wild and domestic bees are important beneficial insects responsible for pollination in forests, field crops and orchards. Diflubenzuron has been tested against honeybees in the field as well as under more controlled situations. High concentrations of diflubenzuron fed to bees in sugar, water or plain water resulted in reduced brood and comb protection.

Pharmacodynamics :-

Toxicity of insecticides is equated to the amount of chemical accumulating at the active site and mode of action. Treatment methods are topical, dietary, contact or direct. Metabolism of an insecticide within insects has a direct relationship with toxicity. Degradation of the active material will decrease the quantity of toxicant available for interaction with the active site. On the other hand, activation of a compound which is structurally in a less active state, will increase the toxicity. Differences in metabolism may also explain the differences in toxicity between strains or species of insects. Degradation of diflubenzuron has been documented for boll weevils by Chang and Stokes (1979). Metabolism of diflubenzuron and penfluron by house flies was measured by Chang (1978) and Chang and Woods (1979). They found that metabolites detected were either conjugates hydroxylated insecticide or the self hydroxylated products.

Resistance, when diflubenzuron is the selection agent, has also been reported in some insects. Pimprikar and Georghious (1979) reported greater than 1000 X resistance with a house fly strain stressed with diflubenzuron.

Recent work done on Benzoylphenyl Ureas

Biochemical findings such as increase in esterase activity, decrease in acid phosphatase and increase in the density of electrophoretic protein bands were recorded in the Dimlin treated American bollworm (Abdeen et al. 1986). The action of acylurea on the cuticle, growth and moulting of insects has been well reported by Reynolds (1987). In a recent carefully controlled study Manduca larvae given an oral LD90 dose of diflubenzuron continued to deposit cuticle matrix essentially normally although the production of chitin microfibrils was completely prevented (Hassan et al. 1987). Hsu et

al. (1987) reported the effect of Dimlin, a chitin synthesis inhibitor on the growth and development of larvae of Aedes albopictus skuse. Tyagi et al.(1987) studied the evaluation of three formulations of chitin synthesis inhibitor (fenoxycarb) against mosquito vectors. Vasuki et al. (1990) reported the effect of IGR on hatching of eggs of three vector mosquitoes.

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CHAPTER TWO

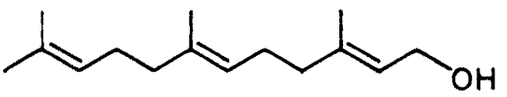
Effects of IGR's on Development and Metamorphosis of *Aedes aegypti*

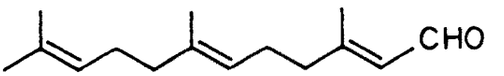
Introduction

In insects, growth and differentiation are regulated by several hormones. The growth and moulting of immature insects is regulated by three main groups of hormones viz. brain hormone, ecdysone and juvenile hormone. Growth in vertebrates and in higher invertebrates is associated with the non-reproductive juvenile stage and in both vertebrates and insects maturation is under hormonal control. As we have seen, juvenile condition in insects depends upon the continued presence of the remarkable chemical given the very appropriate and special name, juvenile hormone, which acts on the cells themselves, and prevents them from maturing. It has already been mentioned in the Historical Review that not only were there different chemical species for the natural juvenile hormone, several different types of synthetic analogues or natural products have also been shown to possess juvenile hormone activity. These various compounds which exhibit properties of natural insect juvenile hormone have been variously termed as Juvenile Hormone Analogues (JHA's), JH mimics, Juveo-mimetic chemicals and Juvenoids. The history of juvenoid begins with Williams (1956) who was the first to obtain juvenile hormone effects on metamorphosis using lipid extracts prepared from abdomens of adult male cecropia moths. The presence of juvenile hormone activity in these extracts was soon confirmed by Wigglesworth (1958).

Bowers (1965) discovered that methyl-10,11-epoxyfarnesate had exceptional activity. Its extraordinary activity led Bowers to speculate that the naturally occuring hormone, when isolated would have a quite similar structure. After extensive research programmes finally he was successful in the isolation and identification of five homologues natural JHs.

Schmialek (1961, 1963) identified farnesol as the active substance from Tenebrio experiments and yeasts. Farnesol was the first pure compound with definite juvenile hormone activity.





FARNESOL **FARNESAL**

Schmialek (1963) was the first to test juvenile hormone activity in the parental alcohol 3,7,11-trimethyl 2,6,10-tridecarterin 1-01 of the second active compound isolated later from the cecropia extracts. The most active component of the preparation with especially high activity on certain Hemipteran was identified by Romonuk et al.(1967) as 7,11 dichlorodihydro-farnesoate. This compound was extensively used in studies on female sterility, ovicidal effects and inhibition of metamorphosis by juvenoids. Paper factor effects were identified as juvabione by Bowers et al.(1966) and dehydrojuvabione by Cerny et al.(1967). The first aromatic juvenoids were prepared by Suchy and Coworkers (1968). They were structurally related to aromatic juvabione. Further progress in the field of aromatic juvenoid chemistry was stimulated by Bowers (1968, 1969) on aromatic terpenoid ethers and insecticide synergists. Finally, the peptide juvenoids introduced by Zaoral and Slama (1970) were essentially aniline or p-aminobenzoic acid derivatives with side chains containing amino acid residues. This encouraged extensive analogue synthesis aimed at the development of selective insecticides with juvenile hormone activity (Henrick, 1982).

These various juvenile hormones and their analogues affect virtually all the insects upon which they have been tested. In immature insects they produce a variety of morphogenetic effects. These also affect the development of internal organs including

the central nervous system, the gonads, and the midgut, where they prevent maturation and metamorphosis (Sehnal 1968). Juvenile hormone blocks not only the metamorphosis of the larva to the adult, but it also blocks the equally profound development of the embryo to the larva. Juvenile hormones also exert a gonadotropic effect, promote the synthesis of yolk protein by the fat body and the accumulation of these proteins in the developing oocytes of many insects. (Engelman 1968, 1970 and Engelmon et al·1971). Juvenile hormone may also activate the prothoracic gland of Lepidopterans and break adult reproductive diapause in insects such as the alfaalfa weevil. In some adult insects these hormones appear to be necessary for the production of pheromones and are involved in various sorts of sexual behaviour.

In all cases of juvenoids for which reliable bioassay data are available, it appears that only one stereoisomer is directly responsible for the morphogenetic activity. Inactive stereo-isomers simply dilute the active principle in the mixtures. Zoecon Corporation have replaced the racemic methoprene (Henrick
and Staal 1988) and hydroprene in many of its products with the S-isomers of high geometrical and optical purity.

The insect growth regulator (IGR), dimlin was chosen for biological assessment in the present work, since it is now well established that these compounds interfere with the cuticle deposition, apparently by the inhibition of chitin synthesis. (Post and Vincent 1973). It was felt that comparison of JHA's with this class of IGR would yield valuable insights into effects and action of the former two.

In the present study the active optical isomers S- methoprene and S-hydroprene, have been examined against different stages (II, III and IV instars) of A. aegypti to assess their persistence and efficacy.

LITERATURE SURVEY

Present knowledge of structure activity relationship of juvenile hormone analogues have been well discussed. (Schneiderman et al. 1965 Crucckshank et al.1971, Pallos et al.1971 Redfern et al. 1971, Slama 1971, Wigglesworth 1969, Zaoral 1970). Sensitive periods of JHA action are always limited but the effects are often delayed and knock-down effects do not occur. JH is a major regulator of the insects development not only of metamorphosis and oogenesis, but also of a variety of other processes. Exposure to exogenous JHA at the moment of low endogenous titer is very disruptive. During the developmental cycle of an insect, biological and physiological changes may influence the morphological characters of its body. Treatment of immature stages of insects at a critical time with juvenile hormones has been reported to interfere with protein synthesis. (Hill 1965, Coles 1965, Minks 1967) and accordingly differences in the sensitivity of head, thorax and abdomen may be found (Serihari 1974, Podufal 1975). Juvenile hormone mimic compounds possessing the biological activity of insect juvenile hormone are known to derange embryogenesis and metamorphosis and under general conditions, to reduce fertility in adults (Bowers 1971 and Slama 1971). Review were done on effect of three juvenile hormone analogues on insects forms. (Gawaad 1976). Saxena and Thorsteinson 1971, Bhaskaran et al. 1971, 1972, Saxena and Sharma 1972 have worked on effect of these JHA on Aedes aegypti, Schaefer and Wilder (1972) on Culex pipiens quinquefasciatus; C. tarsalis and Aedes nigromaculis (Diptera) reported this effect. Naqvi et al. (1976) reported the effect of Altosid (JHA-ZR 515) on Aedes aegypti. Effects of juvenile hormome mimics on larval development and metamorphosis of Drosophila melanogaster have been well studied. (Riddiford and Ashburner 1991). Sehnal and Zelark (1976) studied the action of juvenoids on the metamorphosis of Cyclorrhaphous (Diptera). Action of juvenile hormone on vitellogenin production by the mosquito Aedes aegypti have also been reported (Michael et al. 1988). A newly

synthesized juvenile hormone analogue 2-[1-methyl-2(4-phenoxyphenoxy) ethoxy] pyridine (S-31183) was found to be about 320 times more active than methoprene in Manduca black larva (Hatakoshi et al. 1988). Adems et al. (1989) studied the effect of 20-hydroxyecdysone and a juvenile hormone analogue on vitellogenin production in male houseflies Musca domestica. In most insects female specific egg protein precursors (vitellogenins) are synthesized during pupal or adult stages in response to 20-hydroxyecdysone and/or juvenile hormone. (Davis et al. 1990). Studies were also carried out on the effectiveness of methoprene in water jars in Bangkok, Thailand for the control of Aedes aegypti (Boonluan W.H.O. Bulletin). Insect growth regulators (IGR) induce delayed morphogenetic changes in larvae, pupae and adult insects when they are treated at the larval stage. Widely reported developmental aberrations induced by some IGRs' are the prolongation of the larval stage (Akai and Kobayashi 1971), larval-pupal intermediates, (Spielman and Williams 1966, Varjas and Sehnal 1973), and pupal adult intermediates (Chase 1967, Critchley and Campion 1971 a,b). Regulation of development of mosquitoes exposed to IGR has been reported by several workers. Sacher (1971) showed that in the MON-585 most mortality occured after ecdysis of the 4th instars before the pupal cuticle hardned and melanized. Spielman and Skaff (1967) reported abnormal development in mosquitoes after treatment with farnesoic acid derivatives and categorized these abnormalities in to 10 groups. Spielman and Williams (1966) found developmental intermediates in Aedes aegypti treated with crude synthetic juvenile hormone. Jakob and School (1971, 1972) reported developmental intermediates and anamalous pupae after application of various IGR's. Six species of stored product insects were reared on diets treated with methoprene and hydro-prene to examine the effects on survival, development and or reproduction (Loschiavo 1976).

Juvenile hormone (JH) mimics and insect growth regulators (IGRs) exercise their maximum effect at the time of metamorphosis (Spielmon and Williams 1966, Spielman and Skaff 1967; Jakob and Schoof 1971, 1972; Georghiou and Lin 1974; Astafon 1974).

Dimlin Thompson - Hayward (TH 6040) [1-(4-chlorophenol)-3(2,6 diflurobenzoyl) urea] is an experimental insecticide with a wide range of biological activity. The mode of action of the compound has been reported as the inhibition of chitin synthesis during moulting, thus interfering in the formation of endocuticular deposition. (Mulder and Gijswijit 1973, Post and Vincent 1973). Preliminary studies of its potential use as mosquito larvicide have been reported by Jacob (1973), Schafer et al. (1974). Miura and Takahashi (1974) reported results from the laboratory and limited field studies concerning the effeets of TH 6040 on non-target organisms associated with mosquito breeding habits. A new urea type compound (Van Daalen et al. 1972 Wellinga et al. 1973) showing insect growth regulating properties by inhibiting chitin formation, (Post and Vincent 1973) was recently studied by Mulla et al.(1974 a,b) and Jakob (1973) against mosquitoes, houseflies and midges, dimlin unlike the juvenile hormone type of compounds, produced most of the mortality in the larval stages of the mosquitoes. This compound was also found to have exceptional activity againnst both mosquitoes and midges. (Mulla et al. 1974 a,b). Laboratory tests of dimlin showed a decrease in susceptibility of fourth instar stage as compared to third instar larvae which was not evident in tests with Altosid (Rathburn and Boike 1975). Developmental inhibition of mosquito and the house fly by urea analogues were reported by Jakob (1973). Penfluron and difluron, the disubstituted benzoylphenyl urea compounds reported to cause chitin synthesis inhibition have also been found to affect reproducibility of adult Triboleum castaneum (Saxena and Mathur 1981). A high biological efficacy of diflubenzuron for mosquito larvae of Aedes and Culex genera was detected in laboratory and field

conditions (Rettich 1978). Laboratory selected resistance to diflubenzuron in larvae of Aedes aegypti have been reported (Walker and Wood 1986). Three plastic formulations of both Dursban and Dimlin were tested as controlled release pellets against larvae of C. pipiens and A. aegypti (Saleh et al. 1981). Series of experiments were carried out to investigate the biological effects of TH 6040 on immatures and adults of Culex pipiens fatigans Weid (Sharma et al. 1979). The functions, structure and biochemistry of the insect cuticle in relation to the moulting cycle are briefly reviewed as an introduction to the actions of insecticides that act on the cuticle, particularly acylureas (Reynolds 1987). A number of analogues of the insect growth regulators TH 6038 and TH 6040 were synthesized and tested against four species of insects (Oliver et al. 1976). The efficacy of two chitin synthesis inhibitors viz. diflubenzuron and penfluron was assessed against Aedes aegypti, Culex quinque fasciatus, Anopheles stephensi and A. culicifacies by treating them continuously at second, third or fourth instar larval stage till pupation (Bhakshi et al. 1982). The efficacy and longevity of various formulations of 10 insect growth regulators were investigated against mosquitoes in the laboratory and field (Mulla and Darwazeh 1975).

MATERIALS AND METHODS

The Mosquito Colonies -

Experiments were conducted on different larval instars (II, III and pupa) of laboratory reared strains of Aedes aegypti (L). The culture maintenance regimes followed were based on the protocols suggested by Christopher (1960). Aedes aegypti were reared in a special mosquito insectary maintained at temperature of 28°C ± 1 and relative humidity 80 to 100%. (RH).

The Chemicals :-

The compounds used for experiments in the present work were,

(A) S-Hydroprene (312-006)

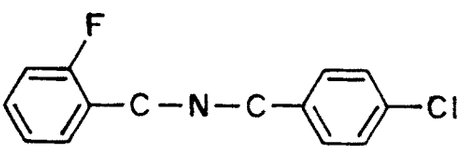
S-Methoprene (312-008)

These compounds were obtained through courtesy of Dr. G.B. Staal of the Zoecon Research Institute, Palo, Alto, California, USA. The Zoecon Corporation has replaced by these as S isomers of high geometrical and optical purity as these are more active (probably about 3 : 1 times) than the (R) isomers.

(B) Diflubenzuron (Dimlin) (Tech)

PHILIPS - DUPHAR, Holland.

Chemical Structure :-



Purity 97.6%

Diflubenzuron (WP) Formulation

25% Wettable Powder.

Experimental Methods :-

Except Dimlin (WP) all test chemicals were dissolved in analytical grade acetone and diluted to desired concentrations. From the latter, 0.05 ml was added to 50 ml. water as per the WHO Standards (WHO- Technical Report 1970). Chosen developmental stages of mosquitoes (different instar larvae) were immersed in the above aqueous solutions as per the designs described below.

Design I : Continuous Exposure :-

In this experimental design larval instars II-IV were used. Each stage was exposed to the test chemicals in desired concentrations right up to emergence of adults. In other words the II instars were exposed for the duration from II through III III to IV and pupa. The III instars were exposed for the duration of III and the IV instars and the pupa, while the IV instars were exposed to the test chemical for the duration of their IV instar and pupal stages only.

Design II :- **Discontinuous Exposure** -

In this experimental design III and IV instar larvae of A. aegypti were exposed for specific periods ranging from 30 and 60 minutes, 8 hrs. and 3 days separately to the test chemicals. Unlike the protocol in design I the test larvae here were transferred to untreated water following completion of predetermined exposure period to the test chemical.

For both designs dose ranges from 1×10^{-6} ppm to 1 ppm were used. Larval and pupal mortality as well as numbers of normal and/or abnormal adults emerging from

the treated larvae were recorded.

Additionally, in the first design incorporating continuous exposure of the experimental larvae to the test chemicals, Larval Growth Index (LGI) and Total Developmental Growth Index (TDGI) were also calculated, as given below :

$$LGI = \frac{\% \text{ pupation}}{\text{Larval period (days)}}$$

$$TDGI = \frac{\% \text{ Emergence}}{\text{Total development period (days)}}$$

Types of developmental inhibition observed :-

Since a fair variety of effects are observed as a consequence of JH/IGR treatment of various larval stages, these have been annotated as below for convenience.

LM :- Represents death during the larval stage, without initiation of pupation.

PM :- The pupa completely escapes from the larval cuticle, but remains partially or totally unmelanised, and eventually dies.

AM :- Here, adults emerge completely but are unable to fly away from the water surface and eventually die.

NA :- No abnormal effect : Full grown normal mosquito adult emerge and survive.

Absolute Potency :-

It may be noted that absolute potency determination of compounds such as test chemicals used here, which have delayed action, requires more complex assessment than with conventional larvicides. The latter can be evaluated by percentage hatch or by deaths at the end of the continuous or short term exposure periods. In the present work, the active JH isomers and dimlin were examined in different larval instars of A. aegypti at different exposure periods. In these tests, larvae and pupae were kept until all had died or emerged as adults. The dose mortality curves (and IC 50 values) and other parameters were calculated on this basis.

RESULTS

Design I :-

(1) Hydroprene :-

Continuous exposure of the test larvae (II and III) did not yield significant larval mortality at doses below 1 ppm. At 1 ppm 71.42% cumulative larval mortality was elicited when continuous exposure was given from II instar onwards. In this treatment, while no pupal mortality was obtained, 14.28% abnormal adult mortality was produced, the remainder being normal adults. Continuous exposure of III instars (through IV) to 1 ppm test concentration produced 31.76% larval mortality, 31.64% pupal mortality, 17.54% abnormal adult mortality and 23.56% normal adults. (Table I and II).

The IV instar larvae presented an altogether different picture. Even at lower dosages (from 1×10^{-6} onwards) larval and pupal mortality as well as effects on adults were obtained, although some normal adult emergence and survival also occured. At 1 ppm treatment the IV instars exhibited 30.0% larval mortality, 60% pupal mortality and 10% abnormal adult mortality. No normal adults emerged or survived at this concentration (Table III).

(2) Methoprene :-

When II and III instar larvae were exposed to methoprene continuously at different range of concentrations, no significant larval mortality occured even at 1 ppm. Pupal mortality (22.29%) in case of II instar started occuring from 1×10^{-1} ppm at continuous

Table I : Effect of S-Hydroprene on different developmental stages, LGI and TDGI of II instar larvae of *Aedes aegypti* (L) on continuous exposure.

Dose (ppm)	LM(%)	PM (%)	AM (%)	L(days)	P (%)	NE(%)	TDP (days)	LGI	TDGI
Control	0	0	0	13	100	100	18	7.692	5.55
1×10^{-6}	0	0	0	15	100	100	20	6.66	5.00
1×10^{-5}	0	0	0	14	100	100	19	7.14	5.26
1×10^{-4}	0	0	0	14	100	100	19	7.14	5.26
1×10^{-3}	0	0	0	12	100	100	18	8.33	5.55
1×10^{-2}	0	14.28 ± 1.21	0	9	85.72 ± 6.50	85.71 ± 5.85	14	9.52	7.14
1×10^{-1}	0	11.11 ± 1.36	22.22 ± 2.12	10	88.89 ± 5.94	66.66 ± 4.29	13	8.88	5.12
1	71.42 ±6.35	0	14.28 ±1.25	10	28.58 ±3.51	14.28 ±2.19	13	2.85	1.09

LM - Larval Mortality
PM - Pupal Mortality
AM - Adult Mortality
P - Pupation
NE - Normal Adult Emergence.

L : Total larval period (days)
TDP : Total developmental period (days)
LGI : Larval growth index
TDGI : Total developmental growth index

Table II :- Effect of S-Hydroprene on different developmental stages, LGI of of III instar larvae of *Aedes aegypti* (L) on continuous exposure.

Dose (ppm)	LM (%)	PM (%)	AM (%)	L	P (%)	NE (%)	TDP (days)	LGI	TDGI
Control	0	0	0	7	100	100	13	14.2	7.69
1×10^{-6}	0	0	0	8	100	100	11	12.5	9.09
1×10^{-5}	0	0	0	7	100	100	11	14.2	9.09
1×10^{-4}	0	0	0	6	100	100	10	16.66	10.00
1×10^{-3}	0	0	0	9	100	100	12	11.11	8.33
1×10^{-2}	5.55 ±1.23	0	5.55 ±1.25	10	94.55 ±2.12	88.88 ±2.34	13	9.45	6.83
1×10^{-1}	14.28 ±3.52	14.28 ±1.25	0	8	71.44 ±5.23	71.42 ±3.46	11	8.93	6.49
1	31.76 ±4.92	31.64 ±2.34	17.54 ±3.58	9 ±4.29	30.6 ±2.39	23.52	12	3.4	1.96

Table III :- Effect of S-Hydroprene on different developmental stages, LGI, TDGI of IV instar larvae of A. aegypti (L) on continuous exposure.

Dose (ppm)	LM (%)	PM (%)	AM (%)	L (days)	P (%)	NE (%)	TDP (days)	LGI	TDGI
Control	0	0	0	5	100	100	8	20.0	12.5
1×10^{-6}	10.75 ±1.23	0	6.45 ±1.25	4	89.25 ±3.89	82.14 ±5.89	7	22.31	11.73
1×10^{-5}	5.55 ±1.29	16.66 ±1.30	61.11 ±3.45	4	77.79 ±4.26	16.66 ±1.89	7	19.44	2.38
1×10^{-4}	13.33 ±2.32	13.33 ±2.22	60.0 ±4.59	3	73.74 ±4.19	13.33 ±1.21	6	24.44	2.22
1×10^{-3}	16.66 ±2.36	5.35 ±1.23	66.66 ±3.83	3	77.99 ±5.21	11.11 ±1.20	6	25.66	1.85
1×10^{-2}	52.0 ±4.50	22.0 ±3.63	25.0 ±1.28	4	26.0 ±2.1	1.0 -	7	6.5	0.142
1×10^{-1}	45.0 ±4.90	45.0 ±3.93	10.0 ±1.20	3	10.0 ±1.23	0	0	3.33	0
1	30.0 ±2.34	60.0 ±6.23	10.0 ±1.29	3	10.0 ±1.95	0	0	3.33	0

larval exposures and increased to 60% at 1 ppm. 10% abnormal adult mortality & 30% normal adult emergence also occured. However, marginal pupal mortality and abnormal adults were found in III instar. Continuous larval treatment at 1 ppm (4.76% pupal mortality, 10.52% abnormal adult mortality and 89.47% normal adult emergence) (Table IV-V).

The IV instars represent a different pattern here also in that larval, pupal mortality and deformed and normal adults are observable from 1×10^{-6} ppm onwards. At 1 ppm, IV instar exhibited 22.22% larval mortality, 77.77% pupal mortality and no adult emergence whatsoever. (Table VI).

(3) Diflubenzuron (Tech) :-

When II instars were treated with this chemical, larval mortality occured even at the lowest dose (1×10^{-3} ppm). Thus 42.8% cumulative larval mortality, 21.42% pupal mortality, 28.57% normal adult emergence were recorded. Above 1×10^{-3} ppm, up to 1 ppm, 100% larval mortality was elicited. (Table VII).

In case of III instar larvae treated at 1×10^{-6} ppm dose, 37.5% larval mortality, 6.25% abnormal adult mortality and 62.5% normal adult emergence was elicited. At all doses above 1×10^{-6} ppm, 100% larval mortality was exhibited (Table VIII).

The IV instars did not show any larval mortality up to the 1×10^{-3} ppm dose. However, 10% pupal mortality and 90% abnormal adult emergence were recorded. Above this concentration 100% larval mortality at all doses was obtained (Table IX).

(4) Diflubenzuron (25 WP) :-

Continuous exposure of III instar to diflubenzuron (WP) induces larval mortality from 1 x 10^{-5} ppm onwards (13.69%). As the concentration was increased there was an increase in larval mortality. Pupal mortality and abnormal adult mortality were not observed. At 1 ppm there was 100% larval mortality, consequently no normal adults emerged. (Table X).

However, when III instars were exposed continuously, the larval, pupal mortality and abnormal adult mortality started appearing from 1 x 10^{-6} ppm concentration onwards. At 1x10^{-3} ppm concentration cumulative larval mortality was 32.50%, pupal mortality 24.07%, abnormal adult mortality 22.34% and normal adult 21.09%. From 1 x 10^{-2} to 1 ppm, 100% larval mortality was obtained (Table XI).

When IV instars were treated with this chemical, larval mortality (8.33%) pupal mortality (12.5%), and abnormal adult mortality (12.5%) was obtained at 1 x 10^{-4}. As the dose was increased there was significant increase in larval mortality resulting in decrease of adult emergence. At 1 ppm 100% larval mortality was recorded (Table XII).

IC50 (50% inhibition concentration) values were calculated for various instars using different chemicals. These are given in Table XIII.

Biological activity of S Hydroprene and S Methoprene has been compared graphically with that of Dimilin in Fig. 1.

Table IV :- Effect of S-methoprene on different developmental stages, LCI and TDCI of II instar larvae of Aedes aegypti on continuous exposure.

Dose(ppm)	LM (%)	PM (%)	AM (%)	L (days)	P (%)	NE (%)	TDP (days)	LCI	TDCI
Control	0	0	0	14	100	100	19	7.14	5.26
1×10^{-6}	4.54 ±1.21	0	0	12	95.45 ±6.85	95.45 ±6.89	17	7.95	5.61
1×10^{-5}	10.0 ±1.35	0	0	13	90.00 ±5.89	90.00 ±3.89	18	6.15	5.58
1×10^{-4}	5.26 ±1.26	0	5.26 ±1.29	12	89.48 ±7.21	89.47 ±5.60	17	7.45	5.26
1×10^{-3}	6.25 ±2.30	0.0	11.11 ±1.89	13	93.75 ±3.51	81.25 ±7.12	18	7.24	5.26
1×10^{-2}	3.59 ±1.21	1.27	21.29 ±2.50	13	96.41 ±2.86	73.85 ±6.23	17	6.75	5.18
1×10^{-1}	10.23 ±2.29	22.29 ±1.80	35.23 ±3.89	12	67.41 ±3.89	32.25 ±4.85	16	5.61	2.01
1	0	60.00 ±3.89	10.00 ±1.21	11	40.00 ±2.41	30.00 ±2.25	15	3.63	2.00

Table V :- Effect of S-methoprene on different developmental stages, LCI, TDCI of III instar larvae of A. aegypti (L) treated on continuous exposure.

Dose(ppm)	LM (%)	PM (%)	AM (%)	L(days)	P (%)	NE (%)	TDP (days)	LCI	TDCI
Control	0	0	0	5	100	100	8	20.0	12.5
1×10^{-6}	0	0	0	8	100	100	11	12.5	9.09
1×10^{-5}	0	0	0	6	100	100	9	16.66	11.11
1×10^{-4}	0	0	0	5	100	100	7	20.0	14.28
1×10^{-3}	0	0	0	6	100	100	7	16.66	14.28
1×10^{-2}	9.09 ±1.25	0	6.06 ±1.35	7	90.91 ±6.89	84.84 ±6.25	10	12.93	8.48
1×10^{-1}	7.31 ±1.29	0	4.87 ±1.26	7	92.69 ±7.21	87.80 ±7.89	8	13.24	10.95
1	4.76 ±1.11	4.76 ±1.29	10.52 ±1.89	6	90.48 ±8.23	89.47 ±8.80	8	15.08	11.18

Table VI :- Effect of S-methoprene on different developmental stages, LGI and TDGI of IV instar larvae of A. aegypti (L) on continuous exposure.

Dose(ppm)	LM (%)	PM (%)	(AM (%)	L(days)	P (%)	NE (%)	TDP (days)	LGI	TDGI
Control	0	0	0	5	100	100	8	20.0	12.5
1×10^{-6}	0	15.35 ±1.21	6.65 ±1.21	5	84.65 ±6.29	78.0 ±6.29	8	16.93	9.75
1×10^{-5}	5.0 ±1.21	20.0 ±3.26	0.15	5	75.00 ±6.29	60.0 ±4.25	9	15.0	4.0
1×10^{-4}	20.0 ±2.30	20.0 ±2.21	30.0 ±3.45	5	60.0 ±7.21	30.0 ±3.41	9	12.0	3.2
1×10^{-3}	0	50.0 ±3.51	20.0 ±2.12	3	50.0 ±4.45	30.0 ±3.89	8	16.6	3.7
1×10^{-2}	23.8 ±2.20	19.0 ±2.25	28.57 ±2.84	6	57.2 ±4.12	28.57 ±2.85	9	9.53	3.17
1×10^{-1}	14.2 ±1.26	14.2 ±1.25	57.14 ±5.21	6	71.44 ±6.24	14.28 ±1.24	8	11.90	1.78
1	22.22 ±3.29	77.77 ±6.97	0	4	0	0	7	0	0

Table VII :- Effect of Dimlin (Tech) on different developmental stages, LCI and TDCI of II instar larvae of A. aegypti (L) on continuous exposure.

Dose(ppm)	LM (%)	PM (%)	AM (%)	L(days)	P (%)	NE (%)	TDP (days)	LCI	TDCI
Control	0	0	0	14	100	100	24	7.14	4.16
1×10^{-6}	46.15 ±4.25	15.38 ±1.29	15.38 ±1.42	15	38.47 ±3.49	23.00 ±2.29	27	2.56	0.85
1×10^{-5}	54.5 ±5.65	3.03 ±1.10	21.21 ±3.40	12	42.2 ±4.26	21.21 ±3.10	18	3.51	1.17
1×10^{-4}	52.12 ±5.29	16.29 ±1.29	19.20 ±3.50	12	31.59 ±6.25	12.39 ±1.29	21	2.63	0.59
1×10^{-3}	42.8 ±3.21	21.42 ±1.40	28.57 ±2.35	9	35.78 ±2.29	7.14 ±2.34	9	3.97	0.44
1×10^{-2}	100	-	-	-	-	-	-	-	-
1×10^{-1}	100	-	-	-	-	-	-	-	-
1	100	-	-	-	-	-	-	-	-

Table VIII :- Effect of Dimlin (Tech) on different developmental stages, LCI and TDCI of III instar larvae of A. aegypti (L) on continuous exposure.

Dose(ppm)	LM (%)	PM (%)	AM (%)	L(days)	P (%)	NE (%)	TDP (days)	LCI	TDCI
Control	0	0	0	10	100	100	18	10.0	5.55
1×10^{-6}	37.5 ±3.56	0	6.25 ±1.29	9	62.5 ±3.89	56.25 ±4.25	15	6.94	3.47
1×10^{-5}	100	-	-	9	-	-	10	-	-
1×10^{-4}	100	-	-	6	-	-	10	-	-
1×10^{-3}	100	-	-	3	-	-	-	-	-
1×10^{-2}	100	-	-	2	-	-	-	-	-
1×10^{-1}	100	-	-	2	-	-	-	-	-
1	100	-	-	2	-	-	-	-	-

Table IX :- Effect of Dimlin (Tech) on different developmental stages, LGI and TDGI of IV instar larvae of A. aegypti (L) on continuous exposure.

Dose(ppm)	LM (%)	PM (%)	AM (%)	L(days)	P (%)	NE (%)	TDP (days)	LGI	TDGI
Control	0	0	0	3	100	100	8	33.33	12.5
1×10^{-6}	5.55 ±1.23	5.55 ±1.29	11.11 ±1.29	4	88.89 ±8.23	77.77 ±8.23	13	22.22	5.09
1×10^{-5}	3.07 ±2.24	18.30 ±3.45	26.29 ±3.25	4	73.29 ±6.93	52.34 ±4.29	10	18.32	5.23
1×10^{-4}	0	40.0 ±4.41	30.0 ±3.86	3	60.0 ±4.49	30.0 ±2.80	9	20.0	3.33
1×10^{-3}	0	10.0 ±1.29	90.0 ±8.29	5	0	0	9	18.0	0
1×10^{-2}	100	-	-	-	-	-	-	-	-
1×10^{-1}	100	-	-	-	-	-	-	-	-
1	100	-	-	-	-	-	-	-	-

Table X :- Effect of Dimlin (25 WP) on different developmental stages, LCI and TDCI of II instar larvae of A. aegypti on continuous exposure.

Dose(ppm)	LM (%)	PM (%)	AM (%)	L(days)	P (%)	NE (%)	TDP (days)	LCI	TDCI
Control	0	0	0	9	100	100	20	11.11	5.0
1×10^{-6}	3.33 ±1.21	3.33 ±1.21	3.33 ±1.21	8	93.34 ±7.89	89.33 ±7.89	17	11.66	5.25
1×10^{-5}	13.69 ±1.29	0	0	8	86.31 ±8.23	86.30 ±8.21	18	10.78	4.79
1×10^{-4}	20.0 ±2.20	0	3.75 ±1.28	9	80.0 ±7.25	75.0 ±4.21	18	8.88	4.16
1×10^{-3}	57.31 ±4.85	0	8.53 ±3.45	7	42.69 ±3.89	32.92 ±2.25	15	6.09	2.19
1×10^{-2}	62.5 ±6.29	0	6.25 ±2.21	7	37.5 ±4.21	31.25 ±4.25	12	5.35	2.60
1×10^{-1}	72.23 ±4.29	10.25 ±1.29	4.28 ±1.28	7	15.72 ±1.25	13.24 ±4.1	13	2.50	1.08
1	100	0	0	0	0	0	0	0	0

Table XI :- Effect of Dimlin (25 WP) on different developmental stages, LCI and TDCI of III instar larvae of *A. aegypti* on continuous exposure.

Dose(ppm)	LM (%)	PM (%)	AM(%)	L(days)	P (%)	NE (%)	TDP (days)	LCI	TDCI
Control	0	0	0	10	100	100	18	10.0	5.55
1×10^{-6}	10.0 ±1.29	10.0 ±1.29	5.0 ±1.21	7	80.0 ±9.29	75.0 ±8.23	13	6.15	5.76
1×10^{-5}	15.25 ±1.29	7.86 ±1.25	10.23 ±2.45	8	76.25 ±6.89	66.66 ±6.23	14	9.53	4.76
1×10^{-4}	20.25 ±2.35	21.12 ±2.85	16.29 ±3.85	7	53.25 ±4.45	42.34 ±5.24	16	7.60	2.64
1×10^{-3}	32.50 ±4.25	24.07 ±2.1	22.34 ±2.29	6	28.25 ±2.35	21.09 ±2.85	13	2.17	1.62
1×10^{-2}	100	-	-	-	-	-	-	-	-
1×10^{-1}	100	-	-	-	-	-	-	-	-
1	100	-	-	-	-	-	-	-	-

Table XII :- Effect of Dimlin (25 WP) on different developmental stages, LCI and TDCI of IV instar larvae of A. aegypti on continuous exposure.

Dose(ppm)	LM (%)	PM (%)	AM (%)	L(days)	P (%)	NE (%)	TDP (days)	LCI	TDCI
Control	0	0	0	7	100	100	11	14.28	9.09
1×10^{-6}	0	0	0	7	100	100	11	14.28	9.09
1×10^{-5}	11.11 ±1.29	0	0	8	88.89 ±10.29	88.88 ±7.64	12	11.11	7.40
1×10^{-4}	8.33 ±2.85	12.5 ±2.12	12.5 ±1.23	7	79.17 ±9.21	66.66 ±8.39	11	11.31	6.06
1×10^{-3}	20.0 ±3.50	0	20.0 ±4.25	6	80.0 ±6.81	60.0 ±4.28	12	13.33	5.0
1×10^{-2}	33.33 ±4.45	11.11 ±2.39	0	8	55.56 ±4.84	55.55 ±4.29	10	6.94	5.5
1×10^{-1}	41.66 ±6.89	8.33 ±1.29	10.66 ±2.25	6	60.0 ±5.23	50.0 ±5.21	10.0	15.27	4.16
1	100	0	0	0	0	0	0	0	0

Table XIII :- Determination of IC50 values (50% inhibition of adult emergence (ppm) of the IGR's treated on II, III and IV instar A. aegypti larvae.

IGR's	INSTARS		
	II	III	IV
S-Hydroprene	1.322×10^{-1}	2.220×10^{-2}	1.06×10^{-5}
S-Methoprene	1.371×10^{-1}	1	9.94×10^{-5}
Dimlin (Technical)	3.477×10^{-6}	1×10^{-6}	1.597×10^{-4}
Dimlin (25 WP)	6.16×10^{-4}	4.70×10^{-6}	1.7×10^{-2}

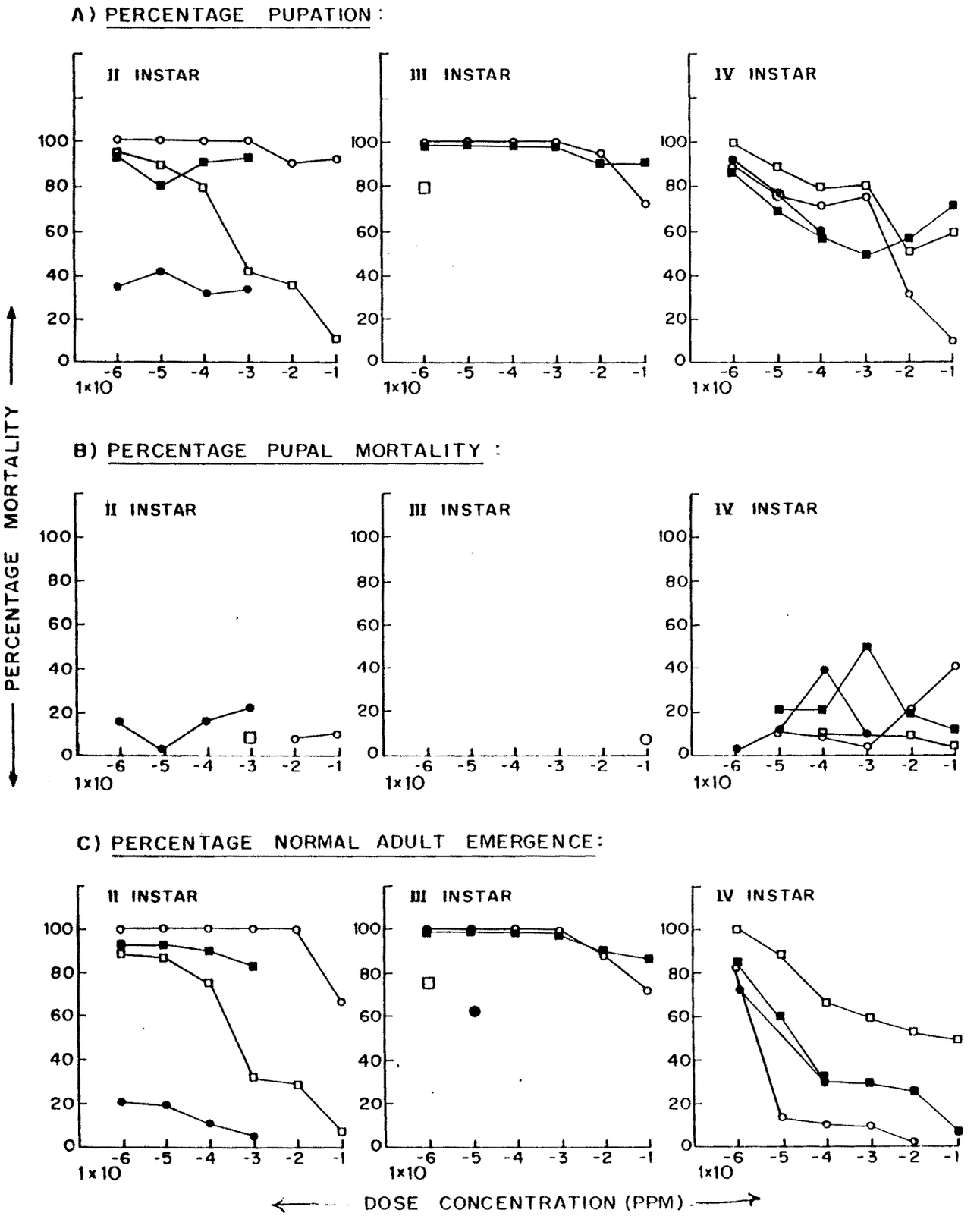


FIG. 1: COMPARISON OF BIOLOGYCAL ACTIVITY OF IGRS ON II, III AND IV INSTAR LARVAE OF A. AEGYPTI (L)
[■-METHOPRENE, o-HYELROPRENE, ●-DIMLIN (Tech.), □-DIMLIN (25 WP)]

Larval Growth Index :- (LGI) (Table I-III) -

(1) Hydroprene :-

The II instar larvae showed more or less similar LGI from 1×10^{-6} ppm (6.5 to 8.8). At 1 ppm the LGI declined substantially (2.85) as compared to other concentrations.

LGI values in III instar exhibited no difference at 1×10^{-6} ppm to 1×10^{-3} ppm (12.5 to 11.11). At higher dosages, LGI was suddenly decreased and finally at 1 ppm it was approximately 3.4 only.

In case of IV instar LGI limits range from 19.44 to 24.44 up to the 1×10^{-4} dose. With further increase in concentration (1×10^{-3} ppm) the LGI increased (25.66), but surprisingly, at higher doses such as 1×10^{-2}, 1×10^{-1} and 1 ppm, the LGI values were 6.5, 3.33 and 3.33 respectively.

(2) Methoprene (Table IV-VI) :-

LGI values for II instars were not significantly different at various dosages. Thus at the lowest concentration (1×10^{-6} ppm) the LGI was 7.95, while at 1 ppm it decreased only to 3.63.

LGI values for III instars exhibited variation. In controls, the value was 20. However, at dosages ranging from 1×10^{-6} ppm to 1 ppm it never exceeded 16.66.

For IV instars, maximum LGI value was obtained at $1x10^{-6}$ ppm (16.93). These values decreased as the concentrations were increased. At 1 ppm, LGI was zero.

(3) Diflubenzuron :- (Tech) (Table VII - IX) -

In case of II instar larvae maximum LGI was recorded at control (7.14). It decreased with increase in concentrations. Finally at 1×10^{-3} ppm it significantly reduced to 3.97.

Only at one concentration i.e. 1×10^{-6} ppm, the III instars exhibited 6.94 LGI. At all other concentrations the LGI was zero.

In case of IV instars the LGI for control larvae was 33.33. However, on treatment, these declined up to 18 at 1×10^{-3} ppm dose level.

(4) Diflubenzuron :- (WP) (Table X-XII) -

As compared to II instar controls (11.11), the LGI values started declining till they reduced to zero at 1 ppm.

In case of III instars, at 1×10^{-6} ppm the LGI was 6.15. At 1×10^{-3} ppm it decreased to 2.17.

LGI values for IV instar remained generally constant (11.11 to 15.27) at 1×10^{-6} ppm concentrations. However, at 1×10^{-2} ppm the LGI was drastically reduced to 6.94. At 1 ppm it was zero.

Total Development Growth Index (TDGI) :- (Table I-IV) -

Hydroprene :-

The TDGI of II instar at all dosages were more or less similar (5.0 - 7.14). Only at 1 ppm it significantly decreased to 1.09..

TDGI in III instar was 9.09 at 1 x 10^{-6} ppm. There was slight variation in TDGI at subsequent concentrations. At 1x10^{-2} ppm, TDGI significantly declined (6.49) and at highest dose of 1 ppm, it was 1.96.

Compared to control (12.5) the TDGI drastically increased with increase in concentration. At 1 x 10^{-2} ppm it was 0.142 and at 1 ppm the value was zero.

Methoprene :- (Table IV - VI) -

In case of II instars, TDGI ranged from 5.26 to 5.0 at all concentrations, except 1 ppm where it was 2.0 only.

With respect to III instars, the TDGI were within the control range, (12.5) from highest to lowest concentrations.

In IV instar, maximum TDGI was observed in control (12.5). It decreased significantly in treated larvae. At 1 x 10^{-1} ppm the TDGI was about 1.785 and with 1 ppm it was zero.

Diflubenzuron :- (Tech) (Table VII - IX) -

With II instars the TDGI was significantly decreased at $1x10^{-6}$ ppm (0.85), compared to control (4.16). From 1×10^{-2} ppm concentration onwards, the TDGI could not be calculated due to larval mortality.

In case of III instar, only at 1×10^{-6} ppm the TDGI was 3.47. At rest of the concentrations it was zero.

TDGI of untreated IV instars was 12.5. At all treatments with test chemical it decreased, reducing to zero at 1×10^{-3} ppm.

Diflubenzuron (WP) :- (Table X - XII) -

Maximum TDGI was obtained in II instar (5.25) at 1×10^{-6} ppm, while at 1 ppm it was zero.

At 1×10^{-6} ppm the III instars TDGI was 5.76 but subsequently it decreased with increase in concentration and at 1×10^{-2} ppm onwards it became zero.

At 1×10^{-6} ppm the IV instars exhibited TDGI of 9.09, which was exactly similar to control. Minimum TDGI was recorded at $1x10^{-1}$ ppm and at 1 ppm it was zero.

Comparative picture of LGI and TDGI in different instars has been depicted graphically in Fig. 2.

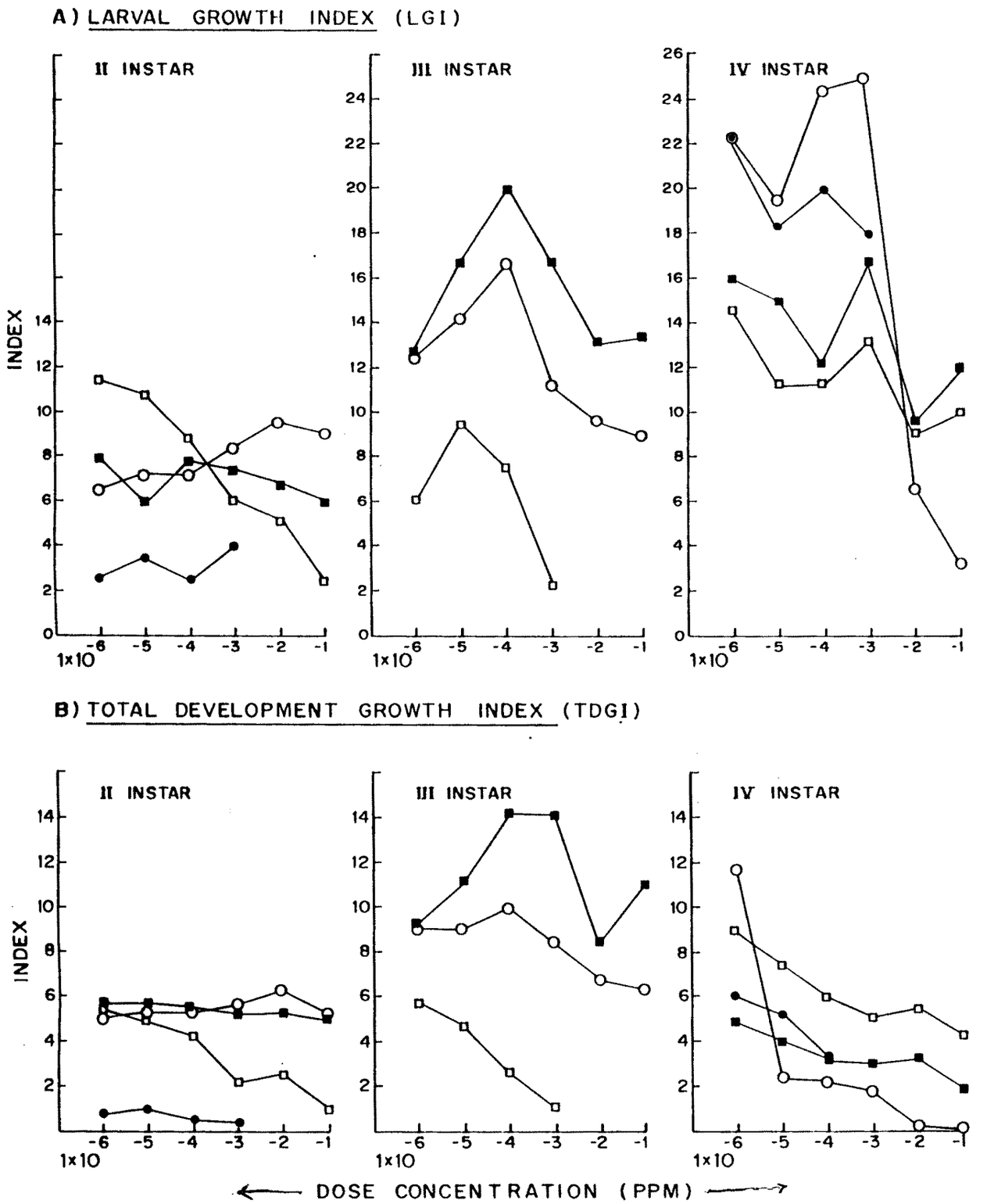


FIG. 2 : COMPARISON OF LGI AND TDGI OF DIFFERENT LARVAL INSTARS OF A. AEGYPTI (L) ON EXPOSURE TO IGR'S
[■-METHOPRENE, o-HYELROPRENE, ●-DIMLIN (Tech.), □-DIMLIN (25 WP)]

Design II

Discontinuous Exposure :-

(1) Thirty Minutes Exposure :-

When III instars were exposed to hydroprene and methoprene at dose ranges from 1 x 10^{-6} to 1 ppm, no deleterious effects were observed and 100% normal adults emerged. With dimlin (WP), 100% adult emergence occured up to 1 x 10^{-4} ppm. At rest of the concentrations up to 1 ppm, 100% larval mortality was obtained. With dimlin (Tech) 100% adult emergence was recorded up to 1x10^{-2} ppm, after which 100% larval mortality occured at all doses (Table XIV and XV).

In case of IV instars both hydroprene and methoprene were ineffective even at 1 ppm dose. With diflubenzuron (WP), at 1 x 10^{-3} reduced adult emergence (74.38%) was observed. At 1 x 10^{-2} ppm it was reduced to 19.02%. At 1 x 10^{-1} and 1 ppm 100% emergence was recorded. Dimlin (Tech) gave 100% normal adult emergence up to 1x10^{-2} ppm dose. However, at 1 x 10^{-1} and 1 ppm doses, larval mortality was 62.38 and 72.39% respectively, while pupal mortality was 37.62% and 27.62% respectively (Table XV and XVII).

(2) Sixty Minutes Exposure :-

Both hydroprene and methoprene failed to adversely affect III instar larvae. Dimlin (WP) also did not affect the test insects up to 1 x 10^{-4} ppm dose. However, 100% larval mortality was recorded at all subsequent concentrations. Dimlin (Tech) did not affect adult emergence up to 1 x 10^{-3} ppm. At 1 x 10^{-2} ppm, 80.95% adult emergence was

Table XIV :- Effect of IGRs (S-Hydroprene and S-Methoprene) on different developmental stages of III instar larvae of Aedes aegypti when exposed for 30 minutes.

	S-HYDROPRENE				S-METHOPRENE			
Dose (ppm)	LM(%)	PM(%)	AM(%)	NA(%)	LM(%)	PM(%)	AM(%)	NA(%)
Control	-	-	-	100	-	-	-	100
$1x10^{-6}$	-	-	-	100	-	-	-	100
$1x10^{-5}$	-	-	-	100	-	-	-	100
$1x10^{-4}$	-	-	-	100	-	-	-	100
$1x10^{-3}$	-	-	-	100	-	-	-	100
$1x10^{-2}$	-	-	-	100	-	-	-	100
$1x10^{-1}$	-	-	-	100	-	-	-	100
1	-	-	-	100	-	-	-	100

Table XV :- Effect of Dimlin (25 WP) and Dimlin (Tech) on different developmental stages of III instar larvae of Aedes aegypti when exposed for 30 minutes.

	DIMLIN (25 WP)				DIMLIN (TECH)			
Dose (ppm)	LM(%)	PM(%)	AM(%)	NA(%)	LM(%)	PM(%)	AM(%)	NA(%)
Control	0	-	-	100	-	-	-	100
$1x10^{-6}$	0	-	-	100	-	-	-	100
$1x10^{-5}$	0	-	-	100	-	-	-	100
$1x10^{-4}$	0	-	-	100	-	-	-	100
$1x10^{-3}$	100	-	-	-	-	-	-	100
$1x10^{-2}$	100	-	-	-	-	-	-	100
$1x10^{-1}$	100	-	-	-	100	-	-	-
1	100	-	-	-	100	-	-	-

Table XVI :- Effect of IGRs (S-Hydroprene and S-Methoprene) on different developmental stages of IV instars larvae of Aedes aegypti when exposed for 30 minutes.

	S-HYDROPRENE				S-METHOPRENE			
Dose (ppm)	LM(%)	PM(%)	AM(%)	NA(%)	LM(%)	PM(%)	AM(%)	NA(%)
Control	-	-	-	100	-	-	-	100
$1x10^{-6}$	-	-	-	100	-	-	-	100
$1x10^{-5}$	-	-	-	100	-	-	-	100
$1x10^{-4}$	-	-	-	100	-	-	-	100
$1x10^{-3}$	-	-	-	100	-	-	-	100
$1x10^{-2}$	-	-	-	100	-	-	-	100
$1x10^{-1}$	-	-	-	100	-	-	-	100
1	-	-	-	100	-	-	-	100

Table XVII:Effect of Dimlin (25 WP) and Dimlin (Tech) on different developmental stages of IV instar larvae of Aedes aegypti when exposed for 30 minutes.

	DIMLIN (25 WP)				DIMLIN (TECH)			
Dose (ppm)	LM(%)	PM(%)	AM(%)	NA(%)	LM(%)	PM(%)	AM(%)	NA(%)
Control	-	-	-	100	-	-	-	100
$1x10^{-6}$	-	-	-	100	-	-	-	100
$1x10^{-5}$	-	-	-	100	-	-	-	100
$1x10^{-4}$	-	-	-	100	-	-	-	100
$1x10^{-3}$	9.29 ±1.29	6.23 ±2.41	10.1 ±2.36	74.38 ±6.89	-	-	-	100
$1x10^{-2}$	8.34 ±2.1	52.32 ±5.35	20.32 ±2.89	19.02 ±2.23	-	-	-	100
$1x10^{-1}$	100	-	-	-	62.38 ±6.36	37.62 ±3.25	0	0
1	100	-	-	-	72.39 ±8.21	27.61 ±2.71	0	0

Table XVIII: Effect of IGRs (S-Hydroprene and S-Methoprene) on different developmental stages of III instar larvae of Aedes aegypti when exposed for 60 minutes

	S-HYDROPRENE				S-METHOPRENE			
Dose (ppm)	LM(%)	PM(%)	AM(%)	NA(%)	LM(%)	PM(%)	AM(%)	NA(%)
Control	-	-	-	100	-	-	-	100
$1x10^{-6}$	-	-	-	100	-	-	-	100
$1x10^{-5}$	-	-	-	100	-	-	-	100
$1x10^{-4}$	-	-	-	100	-	-	-	100
$1x10^{-3}$	-	-	-	100	-	-	-	100
$1x10^{-2}$	-	-	-	100	-	-	-	100
$1x10^{-1}$	-	-	-	100	-	-	-	100
1	-	-	-	100	-	-	-	100

Table XIX : Effect of IGRs Dimlin (25 WP) and Dimlin (Tech) on different developmental stages of III instar larvae of Aedes aegypti when exposed for 60 minutes

	DIMLIN (25 WP)				DIMLIN (TECH)			
Dose (ppm)	LM(%)	PM(%)	AM(%)	NA(%)	LM(%)	PM(%)	AM(%)	NA(%)
Control	-	-	-	100	-	-	-	100
$1x10^{-6}$	-	-	-	100	-	-	-	100
$1x10^{-5}$	-	-	-	100	-	-	-	100
$1x10^{-4}$	-	-	-	100	-	-	-	100
$1x10^{-3}$	100	-	-	-	-	-	-	100
$1x10^{-2}$	100	-	-	-	0	14.28 ±1.21	4.76 ±1.89	80.95 ±8.25
$1x10^{-1}$	100	-	-	-	100	-	-	-
1	100	-	-	-	100	-	-	-

recorded while at the remaining two concentrations 100% larval mortality was obtained (Table XVIII and XIX).

When IV instars were treated with both hydroprene and methoprene, even the 1 ppm dose did not affect the test insects. (Table XX).

Dimlin (WP) also produced 100% adult emergence up to $1x10^{-6}$ ppm, test concentration. Larval and pupal mortality and abnormal adults mortality were recorded at 1 x 10^{-3} ppm (18.29, 28.39, 52.32%). At higher concentrations, 100% larval mortality was elicited. In case of Dimlin (Tech) treatments, 100% normal adults were obtained upto 1 x 10^{-5} ppm dose. Subsequent concentrations reduced adult emergence up to 22.32% (1 x 10^{-2} ppm). At 1 x 10^{-1} and 1 ppm concentration, 100% larval mortality was obtained (Table XXI).

(3) Eight Hrs. Exposure :-

When IV instars were treated with hydroprene for 8 hours, activity started from lower dose onwards, at 1 x 10^{-6} ppm. Here, larval mortality was 18.29% and 81.71% normal adults emerged. As the concentration increased there was increased activity. At 1 ppm, there was 62.34% larval mortality, 18.46% pupal mortality, 15.49% abnormal adult mortality and 3.71% normal adults emerged.

With methoprene, IV instar larvae treated for 8 hours at $1x10^{-6}$ and $1x10^{-3}$ ppm doses exhibited 100% adult emergence. Withfurther increasing concentrations larval/pupal mortality was obtained and abnormal as well as normal adults were produced. At 1 ppm, there was 12.34% larval mortality, 21.34% pupal mortality, 10.29% abnormal adult mortality and 56.03% normal adults emerged.

Table XX :- Effect of IGRs (S-Hydroprene and S-Methoprene) on different developmental stages of IV instar larvae of Aedes aegypti when exposed for 60 minutes.

	HYDROPRENE				METHOPRENE			
Dose (ppm)	LM(%)	PM(%)	AM(%)	NA(%)	LM(%)	PM(%)	AM(%)	NA(%)
Control	-	-	-	100	-	-	-	100
$1x10^{-6}$	-	-	-	100	-	-	-	100
$1x10^{-5}$	-	-	-	100	-	-	-	100
$1x10^{-4}$	-	-	-	100	-	-	-	100
$1x10^{-3}$	-	-	-	100	-	-	-	100
$1x10^{-2}$	-	-	-	100	-	-	-	100
$1x10^{-1}$	-	-	-	100	-	-	-	100
1	-	-	-	100	-	-	-	100

Table XXI: Effect of IGRs Dimlin (25 WP) and Dimlin (Tech) on different developmental stages of IV instar larvae of Aedes aegypti when exposed for 60 minutes.

	DIMLIN (25 WP)				DIMLIN (TECH)			
Dose (ppm)	LM(%)	PM(%)	AM(%)	NA(%)	LM(%)	PM(%)	AM(%)	NA(%)
Control	-	-	-	100	-	-	-	100
$1x10^{-6}$	-	-	-	100	-	-	-	100
$1x10^{-5}$	-	-	-	100	-	-	-	100
$1x10^{-4}$	-	-	-	100	12.30 ±1.21	18.29 ±1.81	0	69.41 ±7.85
$1x10^{-3}$	18.29 ±1.29	28.39 ±3.45	52.32 ±6.34	-	28.39 ±3.25	16.29 ±2.05	8.3 ±1.23	47.02 ±4.25
$1x10^{-2}$	52.32 ±6.89	47.68 ±4.25	-	-	39.29 ±4.36	20.1 ±2.21	18.29 ±3.31	22.32 ±2.21
$1x10^{-1}$	100	-	-	-	100	-	-	-
1	100	-	-	-	100	-	-	-

Table XVII :- Effect of IGRs at different developmental stages on IVth instar larvae of *A. aegypti* when exposed for 8 hours.

Dose (ppM)	S-HYDROPRENE (%)				S-METHOPRENE (%)				DIMLIN (TECH) (%)				DIMLIN (25 WP) (%)			
	LM	PM	AM	NA	LM	PM	AM	NA	LM	PM	AM	NA	LM	PM	AM	NA
Control	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100
$1x10^{-6}$	18.29 ±1.29	0	0	81.71 ±8.21	0	0	0	100	0	0	0	100	0	0	0	100
$1x10^{-5}$	12.34 ±1.35	8.39 ±2.21	0	79.87 ±7.89	0	0	0	100	0	0	0	100	0	0	0	100
$1x10^{-4}$	13.45 ±2.21	10.25 ±1.29	2.34 ±1.56	73.92 ±6.36	10.25 ±1.26	0	0	89.75 ±7.90	18.34 ±2.24	39.29 ±4.36	18.28 ±2.1	24.09 ±2.29	0	0	0	100
$1x10^{-3}$	21.34 ±3.31	3.54 ±1.36	6.29 ±1.21	68.83 ±6.29	16.89 ±1.29	6.58 ±1.89	0	76.63 ±8.23	19.24 ±5.29	58.24 ±6.75	22.52 ±2.21	0	0	0	18.29 ±4.29	89.71 ±1.90
$1x10^{-2}$	38.49 ±4.41	12.34 ±1.29	12.3 ±1.36	36.87 ±2.45	16.85 ±2.10	6.34 ±1.30	4.98 ±1.29	71.83 ±5.25	100	-	-	-	58.23 ±6.29	41.37 ±4.23	-	-
$1x10^{-1}$	45.8 ±5.25	15.39 ±1.10	16.39 ±2.86	23.41 ±2.21	6.82 ±1.21	18.92 ±1.29	4.62 ±1.21	69.24 ±5.89	100	-	-	-	100	-	-	-
1	62.34 ±4.21	18.46 ±2.36	15.49 ±1.91	3.71 ±1.10	12.34 ±1.36	21.34 ±2.26	10.29 ±1.36	56.03 ±4.85	100	-	-	-	100	-	-	-

Table XXIII: Effect of IGRs on III instar larvae of Aedes aegypti at different developmental stages when exposed for 3 days.

Dose (ppm)	S-HYDROPRENE (%)				S-METHOPRENE (%)				DIMLIN (TECH) (%)				DIMLIN (25 WP) (%)			
	LM	PM	AM	NA	LM	PM	AM	NA	LM	PM	AM	NA	LM	PM	AM	NA
Control	-	-	-	100	-	-	-	100	-	-	-	100	-	-	-	100
$1x10^{-6}$	-	-	-	100	-	-	-	100	-	-	-	100	-	-	-	100
$1x10^{-5}$	-	-	-	100	-	-	-	100	-	-	-	100	-	-	-	100
$1x10^{-4}$	-	-	-	100	-	-	-	100	-	-	-	100	8.32 ±1.21	19.34 ±5.26	0	72.34 ±5.29
$1x10^{-3}$	-	-	-	100	-	-	-	100	-	-	-	100	-	-	-	100
$1x10^{-2}$	-	-	-	100	-	-	-	100	100	-	-	-	100	-	-	-
$1x10^{-1}$	-	-	-	100	7.98 ±1.29	8.10 ±1.36	9.11 ±1.29	74.81 ±6.24	100	-	-	-	100	-	-	-
1	100	-	-	-	6.24 ±1.23	28.34 ±3.25	12.11 ±2.1	53.31 ±7.51	100	-	-	-	100	-	-	-

Dimlin (Tech) when tested on IV instar for 8 hours, 1 x 10^{-6} and 1 x 10^{-5} ppm gave rise to 100% normal adult emergence. Subsequent concentrations viz. $1x10^{-4}$ and $1x10^{-3}$ induced larval mortality (18.34% and 19.24%), pupal mortality (39.29 and 58.24%) abnormal adult mortality (18.28% and 22.52%) and normal adults (24.09% and 0%). $1x10^{-2}$, $1x10^{-1}$ ppm and 1 ppm doses gave 100% larval mortality.

When IV instars were exposed to dimlin (WP) for 8 hours, no significant activity was recorded up to 1 x 10^{-4} ppm concentration. At 1 x 10^{-3} and 1 x 10^{-2} ppm concentrations normal adult emergence decreased to 89.71% and 0% respectively. Finally at 1 x 10^{-1} and 1 ppm 100% larval mortality was recorded (Table XXII).

(4) Three Days Exposure :-

Hydroprene was not at all active at 1 x 10^{-1} ppm on III instar larvae on continuous exposure for 3 days. However, at 1 ppm dose all III instar larvae exhibited 100% larval mortality when exposed to the chemical for 3 days. In case of methoprene, 100% adult emergence was recorded at 1 x 10^{-1} ppm for this exposure period. Further increase in dose viz. 1 x 10^{-1} and 1 ppm where larval mortality (7.98 and 6.24%) pupal mortality (8.10% and 28.34%), abnormal adult mortality (9.11% and 12.11%) was observed, resulting in 74.81% and 53.31% normal adults respectively.

Exposure to dimlin (Tech) for 3 days was ineffective up to 1 x 10^{-3} ppm dose. As the concentration was increased from $1x10^{-2}$ to 1 ppm, 100% larval mortality was noted. With dimlin (WP), only two lower concentrations namely 1 x 10^{-6} and 1 x 10^{-5} ppm exhibited 100% normal adult emergence. Rest of the concentrations exhibited 100% larval mortality (Table XXIII).

DISCUSSION

As mentioned, the test compounds namely S-methoprene and S-hydroprene are the more active isomers of methoprene and hydroprene. The latter have been reported to exhibit activity levels of these JH analogues (Kelaea et al. 1980). In comparison in the present work S-methoprene and S-hydroprene exhibited significantly high activity at dose levels of 1×10^{-5} ppm onwards.

Activity levels of IGR dimlin have also been worked out and compared for the technical material and the commercial (WP) formulation.

It may be noted that for the above studies two separate designs entailing continuous larval exposure on the one hand and discontinuous exposure on the other have been used. Test insects ranging from the II to the IV instars were subjected to the above two protocols. The overall objective of these varied experiments was to determine the most effective combination of dose, stage and method of exposure for different test chemicals to get some insight into the problem of field efficacy and application strategy and also to serve as the basis for recommendations of optima for actual field use.

Perusal of results mentioned in the last section reveals that in the continuous exposure experiment of design I, both JHAs S-hydroprene and S-methoprene do not elicit typical JHA effects when treatment commences at the II instar stage and the exposure continues through III to the IV instar and the pupal stage. This evidently indicates that the II instar larvae are not sufficiently susceptible to JHA action. This is well borne out by the relatively high IC50 values obtained (1.32×10^{-1}, and 1.37×10^{-1} ppm for hydroprene and methoprene respectively) for the two test JHAs for this larval stage.

Not very much better was the situation with III instars, where also characteristic JHA effects were not observed at lower dosages and the IC50 values were 2.2 x 10^{-2} ppm for hydroprene and > 1 ppm in case of methoprene.

At the IV instar, however, the JHAs manifested high metamorphic effects. Thus IC50 value of hydroprene was 1.0611×10^{-5} ppm and for methoprene it was 9.94 x 10^{-5} ppm.

From the forgoing it becomes apparent that II and III instar larval stages of the mosquito A. aegypti are not sensitive enough to be the targets for practical application of JHAs.

The IV instar stage on the other hand is obviously the most sensitive to JHA action and may, therefore, be considered ideal for application of these chemicals.

It may be noted here that even for the more sensitive IV instar stage, JHA activity begins to manifest itself only after exposures of 8 hours or more in case of hydroprene and methoprene. Shorter exposures of 30 and 60 minutes are apparently not sufficient to produce metamorphic changes or disruptions by JHAs especially at lower dosages. The failure to elicit metamorphic disturbances by continuous exposure at various dosages from II/III instar onwards can now, in the light of present findings, be attributed to failure of sufficient persistence of JH activity in the test compounds beyond certain time periods equal to or more than the time required for larval development from II to IV instar in case of the test insect, A. aegypti, mosquito.

Some of the findings in the present work with respect to S-isomers of methoprene and hydroprene also hold true for the original racemic version of these compounds

namely methoprene and hydroprene. The latter are also most effective only at the IV instar stage of mosquitoes (Parghal and Temerak 1981, Schaefer and Wilder 1972). Similarly the earlier parental racemic compounds also need an extended minimum time period of exposure before they can exert detectable effects. (Hatakoshi et al. 1986).

The IGR dimlin is reported to exert immediate insecticidal as well as extended growth or development regulatory action even on short term exposure. In the present work therefore, technical and WP formulation of dimlin were used in short term/long term discontinuous treatments only. As anticipated insecticidal as well as other effects were elicited at various doses on exposure at minimum as well as maximum durations. Results in the present investigation also revealed that technical dimlin is unmistakably more active than the commercial wettable powder formulation irrespective of dose or exposure time of the treatment. Presumably deeper and faster penetration of technical dimlin through the deployed carrier solvent, acetone as also slower and dubious contact action of dimlin as a water soluble WP formulation may be implicated.

It emerges from the above that for JHA action to manifest itself effectively a definitive sensitive stage of the target insect has to be aimed at. In field use this is a decided limitation. Coupled with restricted persistence of the JH's, this further reduces the potential of these chemicals since prolonged or continuous exposures also do not help in this case. On the other hand, with the IGR dimlin, short term exposures are also effective (at lower dosages) and at the same time these compounds are apparently more persistence and stable thus extending possible additive value to prolonged or continuous exposure. A possible handicap in case of dimlin is its apparent restrictionon its more effective action through oral route as compared to contact (Gronett J. et al. 1983). This probably accounts for the more superior performance of technical dimlin in the present investigation as compared to its wettable powder counterpart. Recent work seems to

suggest that in certain situations, dimlin is apparently sufficiently active even through contact i.e. cuticular route. (Retnakaran and Smith 1975). However, these surprises probably need further investigation and confirmation.

In summing up, therefore, both JHAs as well as the IGR dimlin can be said to exercise fundamentally non-hazardous, growth and development regulatory or metamorphosis/ecdysis inhibitory effects of restricted application to insect taxa. These chemicals can therefore be appropriately termed as non-hazardous (to non target species) and environment friendly (bio-degradable). These limitations, of specificity sensitive stage dependence, lesser persistence and higher cost notwithstanding, in the present circumstances of high environmental pollution by synthetic organic insecticides and universal concern for more biorational pest management strategies, they must be upheld and projected as very likely candidates of promise in future Integrated Pest Management (IPM) strategies.

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CHAPTER THREE

Effects on Reproduction and Development.

INTRODUCTION

Influence of hormones on insect reproductive physiology and development is well known from the work of Piepho and his colleagues on the wax moth Galleria mellonella (Piepho 1960). Piepho has also reported that the type of cocoon constructed depends on the concentration of JH in the animal. When young male adults of Schistocerca gregaria are allatectomized, the animals subsequently do not display normal sexual behaviour and do not attain the coloration typical of sexually mature animals (Loher 1961, Pener 1965). In the mosquitoes, Lea (1968) has demonstrated that the corpora allata are essential for sexual receptivity and that implantation of active corpora allata into all allatectomized females are not receptive to copulation until some 2 days after about ecdysis, but application of the hydrochlorinated product to newly emerged females rendered them receptive to copulation in 1 day (Craig 1970). In Locusta allatectomy results in less intensive activity (Wajc and Pener 1971) and a reduction in the spontaneous locomotor activity in adult males (Odhimbo 1966a). Perhaps as an indirect result of the observed decrease in sexual behaviour (Strong 1968). Juvenile hormone not only regulates developmental events in insects but is also an important component of reproductive function in almost all the species that have been examined. Juvenile hormone is one of the major hormones that regulates insect metamorphosis and it must decline during the last instar for the larval pupal transformation to begin. The morphogenesis and anatomy of the insect reproductive system have already been reviewed in great detail (Engelman, 1970, Mahowald, 1972, Anderson, 1972a,b, Dewild and Deloof, 1973, Vol.1). Juvenile hormone analogue treatments do not always directly cause death, rather the overall insect population may be reduced by the effect of JHA's upon fecundity and fertility. Research on various adult insects indicates that low quantities of juvenile hormone induce mating activity initial gonad maturation and the develop-ment

of oocytes up to the primary stages of yolk deposition, while Higher concentrations of hormones are required for the completion of egg development (Johansoon 1955, Engelman 1960, Lea 1968). Juvenoids can disrupt a variety of processes in insects, including immature development, metamorphosis and reproduction. (Retnakaran et al. 1985, Slama, 1985).

In the present study oviposition, reproductive behaviour, fecundity and fertility of F_1 and F_2 generations have been examined after the exposure of IV instar larvae of Aedes aegypti mosquitoes to IC50 doses. Studies were also made on newly emerged adults by treating them with JHAs and Dimlin (Technical and 25 WP) offered in dietary sucrose.

Historical Survey :-

In 1936 Wigglesworth (Vol.7) demonstrated that the hormone from the corpora allata (CA) regulated reproduction in Rhodnius prolixus which eventually turned out to be the first unequivocal evidence that the corpora allata had definite role in egg production. He showed that the presence of active CA was necessary for successful yolk deposition and egg maturation. Active CA's are now equated with high JH activity (Doane, 1973). Various responses of the endocrine regulation of growth, metamorphosis and reproduction have been extensively reviewed by Wigglesworth (1964), Engelman (1968), Roller and Dahm (1968) and others. Some of the relationships of juvenile hormone to the sexual behaviour of insects have been shown by Highman (1964). JH plays a central role in vitellogenesis, both in the biosynthesis of vitellogen and in its incorporation into growing oocytes. Lea (1967) first demonstrated the dependency of oogenesis in mosquitoes on secretions from both the brain neurosecretory cells and the corpus allatum. (Gwadz and Spielman 1971). Precocious sexual receptivity induced by a juvenile hormone analogue in females of the yellow fever mosquito Aedes aegypti has also been demonstrated by Gwadz et al. (1971). Post emergence effects of two insect growth regulators on Culex tarsalis have been reported by Arias and Mulla (1975). Reproductive inhibition activity (Wright and Spates 1976) and effects on hatching of mosquito eggs have also been documented in different insects (Miura et al. 1976).

Recent studies have shown that exogenous juvenile hormone or juvenile hormone analogues can promote vitellogenesis in both autogenous and anautogenous mosquitoes (Master et al. 1980, Kelly et al. 1981, Borovsky 1981, Borovsky et al. 1985, Klowden 1987, Martiner and Hagedorn 1987) suggesting that the hormone may also be active after blood is ingested. Exposure to low doses of JHA during the larval stage may allow the insect to develop into the adult form but may still cause subtle effects influencing overall reproductive success.

MATERIALS AND METHODS

LARVA :-

For studying latent and delayed effects of JHAs' on IV instar larvae and adults were chosen as the experimental stages. These were maintained in the insectary at a temperature 28°± 2 and relative humidity (RH) 70-80%. Fourth instar larvae drawn from the mother culture were exposed to respective IC50 dose levels of the test chemicals. Viz.S Hydroprene $1.06x10^{-5}$, S methoprene $9.94x10^{-5}$, dimlin (Technical) $1.597x10^{-4}$ and dimlin (25 WP) $1.7x10^{-2}$ ppm. The larvae were kept continuously in the treated water till adult emergence. Observations were made on the larval and pupal mortality as well as the emerging abnormal or normal adults. The emerging normal adults from these experiments were reared in the usual manner without any subsequent exposure to extraneous JHA. Observations were also made on mating, oviposition, egg hatching and subsequent development right up to F_1 and F_2 generations.

Adult :-

Adults for these experiments were obtained from pupae harvested from mother culture. Freshly emerged adults were offered 5 and 10 ppm doses of test chemicals in 0.05 M dietary sucrose. The offered 0.05 M sucrose solution (10 ml/100 adults) alongwith incorporated JHA in it was replenished after 3 days. This JHA exposure through diet was maintained for 6 days, whereafter the adults were offered mammalian blood meal. No further JHA exposure was made thereafter. In control instead of JHA, carrier solvent i.e. acetone was added.

RESULTS

LARVAE :-

The control larvae treated with the carrier solvent acetone alone produced 100% normal adults. In the F_1 generation 89.23% of eggs laid by them hatched and a total of 96.23% normal adults emerged. From the latter, in the F_2 generation, 91.29% of eggs laid hatched and the normal adult emergence was 100%. There was no significant inhibition of any reproductive function or normal development in the control animals.

In the larvae treated with the respective IC50 doses (1.06×10^{-5} ppm S-hydroprene 9.94×10^{-5}, S-methoprene, 1.597×10^{-4} dimlin (Tech) and 1.7×10^{-2} for dimlin (25 WP), in general approximately 50% normal adult emergence was recorded after the corresponding larval and pupal mortalities of general orders already noted in Chapter I. Remarkably, no detrimental effects were observed and fertility of the surviving normal adults of these larval treatments through F_1 and F_2 generations in respect of all the experimental compounds used was found to be normal (Table I).

ADULTS :-

Control values i.e. egg hatch, survival of adults etc. of normal adults (not treated with any JHAs) were 91.29% and 100% respectively. When adults were offered hydroprene in the diet at 5 ppm they exhibited 86.24% oviposition compared to the untreated controls, which produced 72.39% egg hatching. However, 100% larval mortality occured resulting in 0% normal adult emergence. At 10 ppm the oviposition was 88.29% egg hatchability 70.50% and 100% larval mortality. However, in oviposition rates (62.34% and 61.19%) of methoprene treated animals there was small but significant decline at 5 and 10 ppm doses as compared to hydroprene. At 5 ppm dose the hatching rate was 70.29% larval mortality 10%, pupal mortality 22.32% deformed (malformed) adults 66.45% resulting into 1.23% normal adult emergence. At 10 ppm dose methoprene hatching rate was 68.29% but subsequently 100% larval mortality was observed. With dimlin (Tech) at 5 and 10 ppm dose the oviposition rate was 82.34%

subsequent effects on fecundity, oviposition, hatching in F_1 and F_2 generations of adults of A. aegypti.

IGR	Dose (ppm)	Normal Adult Emergence	Oviposition	Hatching F_1	Adult Emergence	Oviposition	Hatching F_2
Control	-	+	++	+++	++	++	+++
S-Hydroprene	1.06×10^{-5}	+	++	+++	++	++	+++
S-Methoprene	9.94×10^{-5}	+	++	+++	++	++	+++
Dimlin (Tech)	1.597×10^{-4}	+	++	+++	++	++	+++
Dimlin (WP)	1.7×10^{-2}	+	++	+++	++	++	+++

+ - 100%

++ - 80-90%

+++ - 70-80%

Table II : Effect of IGR's treatment on oviposition and viability of fresh adults of *A. aegypti*

IGR's	Dose (ppm)	% Oviposition	% Hatching	← % Mortality → Larval	Pupal	Adult	Normal Adult Emergence
Control	0	89.28 ±7.81	91.29 ±6.29	0	0	0	88.28
S-Hydroprene	5	86.24 ±4.52	72.39 ±6.84	100	-	-	-
	10	88.29 ±6.25	70.50 ±6.25	100	-	-	-
S-Methoprene	5	62.34 ±3.25	70.29 ±6.21	10	22.32 ±2.12	66.45 ±3.59	1.23 ±0
	10	61.29 ±4.21	69.23 ±4.93	100	-	-	-
Dimlin (Tech)	5	82.34 ±6.25	78.26 ±6.25	100	-	-	-
	10	86.29 ±7.29	71.29 ±5.23	100	-	-	-
Dimlin (WP)	5	61.29 ±4.51	65.29 ±4.29	100	-	-	-
	10	63.24 ±4.21	69.26 ±4.23	100	-	-	-

and 86.29% which was almost same as those of the control values but there was marginal decline in hatching rate (78.26% and 71.29% respectively). However, hatched larvae did not develop further. Development at both concentrations resulted in 100% larval mortality thus no normal adults emerged. Oviposition rate and hatching rate at 5 and 10 ppm doses was more or less equal(61.29%, 63.24% and 65.29%, 69.26% respectively). Here again larval development was highly restricted and 100% larval mortality resulting soon after hatching (Table II).

DISCUSSION

Results obtained in the present investigations on the potential of different IGRs' including two JHAs, viz. S-hydroprene and S-methoprene, dimlin (Tech) and dimlin (25 WP) have yielded some very interesting information. It is conclusively established that the demonstrated sensitive IV larval instar's exposure to effective IC50 doses results only in approximately 50% action as expected. However, there is no latent or delayed follow-up activity on subsequent reproductive as well as developmental events and functions for all the test compounds tested.

In case of adults, however, the situation was different. Investigation of latent manifestation of IGR/JHA activity after adult treatment through diet yielded interesting results. There were very definite delayed effects of decided practical importance. Thus, while oviposition and hatching rate were obviously not affected, the hatched larvae from eggs of the treated adults failed to survive. Larval mortality was expressed either in the first instar at both 5 and 10 ppm doses, in the case of hydroprene and the two formulations of dimlin or in the 1st or subsequent instars in case of methoprene. The net results of these delayed activities was complete inhibition of adult emergence in the case of hydroprene and the two formulations of dimlin. Barely significant emergence of approximately 1.23% adults occured in case of methoprene treatment at 5 ppm, while 10 ppm methoprene exposure resulted in 0% normal adult emergence in F_1 generation.

These results clearly establish and indicate that larval exposure is effective only in expression of typical IGR suppressive effects in the particular time and generation of exposure. In other words, larval exposure alone may not be expected to produced any delayed bonus effects in subsequent development or generations. On the other hand, adults exposed to all the IGR's examined in the present work have produced latent or delayed inhibitory effects on reproductive function as well as development in the same

and subsequent generations. Adult decimination occured in the following generations. The same does not hold true for larval exposure.

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CHAPTER FOUR

Effect of Different Temperatures on the Action of IGR's.

INTRODUCTION

Temperature has been demonstrated to have pronounced effects on the potency of various insecticides (Narahonsi 1971a). DDT and pyrethroids are generally more effective in killing insects at low temperatures compared to higher ones. Organophosphate and carbamates, on the other hand, are in many cases more potent at high temperatures than at low temperatures. High DDT and pyrethroid poisoning at low temperatures cannot be attributed to cuticle penetration and detoxification of the insecticides. Penetration capability of insecticides in the cuticle is reduced at lower temperatures. Temperature also plays a role opposite to the negative temperature coefficient of insecticidal activity. Enzymatic detoxification is also decreased by lowering the temperature. This may in part account for the higher insecticidal activity at low temperature (Guthrie, 1950). Although the effect of temperature on the toxicity of insecticides has been studied in many species, very few studies of this type have been reported on the insecticide resistant strains. Any alteration of resistance level produced by temperature changes could provide an insight into the mechanics of evolution of resistance. The relationship between development and temperature has been described recently using a model based on chemical kinetics (Ruedha et al. 1990).

Relatively little work has been done on the effects of different temperature ranges on JH/IGR activity. This may be due to reported faster penetration target site binding and excretion of these chemicals with little or none biologically significant deactivation. However, in view of the reported in vivo metabolism of hydroprene (Tungikar et al. 1978, Hammock, B. and Quistad, G. 1981), methoprene (Hammock, B. and Quistad G. 1981) and for dimlin (Hammock, B. and Quistad, G. 1981 and Maas, W. et al. 1981) and at least one instance of resistance against JH (Rowlands and Dyte 1979), it is possible that factors similar to those operative for various insecticides (cuticular pene-

tration, detoxification = inactivation etc.) may be involved in the insect compound activity paradigm for JHAs and IGRs also.

Attention may also be drawn to the reported inactivation of hydroprene on storage at low temperature (Hebbalkar et al 1979).

The present work was therefore carried out to examine the effect of temperature on the biological activity of JHA and IGR on the metamorphosis of A. aegypti.

LITERATURE SURVEY

Temperature is generally known to have profound effects on biological function. Xenobiotic influences are very often modulated by variable temperatures. Different temperatures also induce insecticidal action or inaction in case of certain toxicants. A classical case is that of DDT which exhibits a negative temperature coefficient in that it is more active at lower than at higher temperature (Guthrie 1950). For most insecticides the reverse is true (Hirano, 1979). As mentioned earlier, surprisingly not much published data exists on the influence of different temperature ranges on the action of JHAs and IGRs.

The influence of post-treatment temperature on the toxicity of 6 insecticides was investigated on a susceptible and 2 resistant strains of Musca domestica L. (Devires and Georghis 1979). The effects of temperature on pre and post adult develop-ment of various mosquito species has been studied by Trips and Schemanchuk (1969). Trips and Schemanchuk (1970), Bar Zee (1958), Brust (1967), Haney and Brust (1967). Shelton (1970) studied the effect of temperatures on development of mosquito species. The impact of temperatures on insects was emphasized by Anderwartha (1971) who stated "Temperature influences the speed of development, the duration of life, the fecundity, and the behaviour of animals especially poikilotherms". Information on the effects of temperature on mosquitoes is available for many species (Huffaker 1944, Bar Zee 1958, Brust 1967, Parker 1979). Influence of temperature is an important consideration in the design of mosquito population and control strategy models (Haile and Weidhaas 1977, Greever and Georghiou 1979). The effect of temperature on the development, growth and survival of Psorphora columbiae are well studied (Mchugh and Olson 1982). Increase in toxicity at lower temperatures is exhibited by DDT (Lindquist et al. 1945), pyrethrum (Chevalier 1930), DDT and methoxychlor (Hoffman and Lindquist

1949, Hoffman et al. 1949). Insecticides most toxic at higher temperatures include lindane, aldrin, dieldrin, toxaphene and parathion. (Hoffman et al. 1949, Hoffman and Lindquist 1949). Guthrie (1950) studied the effect of the temperature on toxicity of certain organic insecticides. Several investigators have studied the effect of temperature on growth rate and survival of the immature stages of Aedes aegypti. Temperature toxicity relationship are also reported for pyrethroids on Heliothis virescens and Anthonomus grandis grandis (Sparks et al. 1983).

Brown (1985) has reported the influence of methoprene at low temperature and starvation on the incidence of diapause in the codling moth. The effect of constant temperatures on the developmental rates, growth and survival of the immature stages of C. quinquefasciatus and A. aegypti were determined at six constant temperatures (Rueda et al. 1990). Toxicity based on knockdown was determined at two post-treatment temperatures for 6 pyrethroids to five species of stored grain pest (Subramanyam and Cutkamp 1987). Tauthong and Brust (1977) studied the effect of temperature on the development and survival of two populations of Aedes compestris. Sparkls et al.(1982) reported the temperature toxicity relationship of pyrethroids on these lepidopterans.

MATERIALS AND METHODS

The fourth instar larvae of Aedes aegypti drawn from the mother culture, as described in Chapter I, were exposed to two different (highest and lowest) temperatures between the observed range at which 100% survival of the test insects could be obtained. These were 25°c to 34°c. At and between these, 100% normal adult emergence was obtained. The JHA/IGR activities of the test compounds at these temperatures were observed for varying degrees of mortality. The pupae were exposed to these high and low temperature extremeties plus 3 intermitant temperatures viz. 25°, 29°, 34° c to study the effects of these temperatures on JHA and IGR action.

Test dosages of different chemicals (S-hydroprene and S-methoprene) and diflubenzuron (Technical and 25 WP) used weresame as those described in Chapter I (Viz. for IV instar larvae $1x10^{-6}$ ppm to 1 ppm and for pupa $1x10^{-6}$ ppm to 10 ppm). After application of desired dose the water medium containing chosen stages of test insects, the holding containers (100 ml capacity glass beaker containing 50 ml. water) were kept at selected experimental temperatures continuously until adult emergence.

For both stages, namely IV instar larvae and pupa, mortality and the same in later instars plus any other abnormalities in adults or their emergence were recorded for each temperature.

For each of the above experiments 10 larva and 10 pupa per replicate were used and all experiments were replicated 5 times. The results were subjected to standard statistical procedure of analysis for calculation of IC50 values of each compound at different temperatures.

RESULTS

LARVA :-

Hydroprene :- (Table I and II) =-

When IV instar larvae of A. aegypti were continuously exposed to JHA, hydroprene, at 25°c temperature, typical JH effects were hardly noticeable at the lowest dose ($1x10^{-4}$ ppm). At this dose i.e. $1x10^{-4}$ ppm the observed larval and pupal mortality was 4.76% and 9.52% respectively and as many as 85.71% normal adults emerged. At subsequent higher doses such as $1x10^{-5}$ ppm onwards there was gradual increase in larval and pupal mortality leading to lesser normal adult emergence. At 1 ppm dose level, 10.52% larval, 42.10% pupal mortality and 47.38% abnormal adults were observed resulting in only 5.29% normal adult emergence.

In contrast, remarkably, hydroprene was totally ineffective in producing any JH effect at all doses when IV instar larvae were continuously exposed at the higher temperature of 34°c.

Methoprene :-

In case of methoprene, exposure of IV instar larvae at lower temperature, 25°c, produced marginal effects only up to $1x10^{-2}$ppm dose level. At this concentration no larval mortality, only 15.78% pupal mortality, 5.26% abnormal adults and 78.94% normal adults emerged. At 1 ppm dose level although larval mortality was observed none, it continued to produce 84.21% pupal mortality and 15.78% abnormal adults resulting in 0% adult emergence.

Table I :- Effect of different IGRs on further developmental stages of IV instar larvae of Aedes aegypti on continuous exposure at 25°C.

Dose (ppm)	S-HYDROPRENE				S-METHOPRENE				DIMLIN (25 WP)				DIMLIN (TECH)			
	LM	PM	AM	NA	LM	PM	AM	NA	LM	PM	AM	NA	LM	PM	AM	NA
Control	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100
$1x10^{-6}$	0	0	0	100	0	11.11 ±1.21	0	88.88 ±6.88	0	0	0	100	61.11 ±5.89	33.33 ±2.25	5.55 ±1.15	0
$1x10^{-5}$	0	0	0	100	0	5.88 ±1.23	11.76 ±1.29	82.35 ±7.29	31.34 ±2.35	21.05 ±2.83	0	47.60 ±4.25	70.58 ±6.89	29.41 ±2.23	0	0
$1x10^{-4}$	4.76 ±1.21	0	9.53 ±1.21	85.71 ±6.89	0	5.88 ±0.89	15.64 ±1.26	78.48 ±8.21	57.14 ±4.89	42.86 ±4.21	0	0	11.11 ±2.23	44.44 ±4.21	44.44 ±3.45	0
$1x10^{-3}$	0	5.4 ±1.21	36.31 ±3.21	58.29 ±5.84	0	6.25 ±1.85	12.5 ±1.21	81.25 ±9.21	25.0 ±2.25	66.66 ±5.89	8.33 ±1.29	0	46.66 ±5.45	40.0 ±2.22	13.33 ±1.25	
$1x10^{-2}$	0	38.88 ±3.35	7.29 ±2.12	53.83 ±4.89	0	15.78 ±1.26	5.26 ±1.28	78.94 ±7.82	100	-	-	-	100	-	-	-
$1x10^{-1}$	0	23.95 ±2.21	47.36 ±4.26	29.14 ±2.23	0	29.35 ±2.34	18.29 ±1.89	52.36 ±5.45	100	-	-	-	100	-	-	-
1	10.52 ±1.29	42.10 ±4.21	47.38 ±4.89	5.29 ±1.25	0	84.21 ±7.89	15.78 ±1.36	0	100	-	-	-	100	-	-	-

Table II :- Effect of different IGRs on further developmental stages of IV instar larvae of *Aedes aegypti* on continuous exposure at 34°C.

DOSE (ppm)	S-HYDROPRENE				S-METHOPRENE				DIMLIN (25 WP)				DIMLIN (TECH)			
	LM	PM	AM	NA	LM	PM	AM	NA	LM	PM	AM	NA	LM	PM	AM	NA
Control	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100
$1x10^{-6}$	0	0	0	100	0	20.0 ±1.89	14.55 ±1.23	65.45 ±6.35	5.56	0	0	94.44 ±8.93	85.7 ±7.89	14.3 ±1.23	-	-
$1x10^{-5}$	0	0	0	100	0	44.44 ±3.48	15.17 ±2.35	40.39 ±3.89	0	11.11	0	88.89 ±7.89	40.0 ±2.12	50.0 ±4.51	10.0 ±1.29	0
$1x10^{-4}$	0	0	0	100	0	33.0 ±4.29	43.0 ±3.65	34.39 ±2.89	0	0	0	88.24 ±7.81	6.25 ±1.21	56.25 ±5.21	36.50 ±2.45	-
$1x10^{-3}$	0	0	0	100	0	43.45 ±5.14	0	56.55 ±5.23	5.88 ±1.21	17.65 ±2.35	11.76 ±1.29	70.59 ±6.25	0	54.55 ±5.29	45.45 ±4.89	-
$1x10^{-2}$	0	0	0	100	0	42.10 ±3.63	11.98 ±1.23	45.92 ±3.89	55.56 ±3.89	44.44 ±3.45	5.88 ±1.23	0	100	-	-	-
$1x10^{-1}$	0	0	0	100	0	27.27 ±2.80	32.34 ±2.89	40.39 ±2.26	100	-	-	-	100	-	-	-
1	0	0	0	100	0	63.89 ±5.35	36.11 ±2.35	0	100	-	-	-	100	-	-	-

On exposure of IV instar larvae at the higher temperature of 34°c to methoprene, JH effects activity began to manifest at the lower doses also. No larval mortality, 20% pupal mortality, 14.55% abnormal adults and 65.45% normal adults were observed at $1x10^{-6}$ ppm. At 1 ppm 0% larval mortality, 63.89% pupal mortality, 36.11% abnormal adults and 0% normal adults were emerged.

Diflubenzuron (TECH) :-

In case of diflubenzuron (Tech) high activity was exhibited for all larval stages exposed at 25°c for all dosages. At $1x10^{-6}$ ppm, no normal adults emerged. From $1x10^{-6}$ to $1x10^{-3}$ ppm larval/ pupal mortality and abnormal adults were 36.84%, 57.14% and 25.0% larval mortality, 21.05, 42.86, 66.66% pupal mortality, 8.33% abnormal adults were obtained respectively. At $1x10^{-2}$ to 1 ppm 100% larval mortality was elicited.

At 34°c also, diflubenzuron (Tech) showed 100% activity. At lowest dose ($1x10^{-6}$ ppm) 85.7% larval mortality and 14.3% pupal mortality resulted into 0% normal adult emergence. At higher concentrations ($1x10^{-2}$ to $1x10^{-1}$ ppm) 100% larval mortality was exhibited.

Diflubenzuron (25 WP) :-

Exposure of IV instar larvae to diflubenzuron wettable powder at 25°c produced little or no effects at lower dosages. (100% normal adult emergence). However, there was drastic reduction in adult emergence at $1x10^{-5}$ ppm (47.60% adult emergence) and from $1x10^{-4}$ ppm onwards, no normal adults emerged. From $1x10^{-2}$ ppm onwards 100% larval mortality was exhibited.

At 34°c diflubenzuron (25 WP) was not at all effective up to $1x10^{-3}$ ppm. At $1x10^{-2}$ ppm however, 55.56% larval mortality and 44.44% pupal mortality was obtained which at $1x10^{-1}$ produced 100% larval mortality.

Comparison of IC50 (ppm) derived upon IGR's treatment on IV instar larvae of A. aegypti at 25°, 29° and 34° c temperature (Table III).

General JH titre in different developmental stages of insects (Fig. I).

PUPA :-

When 0-16 hr. old pupae harvested from the mother culture and treated only with solvent acetone (control) were exposed to different temperatures viz. 20°, 25°, 29°, 34°, 38° c. No deleterious effects were observed and 100% normal adults emerged.

Simultaneously when the pupae from control batch were exposed to 15°C or below, pupal development was inhibited completely resulting in 100% pupal mortality. Similarly at the other higher extreme temperature namely 42°c, once again 100% pupal mortality was obtained.

The development period from pupa to adult at 20°c was 4 days. However, at 25°c the period was 3 days, and this was further reduced to 2 days at 29°, 34° and 38°c temperatures. (Fig.2).

Hydroprene :-

0-16 hr. old pupae when exposed to hydroprene at 20°c were not affected at all at

Table 3 :- Comparison of IC50 (ppm) values derived upon IGR's treatment on IV instar larvae of A. aegypti at 25°, 29°, and 34°C temperature.

	Temperature range		
Test Compound	25°C	29°C	34°C
S-Hydroprene	0.0092	0.0000106	> 1
S-Methoprene	0.0982	0.0000994	0.000929
Dimlin (Technical)	< 0.000001	0.0001597	< 0.000001
Dimlin (25 WP)	0.00001123	0.017	< 0.000001

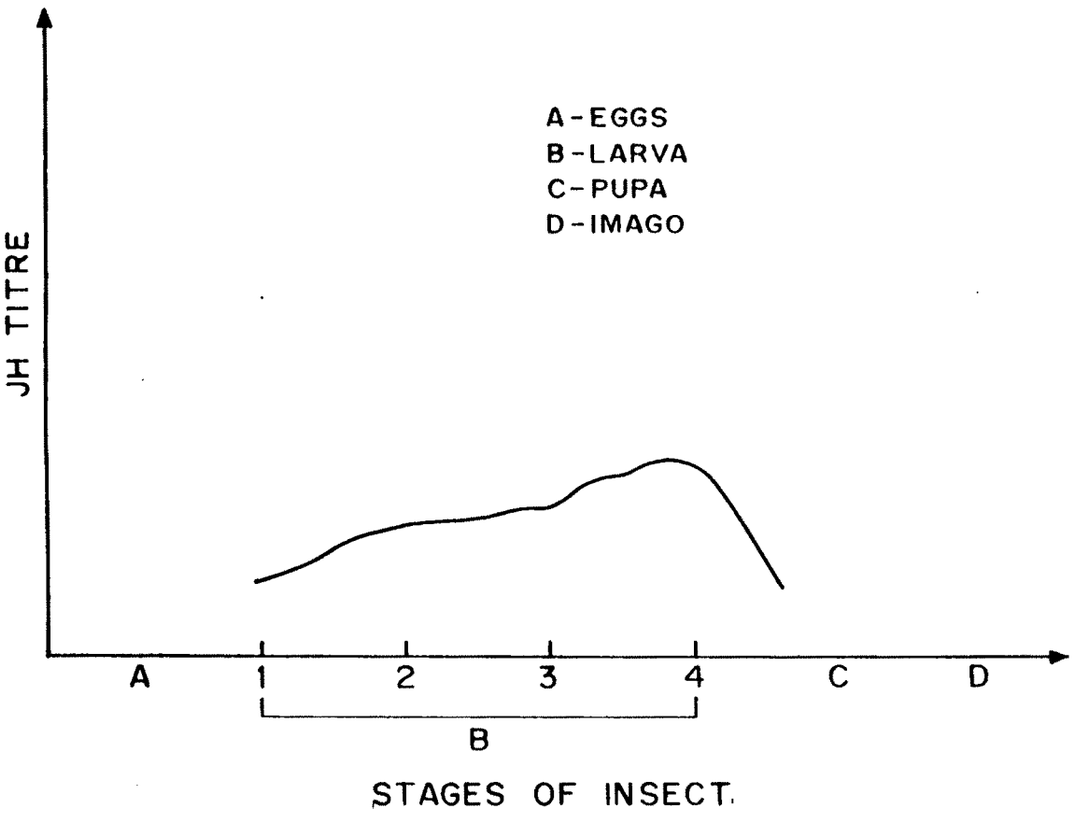

FIG. 1: GENERAL JH TITRE PROFILE IN THE DIFFERENT DEVELOPMENTAL STAGES OF INSECT

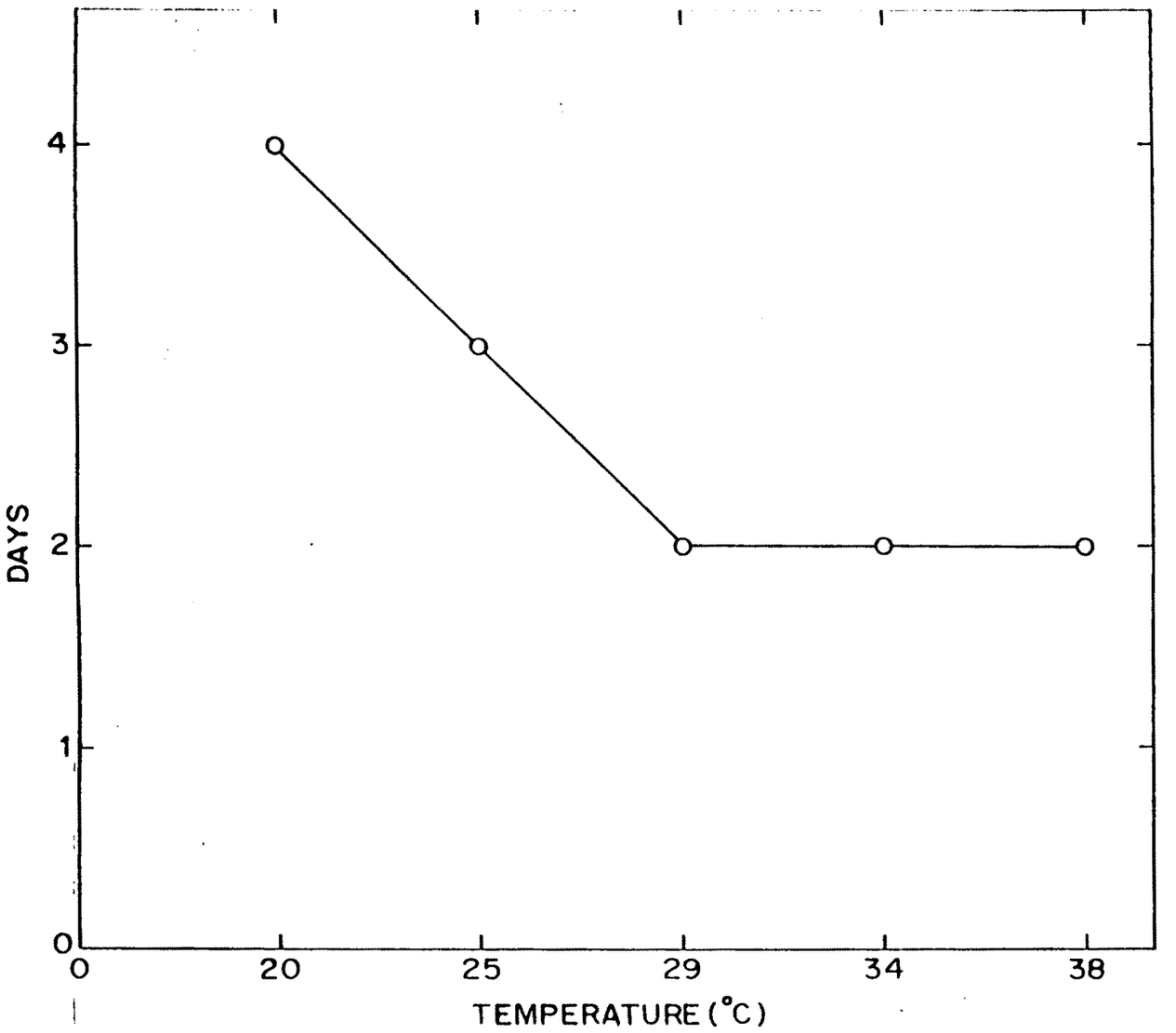

FIG. 2: NUMBER OF DAYS REQUIRED FOR PUPA TO ADULT EMERGENCE OF A. AEGYPTI AT DIFFERENT TEMPERATURES

$1x10^{-1}$ ppm dose level. From 1 ppm onwards, however, there was decline in adult emergence. At 10 ppm there was 9.54% pupal mortality, 82.41% abnormal adults and 8.09% normal adult emergence.

In contrast, the activity of hydroprene at 25°c was higher at lower doses as compared to 20°c. At $1x10^{-3}$ ppm, 100% normal adults were produced. As the concentration increased there was decline in adult emergence. At 10 ppm concentration 100% abnormal adults emerged.

At 29°c temperature, hydroprene caused no effects, when 0 to 16 hr. old pupae were exposed continuously up to 3 ppm dose level. Activity of this compound was observable from 4 ppm onwards. At 10 ppm, 92.34% abnormal adults and 7.76% normal adults were obtained.

When the pupae were exposed to higher temperature of 34°c, the activity of test JHA (hydroprene) manifested was the same as that obtained at 29°c. Nearly same results were obtained at 34°c. At 10 ppm, 35.45% pupal mortality, 55.55% abnormal adults were obtained resulting in 9.0% normal adults.

At 38°c temperature the pupae exhibited 100% adult emergence when exposed to hydroprene at 1 ppm. Lower dosages did not exhibit any JH effects at this temperatures. At 10 ppm, however, 100% pupal mortality was induced.

Methoprene :-

Continuous exposure of pupae to methoprene at 20°c temperature gave 100% adult emergence even at $1x10^{-3}$ ppm. When the dose was increased further, there was decline

in normal adult emergence and increase in pupal mortality as well as abnormal adults. At 10 ppm concentration, 26.23% pupal mortality and 73.6% abnormal adults were recorded.

Methoprene starts eliciting JH activity in the experimental animal from $1x10^{-5}$ ppm concentration at 25°c. At this concen-tration 5.93% pupal mortality and 94.07% normal adult emergence was obtained. At the higher concentrations such as 10 ppm, 100% abnormal adults were obtained.

In contrast, at 29°c methoprene had no deleterious effects even at $1x10^{-1}$ ppm. Activity at this temperature starts from 1 ppm. Here, there was no pupal mortality but 25.12% abnormal adults were produced, and 74.88% normal adults emerged. At 10 ppm concentration 72.39% abnormal adults and 27.61% normal adult emergence was observed.

When pupae were exposed to methoprene at 34°c, no JH effects were observed up to 1 ppm. However, at 10 ppm 35.50% pupal and 64.50% abnormal adults were produced. Consequently no normal adults emerged.

At 38°c temperature pupae exposed to $1x10^{-3}$ ppm did not produce any JH effects. In other words 100% adult emergence occured. Adult emergence declined as the concentration increased. Finally, at 10 ppm, 60% pupal mortality, 31.54% abnormal adults and 8.46% normal adults were recorded.

Diflubenzuron (Technical) :-

At 20°c diflubenzuron (Tech) was effective from $1x10^{-1}$ ppm concentration onwards

upon continuous exposure of pupae. Lower dosages viz. $1x10^{-6}$ ppm to $1x10^{-2}$ ppm produced 100% normal adult emergence. From 5 ppm onwards 100% pupal mortality was exhibited.

In contrast at 25°c, diflubenzuron (Tech) exhibited 100% normal adult emergence at $1x10^{-2}$ ppm concentration. At 4 ppm, 66.57% pupal mortality and 33.43% abnormal adults resulted leading to 0% normal adult emergence. From 6 ppm to 10 ppm concentration, 100% pupal mortality was exhibited.

However, at 29°c, the compound was less effective at lower dosages since as at $1x10^{-2}$ ppm concentration it produced 100% normal adults. JH activity becomes apparent from $1x10^{-1}$ ppm dose with 15% abnormal adult and 85% normal adult emergence. At 10 ppm concentration, 100% pupal mortality was elicited.

The exposure of pupae to diflubenzuron (Tech) at 34°c resulted in 100% normal adult emergence at $1x10^{-3}$ ppm concentration. At $1x10^{-2}$ ppm, 1.75% pupal mortality, 10.32% abnormal adult and 87.71% normal adults were recorded. From 5 ppm onwards 100% pupal mortality was elicited.

At 38°c temperature 100% normal adult emergence was exhibited at $1x10^{-3}$ ppm concentration. On further increase in concentration there was decrease in adult emergence and increase in pupal mortality as well as abnormal adult formation. Only two concentrations viz. $1x10^{-2}$ and $1x10^{-1}$ ppm exhibited 70% and 25% adult emergences respectively. Rest of the concentrations did not show any normal adult emergence. At 8 ppm concentration 100% mortality was recorded.

Diflubenzuron (25% Wettable Powder) :-

Exposure of pupae to diflubenzuron (25 WP) at 20°, 25° and 34°C temperatures produced no effects. 100% normal adults were obtained.

However, at 38° c while the compound still did not show any IGR effects upto $1x10^{-2}$ ppm at 10 ppm, 5.94% pupal mortality and 94.06% abnormal adults were produced resulting in non-emergence of any normal adults.

IC50 values of each compound (S-hydroprene, S-methoprene and diflubenzuron [Tech and 25 WP]) were calculated. These are given in Table IV.

Comparative picture of IC50 values against these compounds has been depicted graphically in Fig. 3.

Developmental inhibition of A. aegypti pupae induced by the IGR's at different temperatures has been depicted graphically in Fig. 4-7.

Table IV:- Determination of IC50 values (50% inhibition of adult emergence) (ppm) of the IGR's treated on 0-10 hr. old pupa of *A. aegypti*.

	Temperature range				
Test Compound	20^{o}	25^{o}	29^{o}	34^{o}	38^{o}
S-Hydroprene	2.914	4.921	6.977	5.178	1.7417
S-Methoprene	0.173	1.756	6.096	4.519	1.322
Dimlin (Technical)	0.972	0.705	0.777	0.115	0.030

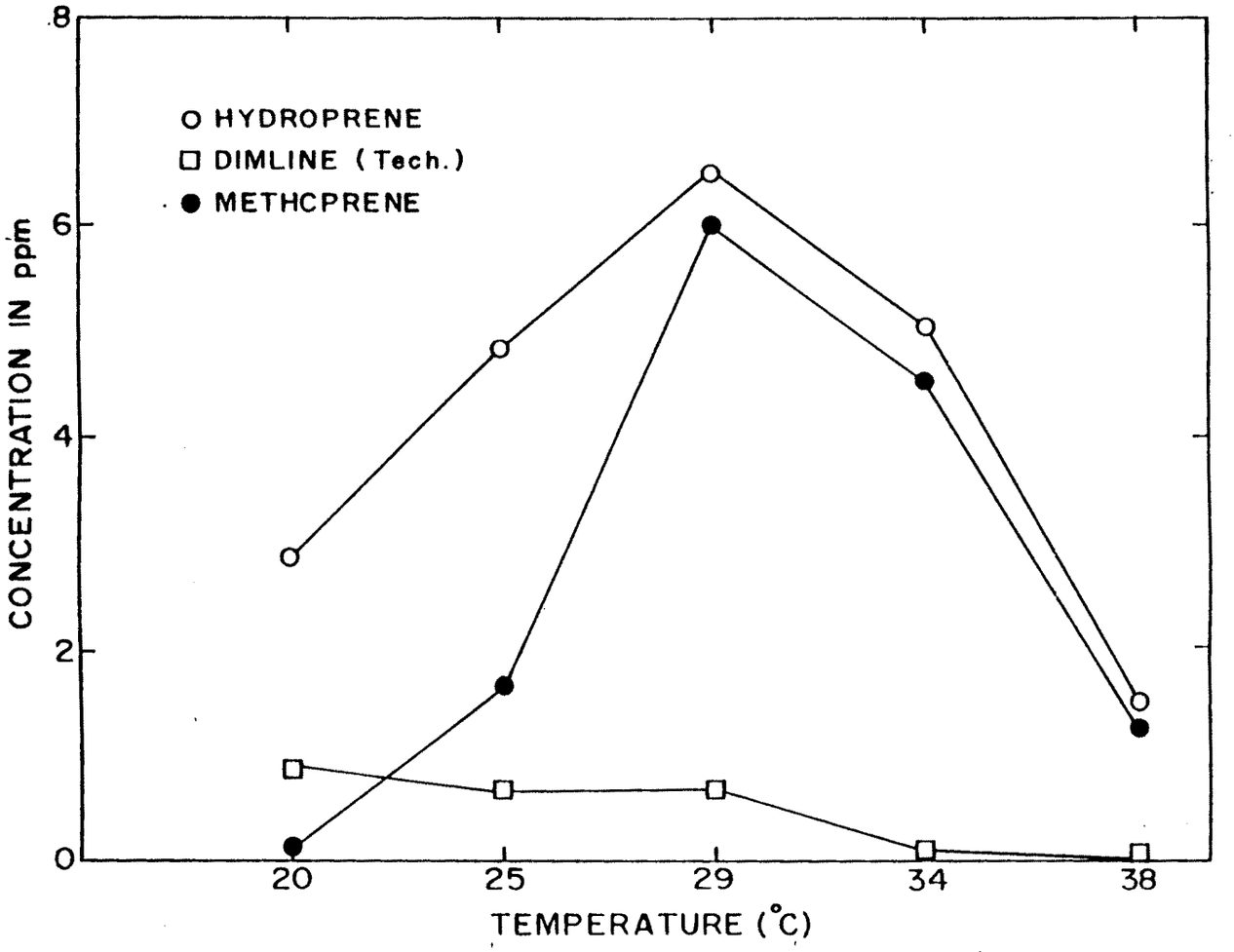

FIG.3 : DETERMINATION OF IC 50 VALUES (50% INHIBITION OF ADULT EMERGENCE) (ppm) OF THE IGR'S TREATED ON 0-10 hr OLD PUPA OF A. AEGYPTI

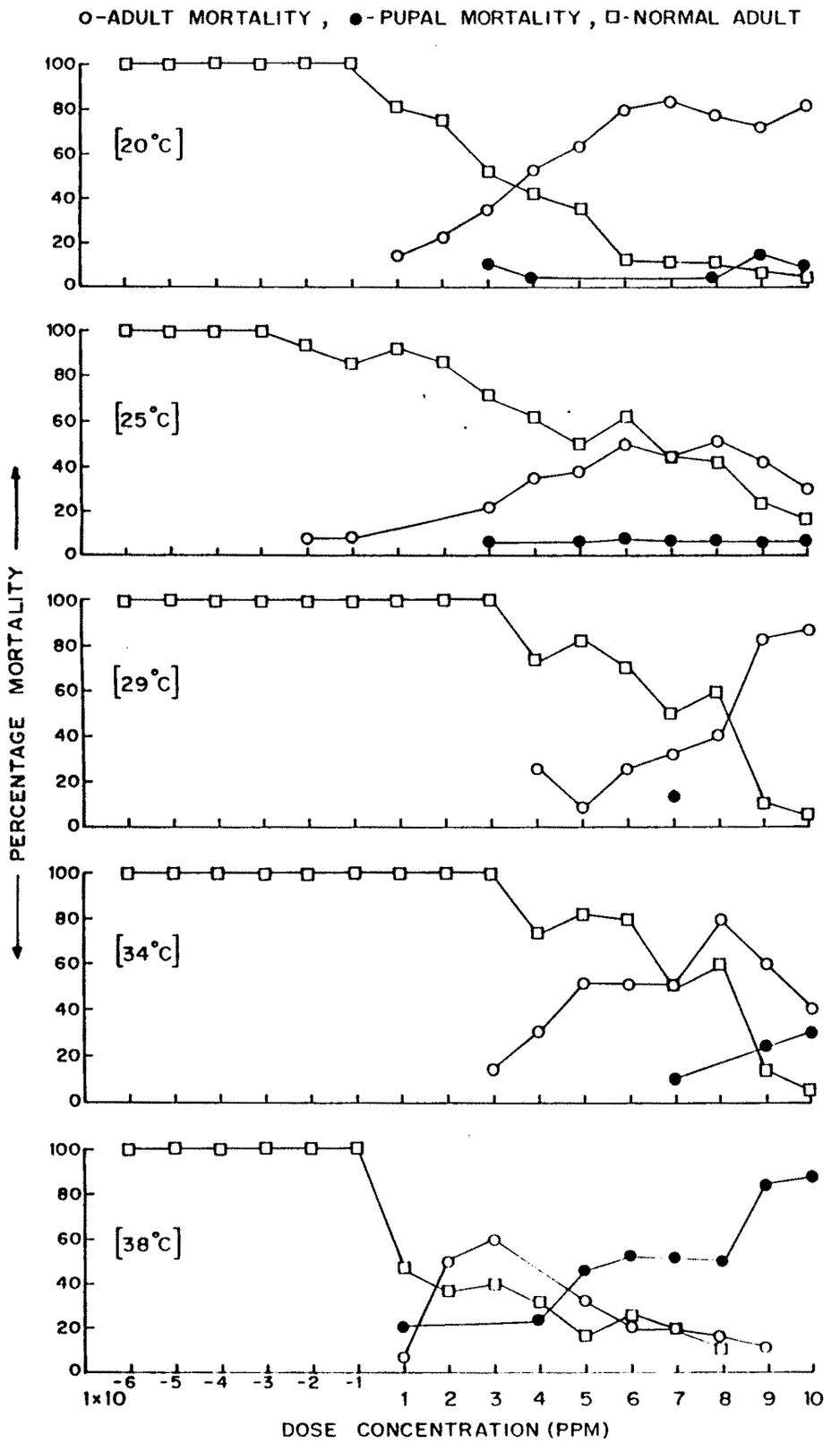

FIG. 4 : INDUCTION OF DEVELOPMENTAL INHIBITION BY THE IGR, S-HYDROPRENE IN A. AEGYPTI PUPAE AT DIFFERENT TEMPERATURES.

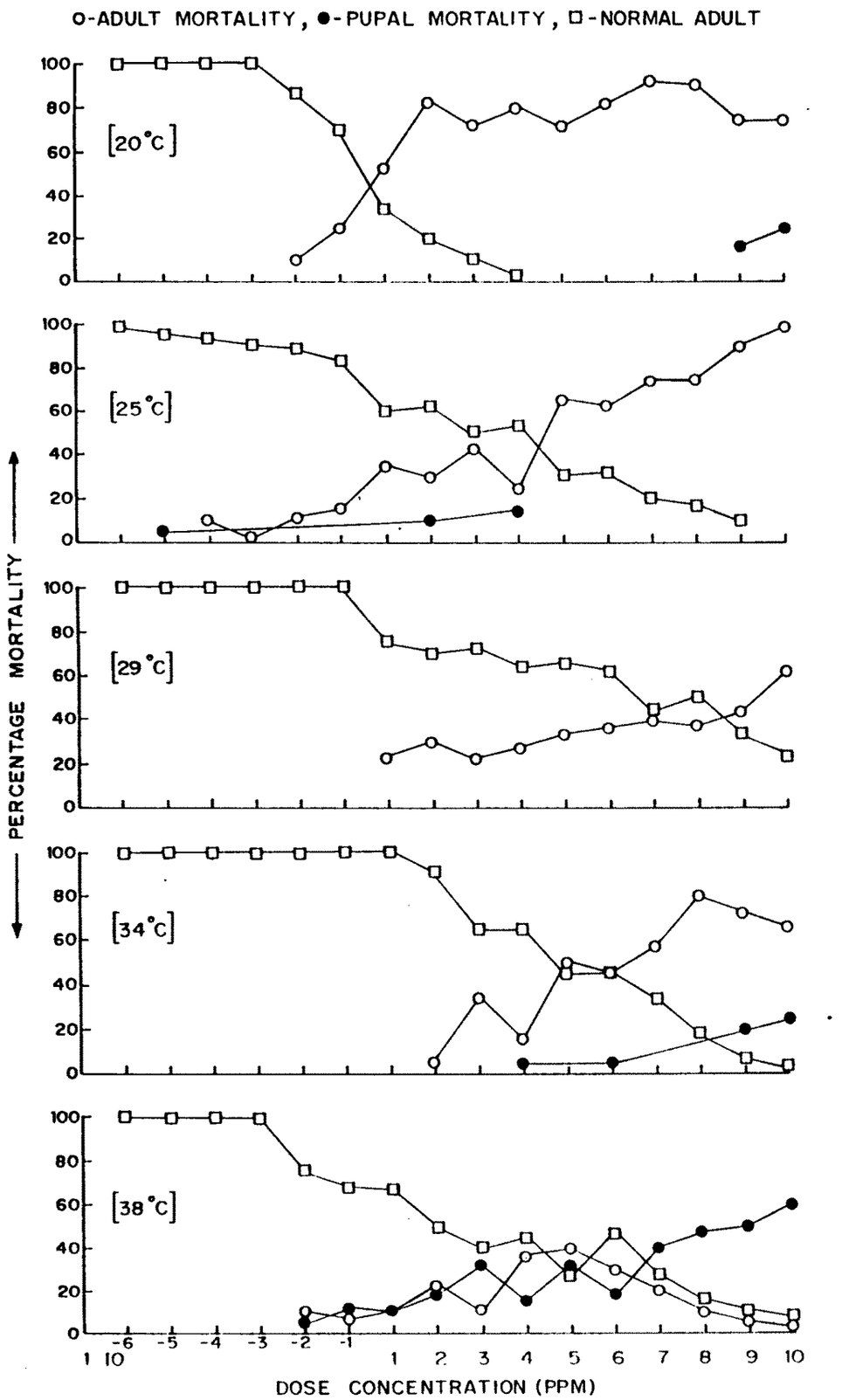

FIG. 5 :INDUCTION OF DEVELOPMENTAL INHIBITION BY THE IGR, S-METHOPRENE IN A. AEGYPTI PUPAE AT DIFFERENT TEMPERATURES.

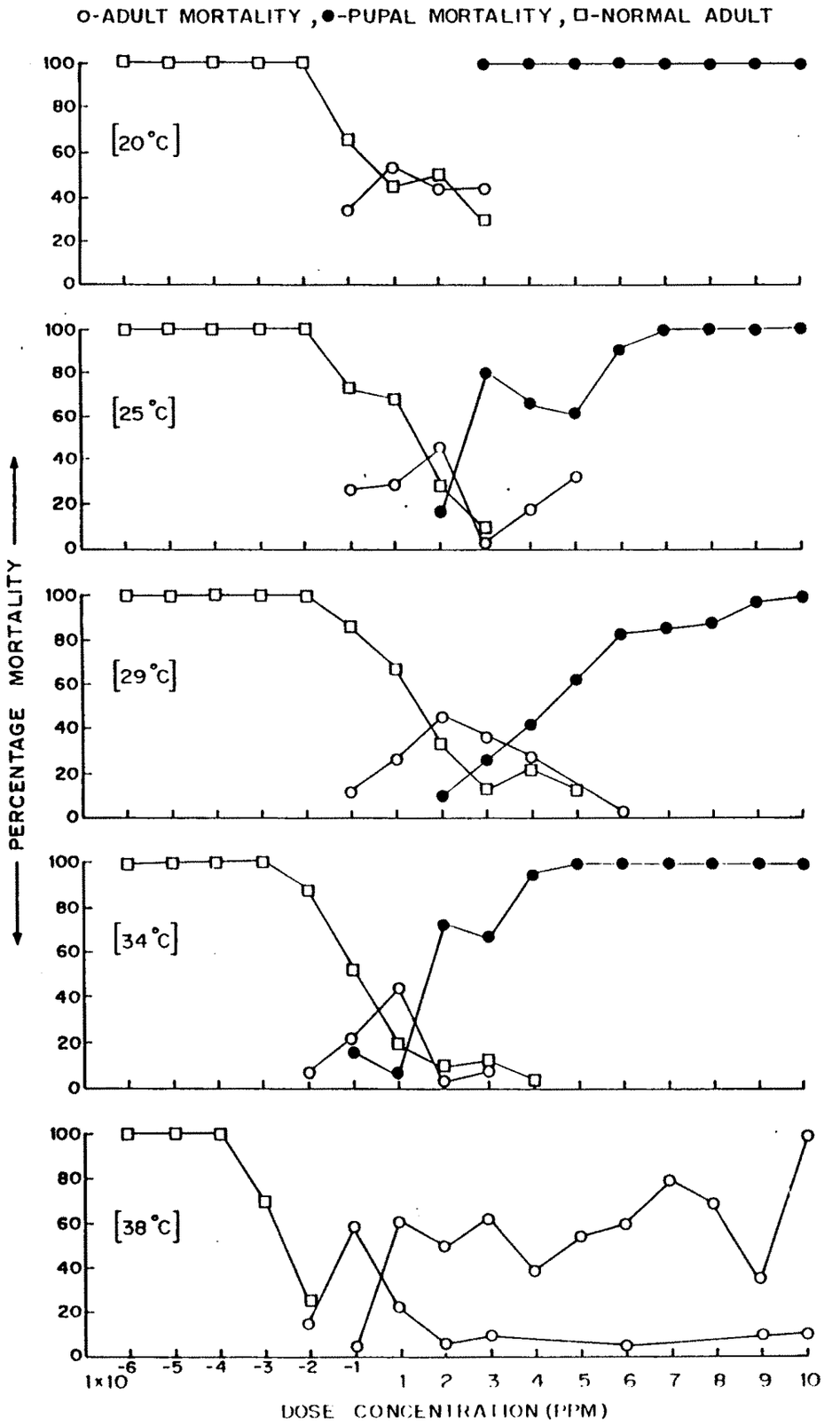

FIG. 6 : INDUCTION OF DEVELOPMENTAL INHIBITION BY THE IGR DIMLIN (TECHNICAL) IN A. AEGYPTI PUPAE AT DIFFERENT TEMPERATURES.

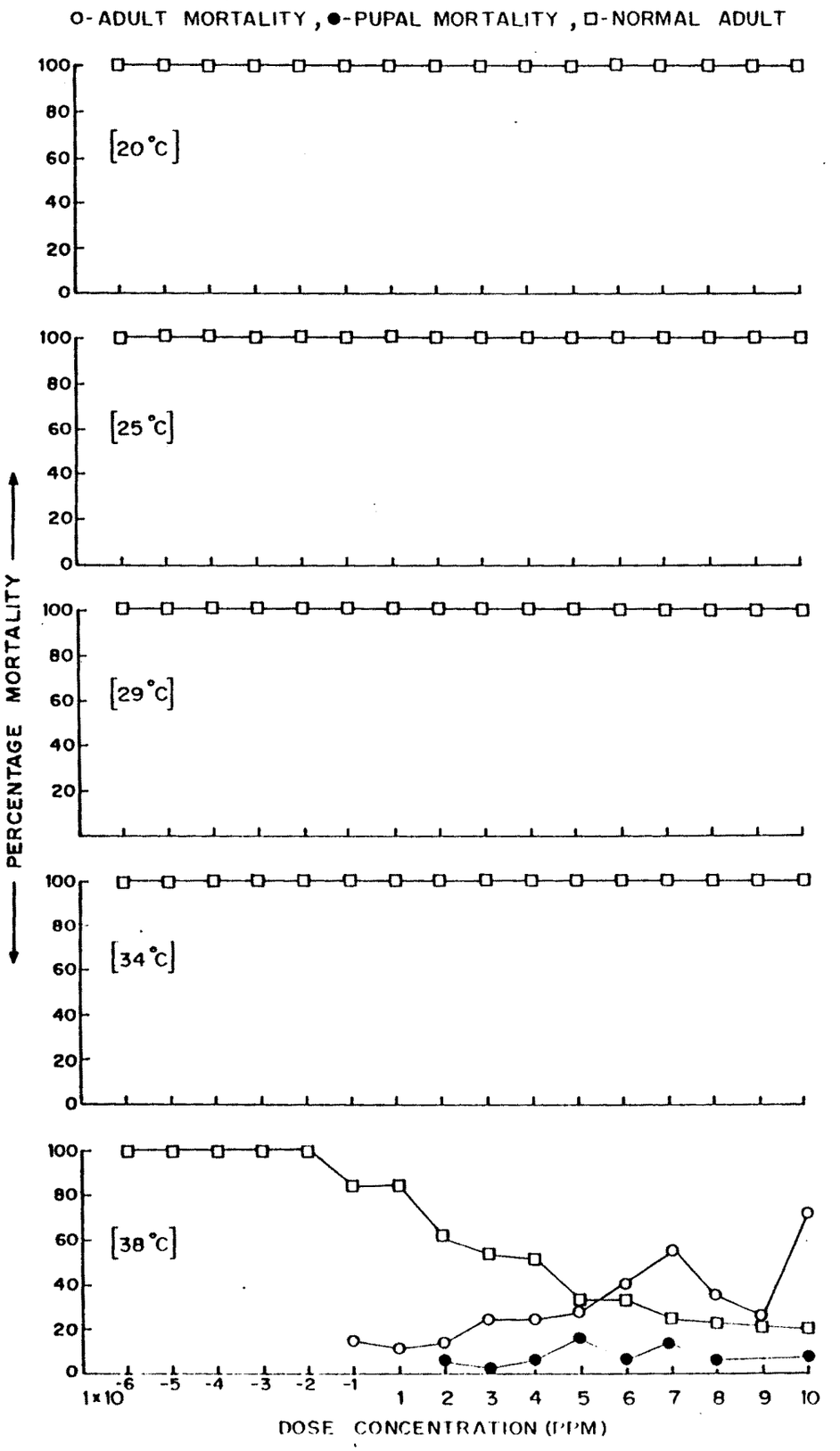

FIG. 7 : INDUCTION OF DEVELOPMENTAL INHIBITION BY THE IGR, DIMLIN (25 WP) IN A.AEGYPTI PUPAE AT DIFFERENT TEMPERATURES.

Table V : Effect• of different IGR's on Aedes aegypti pupae at different temperatures.

Compound	Dose (ppm)	20°C NAE	20°C Treated Vs * Untreated	25°C NAE	25°C Treated Vs * Untreated	29°C NAE	29°C Treated Vs * Untreated	34°C NAE	34°C Treated Vs * Untreated	38°C NAE	38°C Treated Vs * Untreated
Control	-	98.55 ±1.21	-	97.25 ±2.53	-	98.23 ±1.23	-	98.98 ±1.40	-	97.75 ±1.21	-
S-Hydroprene	1	86.64 ±6.45	P<0.001	95.0 ±3.52	NS	99.26 ±2.11	NS	96.67 ±2.31	NS	46.35 ±12.15	P<0.001
S-Methoprene	1	35.86 ±3.59	P<0.001	61.46 ±4.51	P<0.001	81.23 ±6.58	P<0.001	97.91 ±1.22	NS	64.01 ±3.5	P<0.001
Dimlin (Tech)	1	46.12 ±2.83	P<0.001	67.56 ±3.52	P<0.001	65.58 ±3.52	P<0.001	24.23 ±2.11	P<0.001	0	-
Dimlin (25 WP)	1	100	NS	100	NS	100	NS	100	NS	86.70 ±7.89	P<0.001

• All calculations are based on % normal adult emegence.

* Student t-test

NS - Not significant.

NAE - Normal Adult Emergence.

Table VI :- Comparison (ambient 29°C) of normal adult emergence from Aedes aegypti pupae treated with different IGR's at different temperatures.

		29°C	20°C		25°C		34°C		38°C	
Compound	Dose (ppm)	NAE	NAE	Treated Vs* Untreated	NAE	Treated Vs* Untreated	NAE	Treated Vs * Untreated	NAE	Treated Vs * Untreated
Control	0	98.23 ±1.23	-	-	-	-	-	-	-	-
S-Hydroprene	1	99.26 ±2.11	86.64 ±6.45	NS	95.02 ±3.52	NS	96.67 ±2.31	NS	46.35 ±12.15	$P < 0.001$
S-Methoprene	1	81.23 ±6.58	35.86 ±3.59	$P < 0.001$	61.46 ±4.51	$P < 0.001$	97.91 ±1.22	NS	64.01 ±3.5	NS
Dimlin(Tech)	1	65.83 ±3.52	46.12 ±2.83	NS	67.56 ±3.52	NS	24.23 ±2.11	$P < 0.001$	0	-

* Student t-test

NS - Not significant

NAE - Normal Adult Emergence.

Table VII: Comparison of normal adult emergence from S-hydroprene and S-methoprene treated A. aegypti pupae at the lowest and highest experimental temperatures.

	20°			38°		
IGR	Dose (ppm)	NAE		IGR	Dose (ppm)	NAE
S-Hydroprene	1	86.64 ±6.45		S-Hydroprene	1	46.35 ±12.35
			P<0.001			P<0.001
S-Methoprene	1	35.86 ±2.35		S-Methoprene	1	64.01 ±3.5

Table VIII: Comparison of normal adult emergence from S-hydroprene and S-methoprene treated A. aegypti pupae at the lowest and highest experimental temperatures at 1 ppm.

	20°			38°
IGR	Dose (ppm)	NAE		NAE
S-Hydroprene	1	86.64 ±6.45	P<0.001	46.35 ±12.15
S-Methoprene	1	35.86	P<0.001	64.01

DISCUSSION

LARVA :-

The significantly marked effects of temperature on biology in general and entire sequence of developmental and physiological events in particular, have been discussed earlier. It, therefore, follows that chemical stress of any kind must necessarily be influenced by thermal conditions. These effects should naturally become more pronounced at the more extreme values.

In the present work, more commonly investigated larval instar of A. aegypti and comparatively the less studied pupal one have been incorporated to study the temperature IGR interactions.

The results obtained are very interesting and highlight certain pecularities of temperature effects on IGR action. These are particularly valuable in view of the paucity of sufficient information on effects of different temperature ranges on IGR's in general. Thus the JHA S-hydroprene exhibits a remarkable reduction of activity at lower and higher extremes of temperatures imposed on the experimental animals. This is in sharp contrast a fairly high level of activity at the standard/ control/normal ambient temperature level of 29°c. Similar anomalies are also observed for the JHA S-methoprene albeit to a lesser degree (Table III). It is important to note here that on a priori grounds, it may be logically expected that at two extremes of temperatures, (= approaching limits of temperatures capable of inducing mortality in the species) chemical stress would act additively in exhibiting higher bioactivities.

The lesser bioactivities obtained with two potent JHAs S-hydroprene and S-methoprene are therefore extraordinary since they are occuring at both high and low extreme temperatures. It is to be noted here that these results do not correspond to the well known negative temperature coefficient correlations of certain classes of insecticides, notably DDT, pyrethroids (Yates, 1950). In these cases, it may be recalled that the activity is reduced at higher temperature and increased at lower ones. One of the factors possibly responsible for such an effect has been cited as higher metabolism of toxicants at higher temperature. In case of IGR since the phenomenon of reduced bioactivity is being exhibited at both lower and higher extreme temperatures, but not at the normal, usually ambient one for the experimental animals used. Such reasons cannot be adduced here. It is only possible to speculate that reduced cuticular penetration at the lower and higher temperature extremes the metabolic as well as thermal inactivation at the higher one may contribute to the manifestation of lowered bioactivity.

In contrast the antiecdysial IGR dimlin in both its test moieties (Tech. and 25 WP) manifest raised biological activity at either extremes of temperatures (Table III). This may well be expected as a consequence of the additive action of thermal and chemical stress.

PUPA :-

Pupae were used as experimental animals because JH is reportedly absent in this stage (Fig.3). Various reasons such as lower corpora allata activity, higher enzymatic (esterase) degradation etc. have been cited for the latter (Downer et al. 1975). It would be obvious of interest from both academic as well as applied point of view to investigate effects of extraneous JH exposure on the pupa. Exogenous juvenile hormone may interfere with normal pupal physiology/development and thereby cause overall popula-

tion decimination either in the pupal stage or in the emergent adults, thus providing very useful applied potential.

It becomes apparent (Fig.3) that JHA's S-hydroprene and S-methoprene show heightened bioactivity at either extremes of temperatures. On the other hand trace activity is exhibited at the median and ambient thermal values. (Table 3). These results are almost opposite to those obtained with the IV instar (Fig.3). Thermal stress at either extremes of temperature ranges, apparently act additively with the chemical one, unlike the case with the larvae. In case of dimlin too, higher thermal stress at the upper end of the temperature range produces again, presumably by additive action, higher biological action.

The data generated in these experiments has been further utilized for adducing statistical significance of difference between the two means of values at different temperatures as well as with reference to normal, ambient temperatures. The data has been analyzed by subjecting to single tail analysis of independent variables followed by student 't' test (Table V-VIII).

The results obtained in this part of the investigation demonstrate that IGRs' including JHAs' can act on both last larval instar as well as the presumably more resistant pupal instar with fair promise. The latter is positively influenced by temperature gradients in case of pupae the effects often being characteristic for different IGRs.

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CHAPTER FIVE

Selected Biochemical Changes Induced by IGR's at Different Temperatures.

INTRODUCTION

A fair amount of work has been done on different changes, including biochemical effects of IGRs on larval stages in mosquitoes. The pupa, on the other hand, despite being an interesting stage of mosquito life cycle has not been as well investigated. The pupa is a very complicated, closed system in which high amounts of fats and carbohydrates are stored. These are generally consumed at the emergence of the imago. The mosquito pupa is particularly interesting because of its high mobility, indicating the existence of high metabolic activity for the production of high energy levels obviously required for the purpose. The absence of tangible JH function in the pupal stage of the mosquito has already been demonstrated. It has also been shown in the preceeding chapter that the pupa seems to exhibit synchronization of extraneous JH influence with thermal stress. These features make the pupa an exceptionally interesting experimental stage. In this investigation the yellow fever mosquito pupa has been used for examining the possible effects of exogenous IGR applications on selected biochemical parameters at three different temperature levels.

The most immediate energy outputs for intense biological activity such as that exhibited by energetically mobile mosquito pupa is generated by glycogenesis. The carbohydrates, proteins and lipids play especially important roles in the latter. In the present investigation, the biochemical parameters examined have been restricted to carbohydrate and protein as preliminary experimentation did not reveal significant differences in the lipid content spectrum of experimental animals when compared with the untreated controls. It may be remarked that the data on protein content of pupae with or without IGR treatment at different temperatures is being presented for the first time.

SURVEY OF LITERATURE

GENERAL :-

There are three factors which are responsible for intermediary metabolism viz. insect metabolism, greater depth of Trichlorocarboxylic Acid Cycle (TCA) and its regulation in insects. Major work in living organisms was carried out using vertebrates and micro-organisms as experimental subjects. The major pathways such as glycolysis, TCA fatty acid, ß-oxidation, fatty acid synthesis, amino acid metabolism, pentose phosphate nucleotide metabolism have been already established for insect systems (Bursell 1977, Candy 1970, Bursell 1981, Calaby 1951, Candy 1978, Butterworth et al. 1965, Burnet et al. 1963, Bursell 1963). In insects, the most important factor is the inability of dietary steroids to synthesize the steroid ring system. Metabolic implications of insect specialisation such as ecdysis metamorphosis, flight, nutritional behaviour etc. which leads to major biochemical changes (Neville 1975). The biochemical changes associated with metamorphosis in holometabolous insects are well documented (Chen 1971, Agrell and Lindquist 1973). These studies suggest that carbohydrates and lipids are primary energy reserves in these events.

In some species, the major utilization of carbohydrate occurs during the early stages of metamorphosis (Lindh 1967, Tate and Wimer 1971). The rate of lipid utilization is also known to vary during metamorphosis (Ludwig et al. 1964, D'Costa and Birt 1966). Several reviews are available about chemical changes, during metamorphosis (Needhan 1929, Buck 1953, Rockstein 1957, Gilbert and Schneiderman 1961, Fast 1964, Karlson and Sekeris 1964, Chen 1966 and Gilbert 1967). While studying the biochemical processes underlying insect growth and development it has been observed that glycogen and glucose, whose functions are firmly established among almost all

other animals, play equally important roles in the organization and metabolic activity of this largest class of arthropods (Bailey 1975, Candy, D. 1981, Chippendale G. 1978, Steele, J. 1981). Extensive review on insect biochemistry was also reported by Gilmour (1960). The largest stores of carbohydrate for energy metabolism are glycogen and trehalose with its glucose sub unit usually playing a minor role. Glycogen is stored within the cells and can provide substrate directly without the necessity for transport in to the cell. The regulation of glycolysis in insects is controlled on several points along the pathway. As the major polymeric storage form of glucose in animals, glycogen has been shown by histo-chemical methods to be present in a myriad number of insect tissues and it is generally assumed that it is for the most part similar in structure among insect species and across phyla. The only carefully studied insect glycogen is that isolated in its native state form from Phormia regina flight muscle by mild buffer extraction (Childress, C et al. 1970).

The primary role of lipids is in the formation and functioning of insect cuticle and nutritional requirements of insects. In addition to that the biochemistry and physiology of lipoidal hormones and pheromones has been studied extensively. Lipids have a structural role in all membrane systems of the cell. Phospholipids and steroids are important for this function. Lipids also have a role in regulation and information transfer since some hormones (ecdysone and JH) are lipoidal in nature and pheromones are volatile lipid derivatives.

Amino acids and proteins play an important role in different stages of insect life cycle and overall metabolic pathways are largely the same in insects. Amino acids and their derivatives have a number of different functions in insects. The most important of these is that of protein syntehsis for which all 20 common amino acids are required simultaneously. Lack of any one of the essential amino acids prevents protein synthesis

and leads to increased degradation of the other amino acids (Horie, Y. and Inokvehi, T. 1978). The amino acids required for protein syntehsis are derived from hydrolysis of food proteins from turnover of cell proteins and in some insects from the action of symbiotic microorganisms.

IGR ACTION :-

There are a large number of in vivo metabolic effects reported after allatectomy corpora allata implantation, and JH administration (Gilbert 1964). Van Handel (1988) reported the nutrient accumulation in three mosquitoes during larval develop-ment and its effect on young adults. Wigglesworth (1942) had shown with elegant histological evidence that fourth instar larvae of A. aegypti (Linn) synthesize protein, fat and glycogen. Suppression of many species of pest insects (Menn and Beroza 1972) and for the enhancement of the productivity of beneficial species has also been reported (Murakoshi et al. 1972). Effect of an IGR on lipid and carbohydrate reserves of mosquito pupae were studied by Downer et al. (1976). An effect of IGR's on carbohydrate reserves has previously been demonstrated in pupae of the stable fly, Stomoxys calcitrans by Wright and Rushing (1973). IGR's may influence glycogenolysis indirectly by affecting the synthesis and (or) release of neurosecretions from the neuroendocrine system (Wright et al. 1973). Van Handel and Lea (1975) have demonstrated an involvement of neurosecretory cells in facilitating the interconversion of carbohydrate to lipid in adult mosquitoes and the observed results may reflect an imbalance of the factors responsible for this purpose. Lindh (1967) studied same characteristics of glycogen from a fly pupa (Calliphora Erylhrocephala [Meig]). Devi Lemonde et al. (1963) have shown that the nucleic acid and protein content of another insect at various stages of the development will reflect the variations in their rate of synthesis. Effect of apholate and hempa on nucleic acid and protein synthesis in the yellow fever mosquito

have been studied (Pillai and Agarwal 1969). Nucleic acid content per insect follows the growth curve in A. aegypti as in other insects studied so far (Gilbert 1967, Vickers and Millin 1966, Nigon and Daille 1958). Glycogen synthesis is suppressed by a hormone from the medial neurosecretory cell (MNC) in the mosquito (Lea and Handel 1970). Glycogen in pupae of stable flies is affected by a juvenile hormone analogue (Wright and Rushing 1973). A positive correlation between body size and nutritive reserves such as glycogen and lipid has been described for field caught Ae. vexons by Van Handel and Day (1988). Metabolic relationship between female body size reserves and fecundity of Aedes aegypti have also been reported (Briegel 1990).

MATERIALS AND METHODS

0-12 hr. old pupae of A. aegypti were obtained from mother culture as described in Chapter I and exposed to 3 different temperatures viz. 20, 29 and 38°c. IC50 values of different test chemicals [S-hydroprene, S-methoprene, dimlin (Tech) and dimlin (25 WP)] peculiar to the given temperature (Chapter II) were administered in the water containing test pupae. Three different temperature levels were maintained constant for the entire periods of exposure which were 12 and 24 hrs. for 20°c and 24 and 48 hrs. for 29°c and 38°c. The differences in exposure periods were occasioned by commonly observed average duration of pupal stage at different temperatures. Thus, the pupal life extended to 4 days at 20°c, thereby permitting inclusion of a subsequent 48 hr. exposure. On the other hand, at 29°c and 38°c very frequently adult emergences started at 48 hrs. thus obviating the 48 hrs. exposure. At these temperatures, therefore, apart from 24 hr. exposure period common for all temperatures, another one only 12 hrs. was employed in place of 48 hr.

After completion of the selected exposure period for different IC50 dosages, the test animals were sacrificed and tissues were processed for estimation of protein and carbohydrate (glycogen) contents, as described later.

It may be noted that for all estimations at all variables of dose, temperature and exposure comparisons have been made with simultaneous controls treated with the carrier solvent, acetone.

Biochemical Procedures :-

For the estimation of protein and glycogen, from tissues, the procedure of extraction with cold TCA solution was used. (Roe 1961).

Proteins :-

Modified Lowry's (Anon 1983) method was used for estimation of protein levels. Concentrations of BSA protein (Bovine Serum Albumin Powder, Fraction V from Bovine Plasma, Armour Pharmaceutical Company Ltd., Eastbourne, England Batch WH 1370) ranging from 10-50 µg were used for preparing the standard curve (Fig. 1).

Carbohydrates :-

Morris's (1948) procedure was used for estimation of glycogen. The standard curve was prepared from optical density (OD) values obtained against 10-50 µg strength concentrations (Fig. 2).

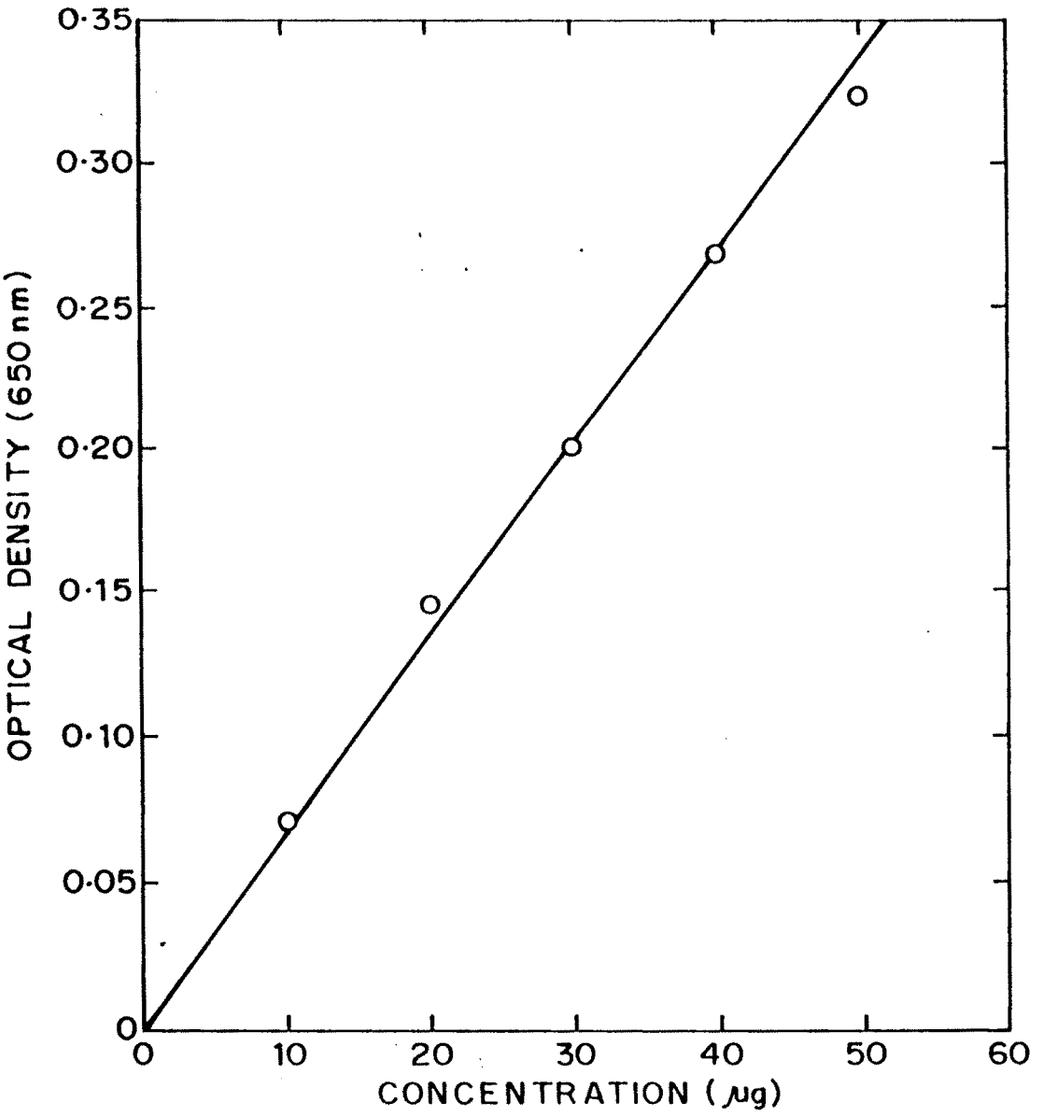


FIG. 1 : STANDARD CURVE FOR BOVINE SERUM AIBUMIN

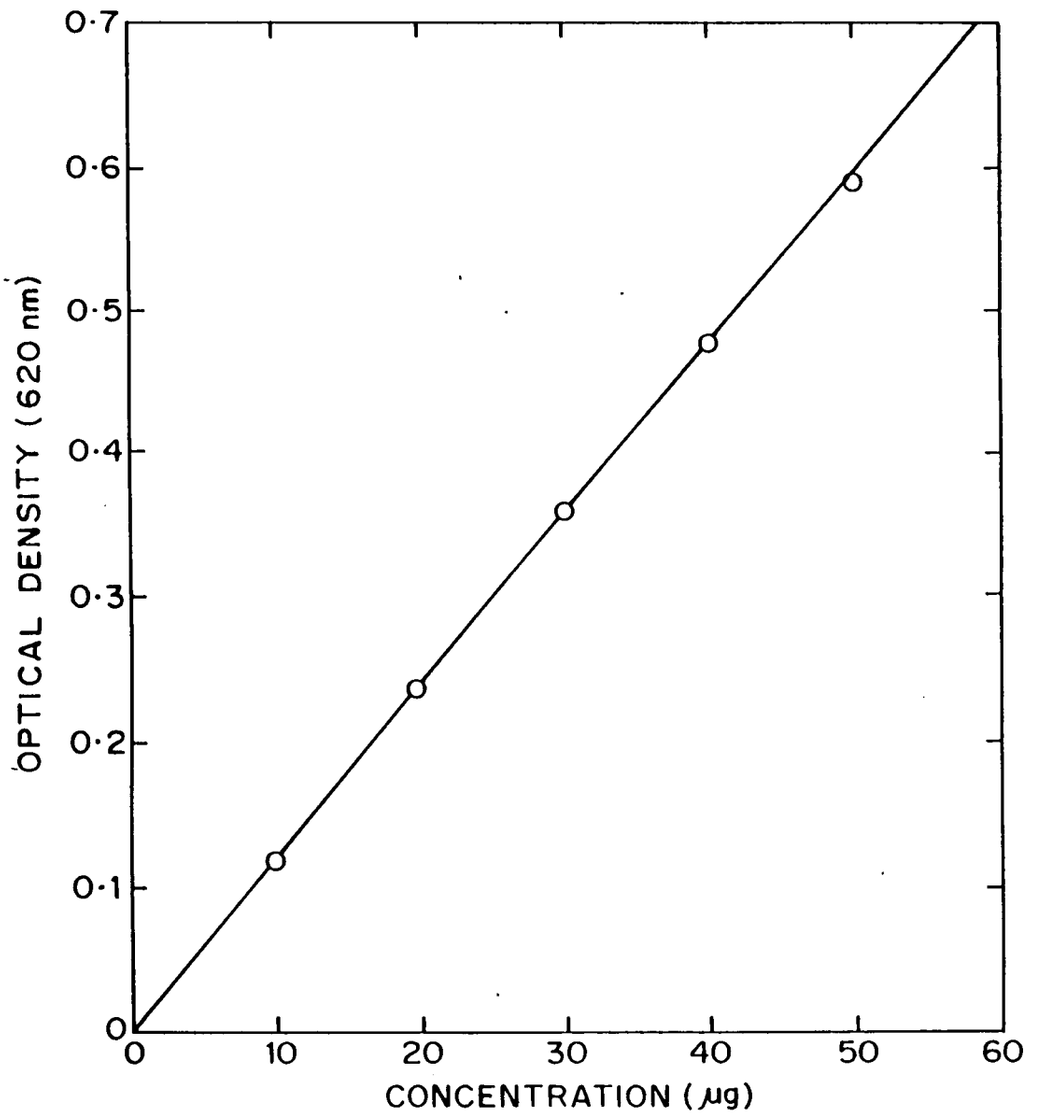


FIG.2:STANDARD CURVE FOR D-GLUCOSE

RESULTS

(A) **PROTEIN** :-(1)

Effect of temperature : -

20_oC :-

When the test pupae were treated with S-hydroprene at 2.914 ppm, the IC50 dose, at this temperature level (Chapter II) the Protein content after 24 hrs. exposure was found to be 36.28 µg/mg body weight. After 48 hrs. exposure this value changed to 42.46 µg/mg body weight. In control animals, the protein content at this temperature after 24 hrs. was 41.78 µg/mg body weight and after 48 hrs., 45.91 µg/mg body weight.

When test pupae were treated with methoprene at the latters IC50 dose at 20°c temperature, protein content after 24 hrs. exposure was found to be 37.04 µg/mg body weight and for 48 hrs. the value was 86.66 µg/mg body weight.

Dimlin (Tech) treated test pupae when exposed to IC50 dose, 0.972 ppm, protein content after 24 hrs. was 37.08 µg/mg body weight and after 48 hrs. the value was 63.04 µg/mg body weight (Table I).

(2) 29°c :-

Test pupae were exposed to the IC50 dose of hydroprene at this temperature level. Protein content after 12 hrs. exposure was 28.63 µg/mg body weight and after 24 hrs. it was 26.76 µg/mg body weight. In control animals the protein content at this tempera-

Table I :- Protein profile of *A. aegypti* pupae on treatment with different IGR's at 20°C.

Compound	Dose (ppm)	Temperature 20°C	
		24 hrs.	48 hrs.
Control	0	41.78 ±1.29	45.91 ±1.30
S-Hydroprene	2.194	36.28 ±2.21	42.46 ±1.39
S-Methoprene	0.173	37.04 ±1.29	86.66 ±2.89
Dimlin (Tech)	0.972	37.08 ±2.34	63.04 ±1.29

* All values are expressed as µg/mg body weight ±SE.

ture after 12 hrs. was 36.71 µg/mg body weight and after 24 hrs. it was 33.47 µg/mg body weight respectively.

Methoprene treated pupae (IC50 dose) exhibited protein content at 12 hrs. 19.96 ug/mg body weight and at 24 hrs. 71.12 µg/mg body weight respectively (Table II).

(3) 38°c :-

Treatment of experimental pupae with S-hydroprene at the IC50 dose at this temperature yielded protein content after 12 hrs. 20.92 µg/mg body weight and after 24 hrs. 48.06 µg/mg body weight respectively. While in control animals the protein content at this temperature after 12 hrs. was 38.96 µg/mg body weight and after 24 hrs. 38.43 µg/mg body weight.

Methoprene treated pupae exhibited 40.04 µg/mg body weight protein content at 12 hrs. and 43.56 µg/mg body weight for 24 hrs.

Dimlin (Technical) treated pupae (IC50 dose) exhibited protein content after 12 hrs., 38.70 µg/mg body weight and after 24 hrs., 49.35 µg/mg body weight (Table III).

(B) **CARBOHYDRATE**

20°C :-

Test pupae were treated with S-hydroprene at the IC50 dose (2.914 ppm) at 20°c. Glycogen content after 24 hrs. exposure was found to be 18.11 µg/mg body weight and after 48 hrs. 6.45 µg/mg body weight. In control animals, the glycogen content at this temperature after 12 hrs. was observed to be 19.16 µg/mg body weight and after 24

Table II : Protein profile of A. aegypti pupae on treatment with different IGR's at 29°C.

Compound	Dose (ppm)	Temperature 29°C 12 hrs.	24 hrs.
Control	0	36.71 ±1.35	33.47 ±1.38
S-Hydroprene	6.977	28.63 ±1.25	26.76 ±1.35
S-Methoprene	6.096	19.96 ±1.21	71.12 ±3.85
Dimlin (Tech)	0.777	18.62 ±2.89	31.34 ±2.36

* All values are expressed as µg/mg body weight ±SE.

Table III : Protein profile of *A. aegypti* pupae on treatment with different IGR's at 38°C.

Compound	Dose (ppm)	Temperature 38°C	
		12 hrs.	24 hrs.
Control	0	38.96 ±1.33	38.43 ±1.89
S-Hydroprene	1.741	20.92 ±1.29	48.06 ±1.92
S-Methoprene	1.322	40.04 ±1.81	43.56 ±2.32
Dimlin (Tech)	0.030	38.70 ±1.39	49.35 ±2.35

* All values are expressed as µg/mg body weight ±SE.

hrs. 7.88 µg/mg body weight.

With methoprene treated (IC50 dose = 0.173 ppm) test pupae the glycogen content at 12 hrs. was 12.45 µg/mg body weight and for 24 hrs. 6.48 µg/mg body weight respectively.

When the test pupae were examined after dimlin (Tech.) treatment at the latter IC50 dose, the glycogen content at 12 hrs. was 11.25 µg/mg body weight and for 24 hrs. it was 3.41 µg/mg body weight (Table IV).

29°c :-

When the test pupae were treated with hydroprene (IC50 dose) at this temperature glycogen content after 12 hrs. was 7.17 µg/mg body weight and at 24 hrs. 17.77 ug/mg body weight. In control animals, the glycogen content at this temperature after 12 hrs. was 6.07 µg/mg and at 24 hrs. 17.68 µg/mg body weight.

Methoprene treated test pupae (IC50 dose) exhibited glycogen content at 12 hrs. as 8.95 µg/mg body weight and at 24 hrs. it was 59.16 µg/mg body weight.

Dimlin (Tech.) treated pupae (IC50 dose) exhibited glycogen content at 12 hrs. as 10.65 µg/mg and after 24 hrs. it was about 16.74 µg/mg body weight (Table V).

38°c :-

When the test pupae were exposed continuously at their IC50 dose for hydroprene at 38°c, glycogen content after 12 hrs. was 8.33 µg/mg body weight and after 24 hrs.

Table IV : Glycogen titre of *A. aegypti* pupae on treatment with IGR's at 20°C.

Compound	Dose (ppm)	Temperature 20°C 24 hrs.	48 hrs.
Control	0	19.16 ±1.35	7.88 ±1.21
S-Hydroprene	2.914	18.11 ±1.21	6.45 ±1.36
S-Methoprene	0.173	12.45 ±1.89	6.48 ±1.01
Dimlin (Tech)	0.972	11.25 ±1.42	3.41 ±1.29

* All values are expressed as µg/mg body weight ISE.

Table V : Glycogen titre of A. aegypti pupae on treatment with IGR's at 29°C.

Compound	Dose (ppm)	Temperature 29°C 12 hrs.	24 hrs.
Control	0	6.07 ±1.10	17.68 ±1.21
S-Hydroprene	6.977	7.17 ±0.89	17.77 ±1.26
S-Methoprene	6.096	8.95 ±1.21	59.16 ±3.56
Dimlin (Tech)	0.777	10.65 ±1.23	16.74 ±1.21

* All values are expressed as µg/mg body weight ±SE.

14.36 µg/mg body weight. On the other hand in control animals the glycogen content at 38°c after 12 hrs. was 8.51 µg/mg body weight and after 24 hrs. it was 4.72 µg/mg body weight.

Methoprene treated pupae (IC50 dose) exhibited glycogen content after 12 hrs. was 7.14 µg/mg body weight and after 24 hrs. 12.91 µg/mg body weight.

With dimlin (Tech.) treatment at IC50 dose, the exhibited glycogen content after 12 hrs. as 11.94 µg/mg body weight and after 24 hrs. it was 5.27 µg/mg body weight (Table VI).

DISCUSSION

As remarked earlier natural juvenile hormone does not seem to be present in the pupal stage. The absence of this vital chemoregulator in the pupal stage may be as a result of decreased activity of the corpora allata (CA) and or increased degradation of the hormone e.g. by enzyme such as carboxylesterase. It has been already shown in the present work (Chapter II) that treat-ment of the pupal stage of mosquitoes with extraneous IGRs, especially a JHA, causes definitive disruption of development and metamorphosis resulting in overall reduction of viable normal adult emergence.

The results obtained in the present investigation reveal that extraneous IGR application seems to influence both protein and carbohydrate (glycogen) levels albeit selectively both in terms of the test chemical used and temperature of exposure. Stimulation of protein synthesis under the influence of various JH analogues has been reported in various insect species. (Sroka and Gilbert 1974, Benskin and Vinson 1973, Elliott

Table VI : Glycogen titre of *A. aegypti* pupae on treatment with IGR's at 38°C.

Compound	Dose (ppm)	Temperature 38°C 12 hrs.	24 hrs.
Control	0	8.51 ±1.10	4.72 ±0.81
S-Hydroprene	1.741	8.33 ±1.11	14.36 ±1.21
S-Methoprene	1.322	7.14 ±1.29	12.91 ±1.35
Dimlin (Tech)	0.030	11.94 ±1.29	5.27 ±1.32

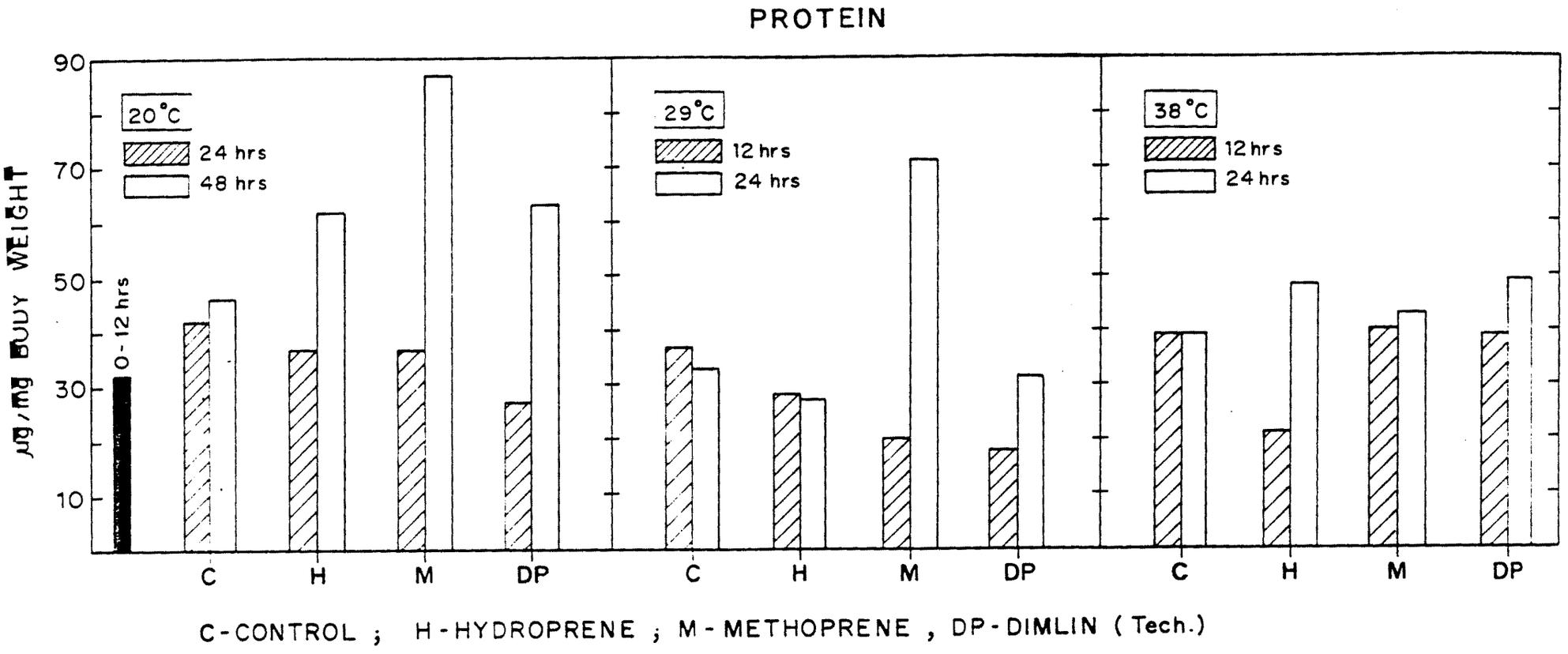
* All values are expressed as µg/mg body weight ±SE.

1978, Koeppe et al. 1981). Thus it may be seen that the JHA methoprene most conspicuously affects protein as well as glycogen levels after 48 hrs. exposure at 20°c and 29°c temperatures. The JHA hydroprene, follows methoprene closely in exhibiting similar ostensible effects on protein contents especially after 48 hr. exposure at 20°c and 38°c and on carbohydrate (glycogen) at 29°c and 38°c after 24 hr. exposure (Fig.3).

The anti ecdysial IGR, dimlin also exhibits significantly elevated levels of protein at 20°c after 48 hr. exposure and at 38°c after 24 hr. exposure. Strangely glycogen levels are not significantly affected by dimlin at 20° and 29°c. However, there is a percetable decline in glycogen levels at 38°c after 12 hrs. exposure (Fig.4).

From the foregoing it would appear that the JHA methoprene is the most potent IGR affecting fluctuations in vital biochemical parameters investigated. This is not surprising in view of the known excellence and high potency of methoprene. The latter is closely followed by hydroprene in both JH activity as well as influence on the biochemistry of test animals, as investigated herein. The anti-ecdysial dimlin is a poor third in terms of its effects on the selected biochemical parameters. It may be recalled here that apart from the per se effects on the selected biochemical elements namely proteins and glycogens, the variable of temperatures has also been incorporated in the overall design of experiments.

The information gleaned from the data obtained indicates that the length of exposure as well as level of temperature seem to combine forces in additively affecting the biochemistry of test insect except that at the nearly lethal, high stress temperature level of 38°c, these effects seem to tone down presumably as a consequence of extreme stress approaching termination of life.



C-CONTROL ; H-HYDROPRENE ; M-METHOPRENE , DP-DIMLIN (Tech.)

FIG.3 : COMPARATIVE PROTEIN LEVELS IN A. AEGYPTI PUPAE ON EXPOSURE TO IGRS AT DIFFERENT TEMPERATURES.

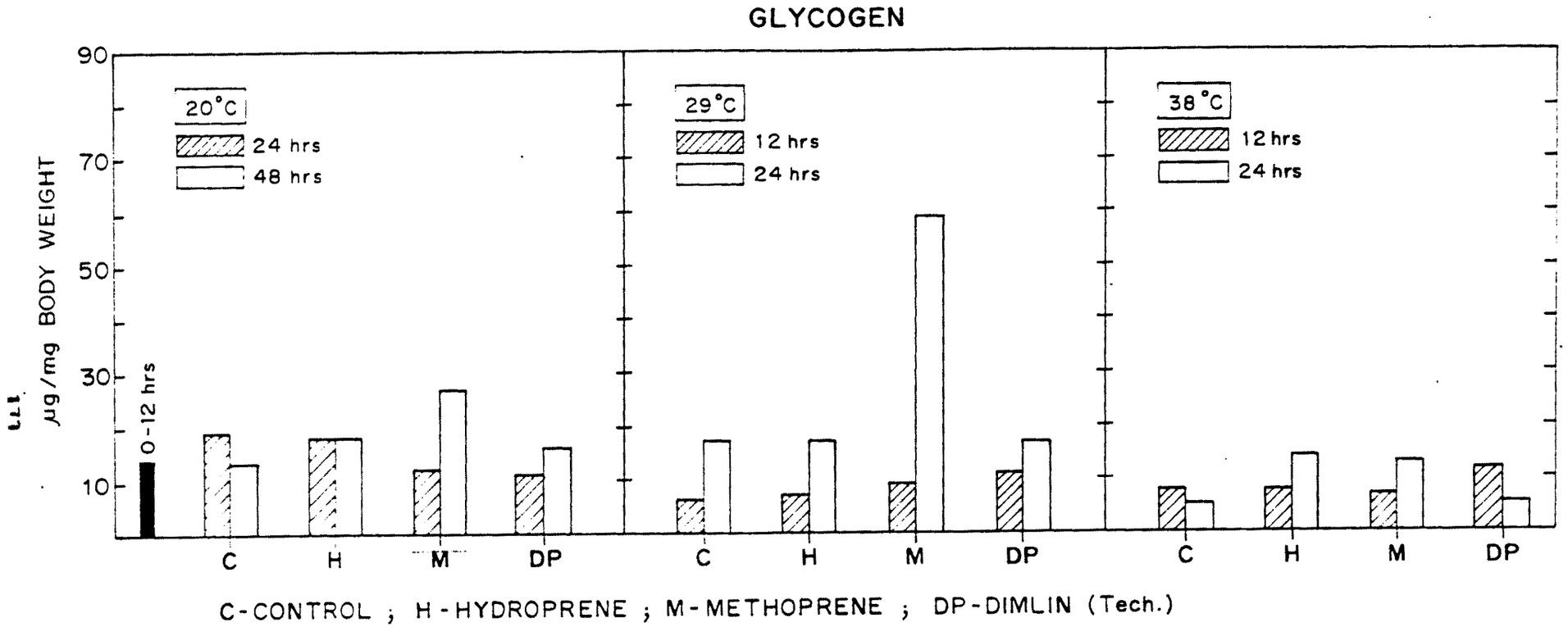


FIG. 4: COMPARATIVE GLYCOGEN LEVELS IN A. AEGYPTI PUPAE ON EXPOSURE TO IGRS AT DIFFERENT TEMPERATURES.

The effects on the selected biochemical parameters namely protein and glycogen contents, targeted for the present investi-gation reveal that various IGRs especially the more potent JHAs are definitively potent enough to disrupt metamorphic events and thereby cause vital alterations in important biochemical ingredients of the test organisms. The net result of these is overall population reduction. The present investigation is thus able to provide one more criterion for potent IGR action, namely changes in protein and carbohydrate levels in IGR treated animals. Additionally the present data also indicate that temperature levels as well as exposure time also act in tandem to produce the results, which are peculiar to the experimental design incorporating these variables, as in the present investigation.

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CHAPTER SIX

Bioactivity of Ageing Residues of IGR's at Different Temperatures.

Introduction :

Stability and persistence of activity of agrochemicals, particularly those intended to affect pests/vectors, is an important consideration in assessment of field/commercial potentials. Older conventional insecticides - the hydrocarbons, cyclodienes etc. had little handicaps in this respect. In fact, very high persistences have been the ultimate cause of their doom. In contrast, the modern Third and Fourth generation pesticides, notably the IGR's, unfortunately suffer greatly on this count. Thus, most juvenoids are dramatically unstable, photo and thermo- decompositions are the primary labilities though other types of degradation may also occur (Gill et al. 1972). An important practical photochemical reaction is the isomerization of 2E, 4E methoprene to the biologically inactive 2Z, 4E isomer. Photodegradation products exhibit greatly reduced biological activity (Singh 1973, Gill et al. 1974, Hammock et al. 1974a).

Studies undertaken for determination of diflubenzuron residues in a variety of agricultural and non agricultural crops, aquatic vegetation, forest products, cow and poultry tissues milk eggs have been well summarized by Carlson (1980).

Curiously, biological activity of some IGRs viz. methoprene and dimlin continues even when their chemical residues cease being detected by GLC techniques (Madder and Lockhart 1980). It is possible that unknown or undetectable minor components may be the causes of this continuing bioaction. Information on these aspects is particularly scanty with respect to the pupal stage of the mosquito. The pupa, of course is reportedly the most hardy or resistant stage in the life cycle of mosquito. It, therefore, follows that bioactivity obtained on the pupa must necessarily accrue to the larval stages also. In other words, data on bioactivity of aged residues of IGR's on

the pupa would be of enormous relevance for practical application.

The present chapter incorporates results of such an investigation.

Literature Survey :

Methoprene is one of the first juvenile hormone mimics to gain field and commercial acceptance. Its persistence has been extensively studied in laboratory and field experiments. Sophisticated chemical methods for administration of residues of methoprene have been done and used in the Western World fairly extensively (Wright and Bowman 1972). Miller et al. (1975) have published a comprehensive paper on the determination of residues at ppb level in water, soil, plant and animal samples. Hunt and Gilbert (1976) developed a method for determining this insect growth regulator using both a small sample size of beef fat and HPLC column of parasi. The sensitivity limit was estimated as 8 ng/g. Methoprene is effectively separated on a 25 x 0.46 cm. column of Zorbox SIL (Dupont) with a solvent system such as hexane-diethyl ether (97:3). The lower limit of sensitivity is about 10 ng (D.A. Schooley). Environmental degradation of methoprene with respect to photodecomposition has been studied by Quistad et al. (1975). Studies were conducted (Madder and Lockhart 1980) on the dissipation of diflubenzuron and methoprene from shallow prairie pools. Methoprene is considerably more stable to degradation under conditions necessary for stored products pest control.

However, due to its exorbitant costs methoprene has never been used in the field in developing world including India. As such, there has been little or no work on methoprene or other IGR residues in this part of the world. However, reports on e.g. photodecomposition (Gill, et al. 1972) etc. of various IGRs including methoprene and

diflubenzuron are important indicators and guideline for evaluation of their stability and commercial/ field feasibility. In the present study more active isomers of methoprene and hydroprene have been used apart from chitin inhibitor diflubenzuron. Review of literature on these products has revealed useful information on stability, limits of sensitivity, persistence etc.

Factors affecting the stability of dimlin in water and its persistence in field waters were studied by Schaefer and Dupras (1976). High temperature and elevated pH enhanced instability (Ivic et al. 1979, 1980a). Half life of diflubenzuron in water was found as being 56, 7 and < 3 days for pH 4,6 and 10 respectively. The degradation of diflubenzuron by aquatic organisms has been reviewed by Schooley and Quistad (1979). Aquatic microbial metabolism has been reported by Metcalf et al. (1975), Schaefer and Dupras (1976, 1977) and Booth and Ferrell (1977). The degradation of diflubenzuron by fish as well as other components of an aquatic eco-system was detailed by Metcalf et al. (1975) and Booth and Ferrell (1977).

Since hydroprene is not as commercially popular as methoprene there is a paucity of information concerning its environmental degradation.

It may be concluded that on the whole methoprene seems to be considerably more stable to degradation in storage condition as opposed to open field conditions. It is also important to note that chemical instrumentational analysis of residues as e.g. by GLC may not always be correct indicators of continuing or totally absent biological activity (Madder 1978, 1980).

It is on the basis of such report that biological assessment of residual activities on crucial or pivotal stages of life cycle becomes important.

Materials and Methods

Methods for examining effects of test chemicals used in the present work on Aedes aegypti pupa for 24 hr. exposure period have been described in Chapter III. For assessing loss of activity on aging the following procedures were adopted.

0-16 hr old Aedes aegypti pupae were collected from laboratory culture. IGR's viz. s-Hydroprene, s-Methoprene and Dimlin (both technical as well as 25 wp formulation) were used at the concentration level of 1,3 and 5 parts per million (ppm). These chemicals were introduced in 50 ml water in 100 ml beaker and kept in BOD incubator at different temperatures i.e. 20, 25, 29, 34 and 38°c. On 3rd, 5th, 7th and 15th day the test animals (0-16 hr old pupae) were introduced in these concentrations of the test chemicals and kept there till adult emergence. Pupal mortality, abnormal adults and normal adult emergences were recorded to analyse persistence of bioactivity and its implications.

In order to countercheck and supplement the above, similar experiments were conducted, albeit at ambient temperature (29°c) only, with the IV instar larvae also. All procedures remained the same as described earlier and above.

Results :

Results in terms of bioactivity obtained (or not obtained) as a consequence of exposure of test animals (0-16 hr old pupae) to IGR residues aged for 3,5,7 and 15 days at different temperatures are given below. The results are arranged according to the temperatures at which the IGR residues were aged.

(A) **Effect of 20°c temperature** - (Table - I)

Hydroprene :

1 and 3 ppm concentrations aged at 20°c for 3,5,7 and 15 days failed to produce any observable effects on test pupae. Residues of 5 ppm kept for 3 days at 20°c, however, induced 10.52% pupal mortality, 52.63% mortality of emergent abnormal adults and 36.84% normal adults were produced. On exposure of test pupae to 5 days old residues of 5 ppm concentration, adult emergence increased to 45%. In 7 days aged residues of 5 ppm dose, inhibition of adult emergence was reduced to 44.45% i.e. 55.55% normal adult emerged and after 15 days, none of the dosages including 5 ppm was effective i.e. 100% normal adults emerged from the exposed pupae.

Methoprene :

Exposure of test pupae to 3 day old 1 ppm dose residues caused 42.11% adult mortality. Similarly aged 3 ppm residues produced 50% mortality of emergent abnormal adults while the remaining adults emerged normal and survived. 3 day old 5 ppm doses gave 9.54% pupal mortality. 66.66% emergent abnormal adult mortality and 23.80% normal adult emergence. 5 day old 1 ppm residues gave 5.55% pupal mortality, 44.45% emergent abnormal adult mortality and 50.0% normal adult emergence. Curiously, 5 ppm aged residues resulted into 85% emergent abnormal adult and 15% normal adult emergence. Again 7 day aged 1 ppm residues produced 10% pupal mortality and 90% abnormal adults. 5 ppm caused 85% emergent abnormal adult mortality and 25% normal adults. 1 and 3 ppm dosages failed to produce any IGR effects on the test pupae after 15 days aging. However, 40% abnormal adult mortality and 60% normal adult emergence were recorded in 5 ppm concentration aged for 15 days.

Table I : Biological activity of different aged residues of different IGRs on *Aedes aegypti* pupae at 20°c

Compound	3 DAY			5 DAY			7 DAY			15 DAY		
Dose (ppm)	PM	AA	NA	PM	AA	NA	PM	AA	NA	PM	AA	NA
Control	0	0	100	0	0	100	0	0	100	0	0	100
S-Hydroprene												
1	0	0	100	0	0	100	0	0	100	0	0	100
3	0	0	100	0	0	100	0	0	100	0	0	100
5	10.53 ±1.21	52.63 ±4.56	36.84 ±2.34	0	55.0 ±3.89	45.0 ±4.52	0	44.45 ±3.89	55.55 ±2.30	0	0	100
S-Methoprene												
1	0	42.11 ±2.12	57.89 ±4.62	5.55 ±1.21	44.45 ±2.32	50.0 ±4.29	10.0 ±1.21	0	90.0 ±3.45	0	0	100
3	0	50.0 ±3.12	50.0 ±4.21	5.26 ±2.10	68.43 ±2.89	26.31 ±1.21	10.55 ±1.23	47.35 ±2.32	42.10 ±2.89	0	0	100
5	9.54 ±1.23	66.66 ±4.56	23.80 ±2.22	0	85.0 ±2.39	15.0 ±1.23	0	75.0 ±3.85	25.0 ±2.32	0	40.0 ±3.89	60.0 ±4.45
Diflubenzuron (T)												
1	0	47.38 ±1.23	52.62 ±4.36	0	28.52 ±2.22	71.48 ±5.23	5.0 ±1.1	35.0 ±1.29	60.0 ±4.53	50.0 ±4.63	25.0 ±2.25	25.0 ±2.22
3	0	55.0 ±3.33	45.0 ±4.41	5.0 ±1.21	40.0 ±2.22	55.0 ±3.49	0	39.14 ±2.23	60.86 ±4.49	80.0 ±6.21	0	20.0 ±1.29
5	4.34 ±2.89	56.52 ±5.34	39.14 ±4.20	0	50.0 ±4.89	50.0 ±3.89	0	52.93 ±4.28	47.07 ±3.89	55.45 ±4.89	27.29 ±2.11	17.17 ±1.33
Diflubenzuron (25 wp)												
1	0	0	100	0	0	100	0	0	100	0	0	100
3	0	0	100	0	0	100	0	0	100	0	0	100
5	0	0	100	0	0	100	0	0	100	0	0	100

Dimlin (Tech). :

Diflubenzuron (Tech.) 1 ppm after 3 day aging leads to 47.38% emergent abnormal adult mortality and 52.62% normal adults. In 5 ppm concentration, 3 day aged residues inflicted 4.34% pupal mortality, 56.52% emergent abnormal adult mortality and 39.14% normal adult emergence was obtained. After 7 day aging 28.52% emergent abnormal adult mortality accrued and 71.48% normal adults were emerged at 1 ppm. Distribution of abnormal adult mortality and normal adult emergence were equal in 5 ppm residues aged for 5 days. However, 15 day aging of 1 ppm residues gave 50% pupal mortality, 25% emergent abnormal adult mortality and 25% normal adults. Similarly aged residues of 5 ppm concentration produced 55.45% pupal mortality, 27.29% emergent abnormal adult and 17.17% normal adults.

Dimlin (25 wp) :

Different (3,7,5 and 15 day) aged residue of 1,3 and 5 ppm concentrations failed to produce any morphological effects on the test pupae.

(B) **Effect of 25°c temperature** - (Table II)

Hydroprene :

1 and 3 ppm Hydroprene on 3 days aging did not cause any morphological abnormalities in the test animals. However, same aging of 5 ppm doses produced 55% emergent abnormal adult mortality and 45% normal adults. After 5,7 and 15 day aging, all dosages were found ineffective.

Table II : Biological activity of different aged residues of different IGRs on *Aedes aegypti* pupae at 25°c

Compound Dose (ppm)	3 DAY PM	3 DAY AA	3 DAY NA	5 DAY PM	5 DAY AA	5 DAY NA	7 DAY PM	7 DAY AA	7 DAY NA	15 DAY PM	15 DAY AA	15 DAY NA
Control	0	0	100	0	0	100	0	0	100	0	0	100
S-Hydroprene												
1	0	0	100	0	0	100	0	0	100	0	0	100
3	5.26 ±1.21	15.78 ±1.23	78.96 ±7.34	0	0	100	0	0	100	0	0	100
5	0	55.0 ±4.45	45.0 ±3.33	0	0	100	0	0	100	0	0	100
S-Methoprene												
1	5.0 ±1.10	10.0 ±1.29	85.0 ±7.23	0	22.22 ±2.22	77.78 ±4.89	0	0	100	0	0	100
3	0	52.38 ±6.23	47.62 ±3.29	0	41.18 ±2.22	58.82 ±4.49	0	15.0 ±1.29	85.0 ±7.33	0	0	100
5	5.28 ±1.11	47.36 ±3.33	47.36 ±4.42	0	63.15 ±4.36	36.85 ±2.29	0	25.0 ±4.45	75.0 ±3.34	0	15.0 ±1.21	85.0 ±4.34
Diflubenzuron (T)												
1	0	36.85 ±1.21	63.15 ±3.33	0	15.78 ±1.33	84.22 ±4.32	5.0 ±1.10	25.0 ±1.29	70.0 ±5.21	5.0 ±2.12	5.0 ±2.84	90.0 ±4.45
3	0	63.15 ±4.33	36.85 ±3.15	0	20.0 ±1.89	80.0 ±3.89	0	35.0 ±3.50	65.0 ±4.89	0	20.0 ±2.13	80.0 ±3.50
5	10.52 ±2.89	52.64 ±5.34	36.84 ±2.39	0	50.0 ±4.23	50.0 ±5.89	9.53 ±1.89	28.57 ±3.89	61.90 ±4.45	4.76 ±2.21	23.76 ±4.35	71.48 ±3.89
Diflubenzuron (25 wp)												
1	0	0	100	0	0	100	0	0	100	0	0	100
3	0	0	100	0	0	100	0	0	100	0	0	100
5	0	0	100	0	0	100	0	0	100	0	0	100

Methoprene :

1 ppm methoprene after 3 days aging caused only 5% pupal mortality and 10% emergent abnormal adult mortality, resulting in overall 85% normal adult emergence from exposed pupae. Similarly aged 5 ppm residues produced 5.28% pupal mortality, 47.36% emergent abnormal adult mortality and 47.36% normal adults. Exposure of the test pupae to 5 day old residues produced 22.22% emergent abnormal adult mortality and 77.28% normal adult emergence at 1 ppm. However, similarly aged 5 ppm residues produced 63.15% emergent abnormal adult mortality and 36.85% normal adults. 1 ppm, 7 day residues failed to produce any observable effects. However, week old 5 ppm residues caused 25% emergent abnormal adult mortality and 75% normal adult emergence. After 15 day aging, both 1 and 3 ppm failed to produce any IGR effects. However, 2 week old 5 ppm residues produced 15% emergent abnormal adult mortality and 85% normal adults.

Dimlin (Tech.) :

Exposure of test pupae to 3 day old 1 ppm dose residues caused 36.85% emergent abnormal adult mortality and 63.15% normal adults emergence. However, 5 ppm residues gave 10.52% pupal mortality, 47.64% emergent abnormal adult mortality and 36.84% normal adult emergence. Exposure, to 5 day old residues produced 15.78% emergent abnormal adult mortality and 84.21% normal adults. At 5 ppm, distribution of emergent abnormal adult mortality and normal adult emergence (50%) were equal. On exposure of test pupae to 7 day old residues 5% pupal mortality, 15% emergent abnormal adult mortality and 70% normal adults were produced. Week old 5 ppm residues gave 9.53% pupal mortality, 28.57% emergent abnormal adult mortality and 61.90% normal adults. Again, 15 day aged residues produced 90% normal adult emergence. However, same aging of 5 ppm dose caused 4.76% pupal mortality,

23.76% emergent abnormal adult mortality and 71.48% normal adults emerged.

Dimlin (25 WP) :

1,3 and 5 ppm doses were not effective i.e. 100% normal adults emerged even with fresh doses.

(c) **Effect of 29°c temperature** - (Table-III)

Hydroprene :

Exposure of test pupae to all doses (1,3 and 5 ppm) failed to exhibit any observable effects on the test pupae even with freshly applied doses.

Methoprene :

At all doses methoprene was also ineffective even on fresh application.

Dimlin (Tech.) :

Exposure of test pupae to 3 day aged residues caused 60% abnormal adult mortality and 40% normal adults emerged at 1 ppm. dose. Similarly aged 5 ppm residues resulted in 5.26% pupal mortality and 36.86% adult emergence. Again 5 day aged 1 ppm doses produced 41.17% emergent abnormal adult mortality and 58.83% normal adult emergence. 7 day aged 1 ppm residues produced 50% emergent abnormal adult mortality and 45% normal adults. Similarly aged 5 ppm residues gave 88.24% emergent abnormal adult mortality and 11.76% normal adults. 15 day aged residues

Table III : Biological activity of different aged residues of different IGRs on *Aedes aegypti* pupae at 29°c

Compound and Dose (ppm)	3 DAY PM	3 DAY AA	3 DAY NA	5 DAY PM	5 DAY AA	5 DAY NA	7 DAY PM	7 DAY AA	7 DAY NA	15 DAY PM	15 DAY AA	15 DAY NA
Control	0	0	100	0	0	100	0	0	100	0	0	100
S-Hydroprene												
1	0	0	100	0	0	100	0	0	100	0	0	100
3	0	0	100	0	0	100	0	0	100	0	0	100
5	0	0	100	0	0	100	0	0	100	0	0	100
S-Methoprene												
1	0	0	100	0	0	100	0	0	100	0	0	100
3	0	0	100	0	0	100	0	0	100	0	0	100
5	0	15.0 ±2.32	85.0 ±4.5	10.52 ±1.29	5.26 ±2.32	84.22 ±2.42	0	26.31 ±2.45	73.69 ±3.85	0	0	100
Diflubenzuron (T)												
1	0	60.0 ±2.9	40.0 ±3.5	0	36.85 ±4.5	63.15 ±3.89	5.0 ±1.28	50.0 ±5.23	45.0 ±3.30	0	0	100
3	0	55.0 ±3.25	45.0 ±4.32	0	38.88 ±3.30	61.12 ±4.50	0	75.0 ±2.80	25.0 ±3.50	0	0	100
5	5.26 ±1.28	57.88 ±5.38	36.86 ±4.82	0	41.17 ±3.33	58.83 ±4.50	0	88.24 ±4.50	11.76 ±1.50	0	25.0 ±1.28	75.0 ±2.30
Diflubenzuron (25 wp)												
1	0	0	100	0	0	100	0	0	100	0	0	100
3	0	0	100	0	0	100	0	0	100	0	0	100
5	0	0	100	0	0	100	0	0	100	0	0	100

failed to produce any IGR effects at 1 ppm. However, at 5 ppm these resulted into 25% emergent abnormal adult mortality and 75% normal adult emergence.

1,3 and 5 ppm failed to produce any morphological effects to test pupae even on fresh application of these doses.

(D) **Effect of 34°c temperature** - Table IV

Hydroprene :

Hydroprene at 1,3 and 5 ppm also did not exhibit any observable effects to test pupae even with fresh applications.

Methoprene :

Exposure of test pupae to 3 day old 1 ppm residues produced 18.18% emergent abnormal adult mortality and 81.82% normal adults. However, similarly aged 5 ppm residues lead to 33.34% emergent abnormal adult mortality and 66.66% normal adults. 5,7 and 15 day old residues failed to produce any IGR effects even at 5 ppm.

Dimlin (Tech.) :

3 day old 1 ppm Dimlin (Tech.) produced 63.63% emergent abnormal adult mortality and 36.37% normal adults emerged. Similarly aged 5 ppm dose caused 100% pupal mortality. 5 day aged 1 ppm residues caused 5.55% pupal mortality 61.10% emergent abnormal adult mortality and 33.33% normal adult emerged. However, similarly aged 5 ppm dose caused 50% pupal mortality and 50% emergent abnormal

Table IV : Biological activity of different aged residues of different IGRs on *Aedes aegypti* pupae at 34°c

Compound and Dose (ppm)	3 DAY			5 DAY			7 DAY			15 DAY		
	PM	AA	NA	PM	AA	NA	PM	AA	NA	PM	AA	NA
Control	0	0	100	0	0	100	0	0	100	0	0	100
S-Hydroprene												
1	0	0	100	0	0	100	0	0	100	0	0	100
3	0	0	100	0	0	100	0	0	100	0	0	100
5	0	0	100	0	0	100	0	0	100	0	0	100
S-Methoprene												
1	0	18.18 ±1.28	81.82 ±6.89	0	0	100	0	0	100	0	0	100
3	0	25.0 ±2.80	75.0	0	0	100	0	0	100	0	0	100
5	0	33.34 ±2.39	66.66 ±5.35	0	0	100	0	0	100	0	0	100
Diflubenzuron (T)												
1	0	63.63 ±5.23	36.37 ±3.85	5.55 ±2.89	61.12 ±5.20	33.33 ±3.29	0	22.23 ±4.50	77.77 ±6.89	0	11.2 ±2.89	88.8 ±6.89
3	75.0 ±5.28	25.0 ±2.89	0	42.10 ±4.32	36.85 ±4.50	21.05 ±5.28	0	25.0 ±3.89	75.0 ±3.25	0	11.2 ±2.32	88.8 ±4.50
5	100	0	0	50.0 ±5.20	50.0 ±3.89	0	10.52 ±6.28	26.33 ±4.50	63.15 ±3.45	0	40.0 ±5.28	60.0 ±6.89
Diflubenzuron (25 wp)												
1	0	0	100	0	0	100	0	0	100	0	0	100
3	0	0	100	0	0	100	0	0	100	0	0	100
5	0	0	100	0	0	100	0	0	100	0	0	100

adult mortality. 7 day aged 1 ppm residue lead to 22.22% emergent abnormal adult mortality and 77.77% normal adults. However, 7 day old 5 ppm dose caused 10.52% pupal mortality, 26.33% emergent abnormal adult mortality and 63.15% normal adults emerged. 15 day aged residues of 3 ppm dose caused 11.2% emergent abnormal adult mortality and 38.8% normal adult were recorded. 15 day old 5 ppm residues caused 40% emergent abnormal adult mortality and produced 60% normal adults.

Dimlin (25 WP) :

1,3 and 5 ppm doses failed to produce any observable effects on test pupae even on fresh application of these doses.

(E) **Effect of 38°c temperature** - (Table V)

Hydroprene :

3 day old 1 ppm dose did not produce any morphological effects on test pupae. Similarly aged 5 ppm lead to 80% normal adult emergence and 20% emergent abnormal adult mortality. In 5,7 and 15th day old residues even 5 ppm dose did not produce any IGR effects.

Methoprene :

1 ppm methoprene after 3 day aging leads to 4.76% pupal mortality, 23.80% emergent abnormal adult mortality and 71.44% normal adults. However, similarly aged residues 5 ppm gave 40% emergent abnormal adult mortality and 60% normal adults. Exposure of test pupae to 5 day old 5 ppm residue produced 16.68% pupal

Table V: Biological activity of different aged residue of different IGRs on *Aedes aegypti* pupae at 38°c

Compound and Dose (ppm)	3 DAY PM	3 DAY AA	3 DAY NA	5 DAY PM	5 DAY AA	5 DAY NA	7 DAY PM	7 DAY AA	7 DAY NA
Control	0	0	100	0	0	100	0	0	100
S-Hydroprene									
1	0	0	100	0	0	100	0	0	100
3	0	0	100	0	0	100	0	0	100
5	0	20.0	80.0	5.26	5.26	89.48	0	0	100
S-Methoprene									
1	4.76 ±1.28	23.80 ±4.52	71.44 ±5.89	0	0	100	0	0	100
3.	15.0 ±3.89	15.0 ±5.2	70.0 ±3.89	0	0	100	0	0	100
5	0	40.0 ±3.89	60.0 ±2.80	16.68 ±2.32	16.66 ±3.85	66.66 ±4.5	0	10.0 ±1.28	90.0 ±5.23
Diflubenzuron (T)									
1	55.55 ±2.89	11.10 ±3.89	33.35 ±4.50	73.68 ±5.89	15.78 ±3.89	10.54 ±4.50	23.5 ±2.89	47.05 ±5.25	29.45 ±5.23
3	73.68 ±4.52	26.32 ±3.89	-	95.0 ±2.90	5.0 ±1.28	-	100	-	-
5	90.47 ±4.52	9.53 ±3.89	-	100	-	-	100	-	-
Diflubenzuron (25 wp)									
1	0	52.63 ±3.89	47.37 ±5.28	0	15.78 ±3.82	84.22 ±4.82	0	50.0 ±2.89	50.0 ±5.28
3	15.78 ±5.23	42.12 ±4.23	42.10 ±5.2	5.0	35.0 ±3.80	60.0 ±4.50	0	63.15 ±3.89	36.85 ±4.28
5	10.52 ±4.50	68.43 ±2.32	21.05 ±4.50	0	57.88 ±6.89	42.21 ±5.28	10.52 ±2.89	63.15 ±4.82	26.33 ±4.38

mortality and 66.66% normal adults. Week or fortnight old 1,3 and 5 ppm doses were not effective i.e. 100% normal adults emerged.

Dimlin (Tech.) :

Exposure of test pupae to 3 day old 1 ppm residues lead to 55.55% pupal mortality, 11.10% abnormal adult mortality and 33.3% normal adults. However, similarly aged 5 ppm residues gave 90.47% pupal mortality and 9.53% abnormal adults. Exposure of test pupae at to 5 day old 1 ppm residues gave 73.68% pupal mortality, 15.78% abnormal adult mortality and 10.54% normal adults. However, similarly aged 5 ppm residues gave 100% pupal mortality. 7 day old 1 ppm residues produced to 23.5% pupal mortality, 47.05% abnormal adult mortality and 29.45% normal adults. 3 and 5 ppm 7 day old residues gave 100% pupal mortality. Data on 15th day aged residue could not be recovered due to 60-80% evaporation of water at this temperature.

Dimlin (25 WP) :

Exposure of test pupae to Dimlin (25 WP) 3 day old 1 ppm residue produced 52.63% abnormal adult mortality and 47.36% normal adults. However, similarly aged 5 ppm residues gave 10.52% pupal mortality, 68.43% abnormal adult mortality and 21.05% normal adults. 5 day old 1 ppm residues caused 15.78% abnormal adult mortality and 84.21% normal adults. Similarly aged 5 ppm gave 57.88 abnormal adult mortality and 42.21% normal adults. 7 day old 1 ppm residues gave 50% each abnormal adult mortality and normal adult emergence. Week old 5 ppm residue caused 10.52% pupal mortality, 63.15% abnormal adult mortality and 26.33% normal adult emergence.

Observations on 15 day old residue of these IGRs i.e. Hydroprene, Methoprene, Dimlin [Tech.] and Dimlin [25 wp] could not be made at 38°c temperature due to substantial (50%) evaporation of water.

Biological activity (Dose Mortality response) of IGR's at different time intervals on A. aegypti at different temperatures has been depicted graphically in Fig. 1-5.

When IV instar larvae were exposed at ambient temperature to various test chemicals, 3,5 and 7 day old residues reduced 100% larval mortality at 1,3,5 ppm with all the IGR's. Compound possessing biological activity even at 7 day observations were also studied. Even 15 day old residues of all compound produced 100% larval mortality at all doses. Only after aging for 1 month activity of methoprene declined to 60% and 80% larval mortality at 1 ppm was obtained (Table VI) while at rest of the doses, activity was not reduced. Aging beyond 1 month at $28^{o}\pm$c temperature resulted in almost 50% evaporation of initial water volume rendering these samples unfit for incorporation in these studies.

Discussion

Reports (Schaefer and Dupras 1976) indicate that diflubenzuron rapidly hydrolyses into chlorophenylurea in pond water. However, chlorophenylurea concentration was observed for several days after treatment. It also apparently diminishes below GLC detection limits (1 g/l) within 24 hr. of application. Methoprene also disappears rapidly, possibly due to photoisomerism and microbial degradation (Schooley et al. 1975). Half life of methoprene is reported to be < 1 hr. (Malder and Lockhart 1980).

Table VI: Biological activity of different aged residues of different IGRs on *Aedes aegypti* larvae at 29°c

Compound & Dose (ppm)	3 DAY			5 DAY			7 DAY			15 DAY			30 DAY		
	LM	PM	NA	LM	PM	NA	LM	PM	NA	LM	PM	NA	LM	PM	NA
Control Hydro-prene	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
1	100	-	-	100	-	-	100	-	-	100	-	-	63.29 ±2.45	36.71 ±4.5	-
3	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
5	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
Metho-prene															
1	100	-	-	100	-	-	100	-	-	100	-	-	80.30 ±6.89	19.70 ±2.34	-
3	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
5	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
flu-nzuron T)															
1	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
3	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
5	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
flu-nzuron 5 wp)															
1	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
3	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
5	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-

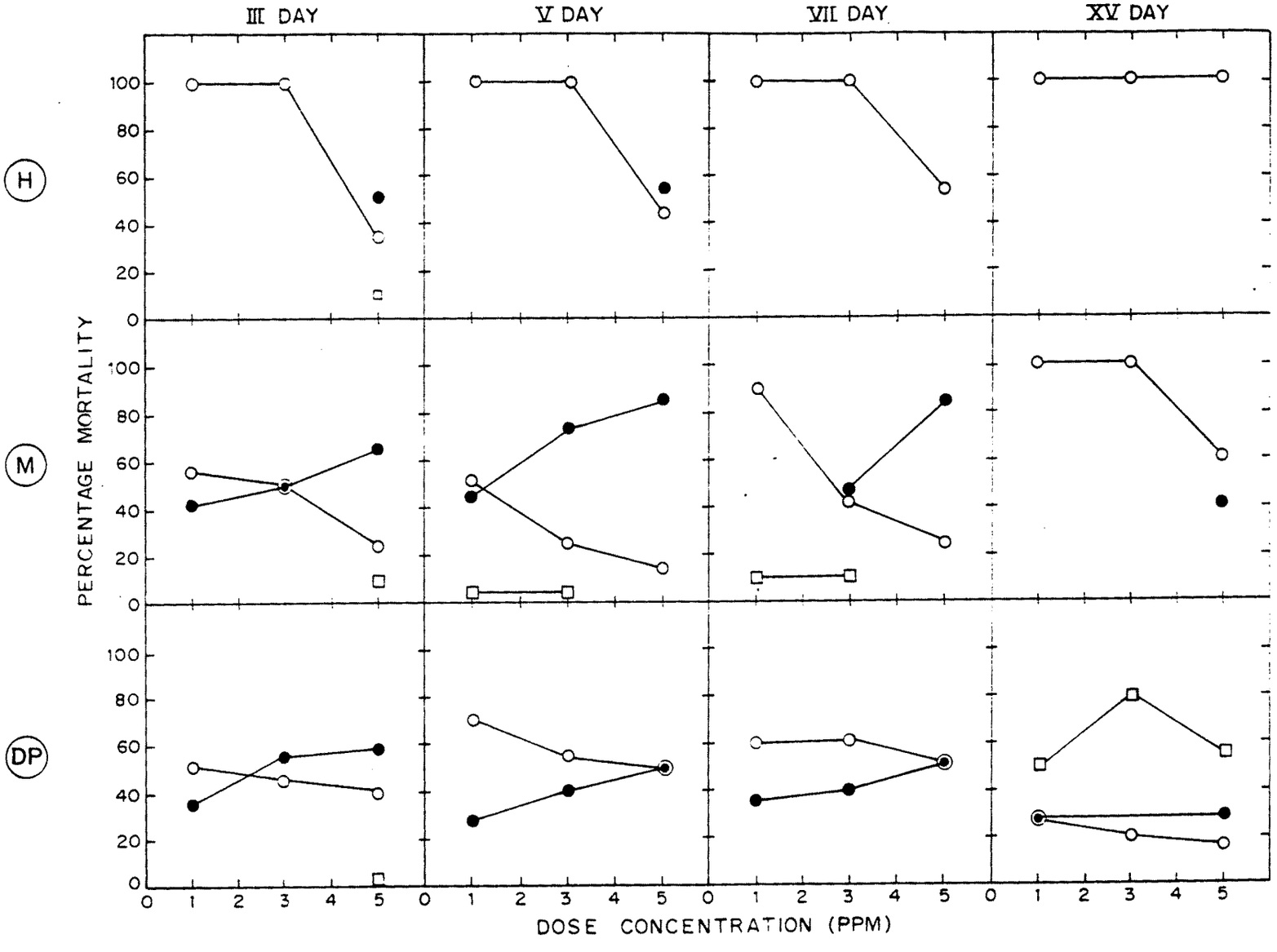

FIG. 1 : BIOLOGICAL ACTIVITY (DOSE-MORTALITY RESPONSE) OF IGR'S AT DIFFERENT TIME INTERVALS ON A. AEGYPTI PUPAE AT 20°C TEMPERATURE.

[H-HYDROPRENE ; M-METHOPRENE ; DP-DIMLIN (Tech.) ; □-PM-PUPAL MORTALITY ; ••• ADULT MORTALITY ○-NA-NORMAL ADULT]

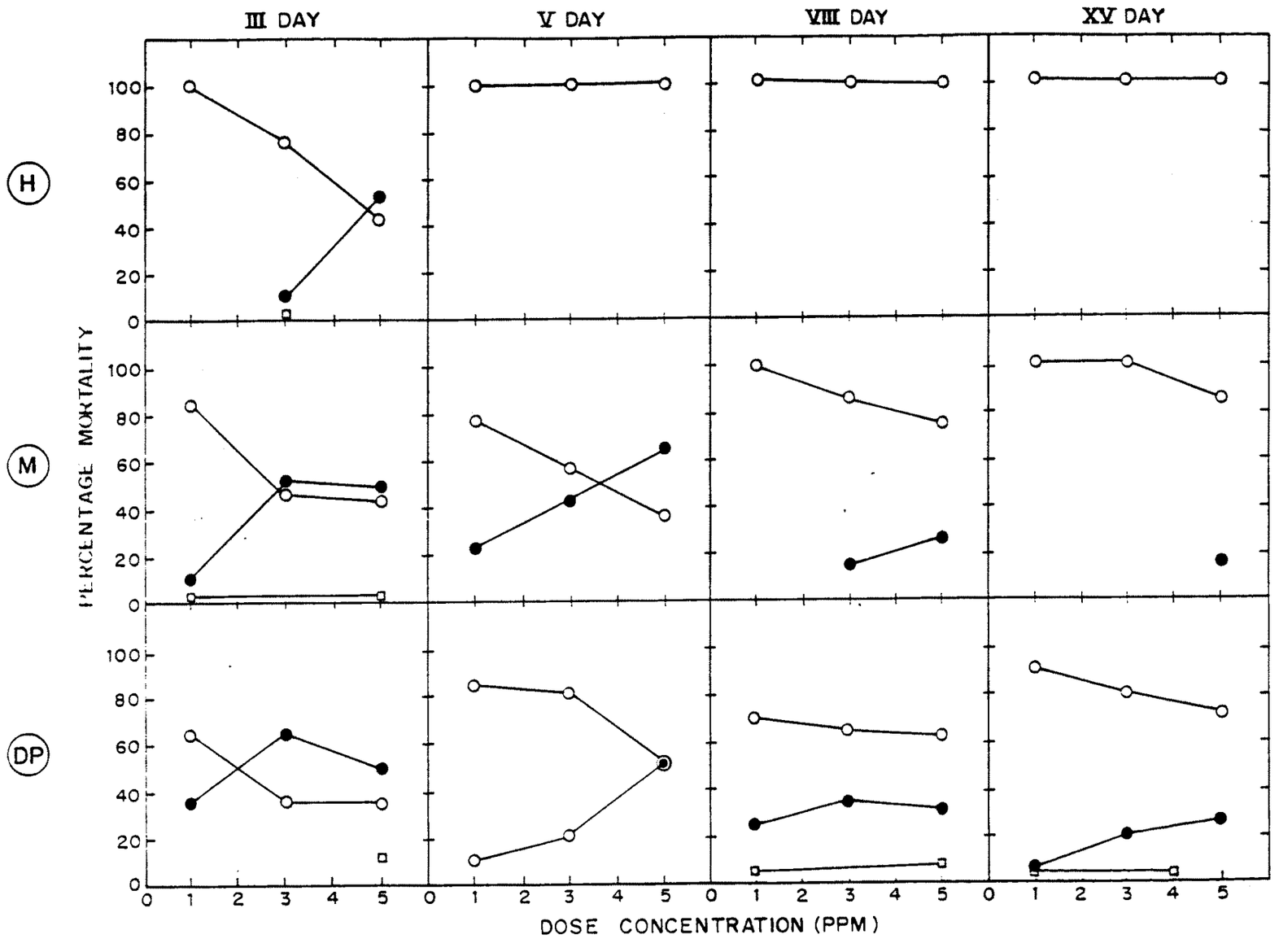

FIG. 2 : BIOLOGICAL ACTIVITY (DOSE-MORTALITY RESPONSE) OF IGR'S AT DIFFERENT TIME INTERVALS ON A. AEGYPTI PUPAE AT 25°C TEMPERATURE.

[H-HYDROPRENE ; M-METHOPRENE ; DP-DIMLIN (Tech.) ; □-PM-PUPAL MORTALITY ; ●-AM-ADULT MORTALITY ; O-NA-NORMAL ADULT]

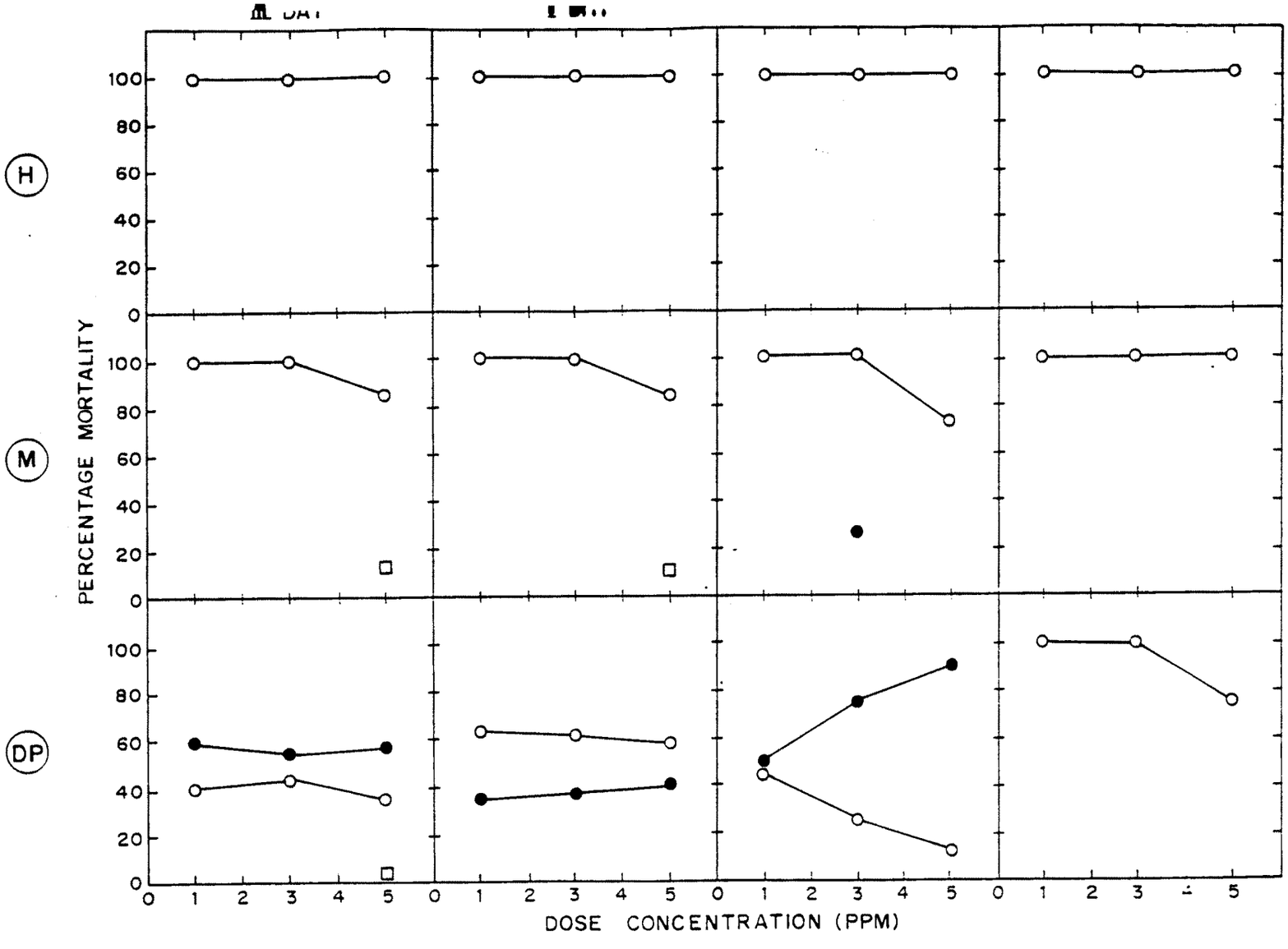

FIG. 3 : BIOLOGICAL ACTIVITY (DOSE-MORTALITY RESPONSE) OF IGRS AT DIFFERENT TIME INTERVALS ON A. AEGYPTI PUPAE AT 29°C TEMPERATURE.

[H - HYDROPRENE ; M - METHOPRENE ; DP - DIMILIN (Tech.) ; □ - PM - PUPAL MORTALITY ; ● - AM - ADULT MORTALITY ; ○ - NA - NORMAL ADULT]

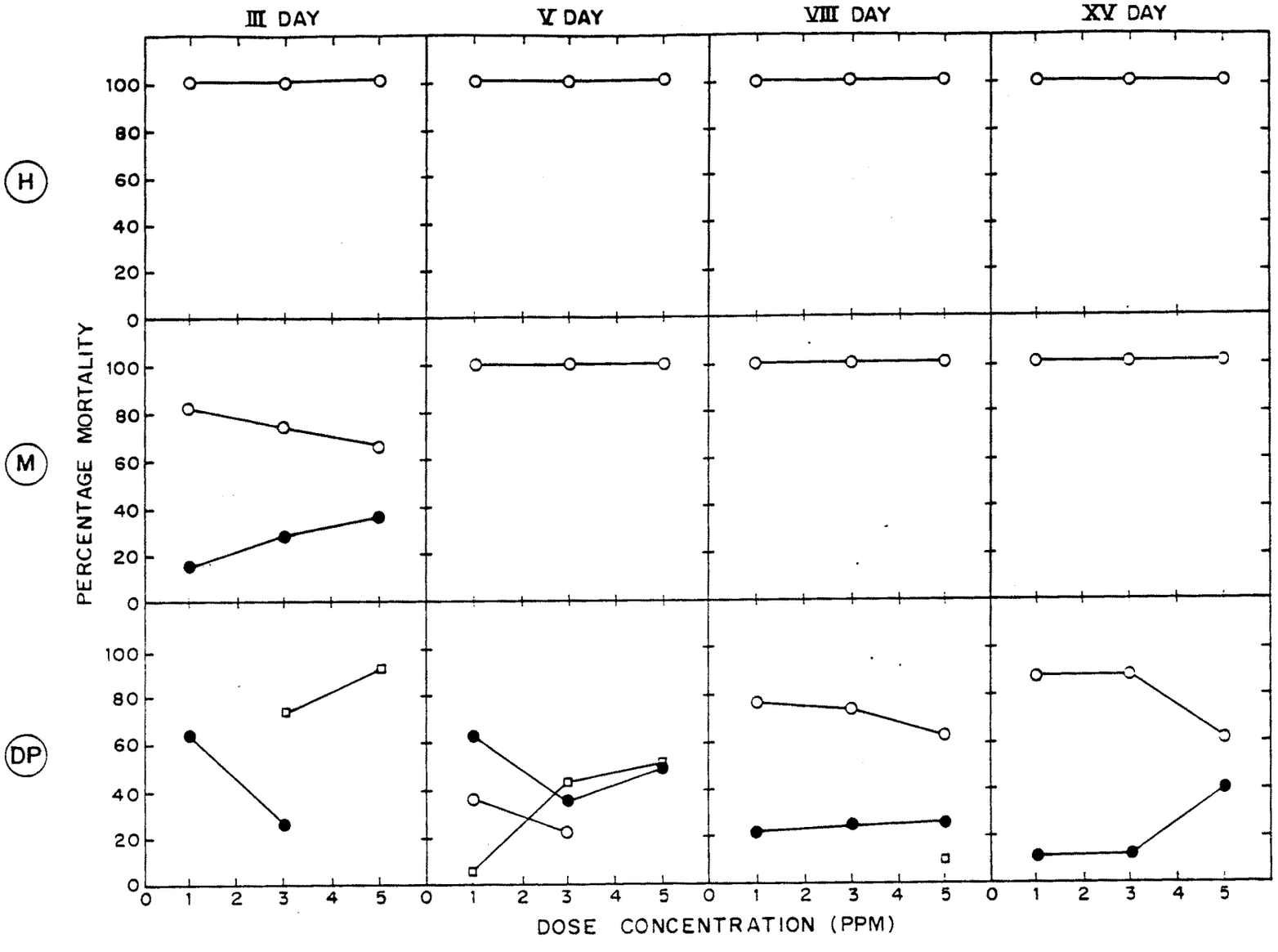

FIG.4 : BIOLOGICAL ACTIVITY (DOSE-MORTALITY RESPONSE) OF IGRS AT DIFFERENT TIME INTERVALS ON A. AEGYPTI PUPAE AT 34°C TEMPERATURE.

[H-HYDROPRENE ; M-METHOPRENE ; DP-DIMLIN (Tech.) ; □-PM-PUPAL MORTALITY ; ●-AM-ADULT MORTALITY ; ○-NA-NORMAL ADULT]

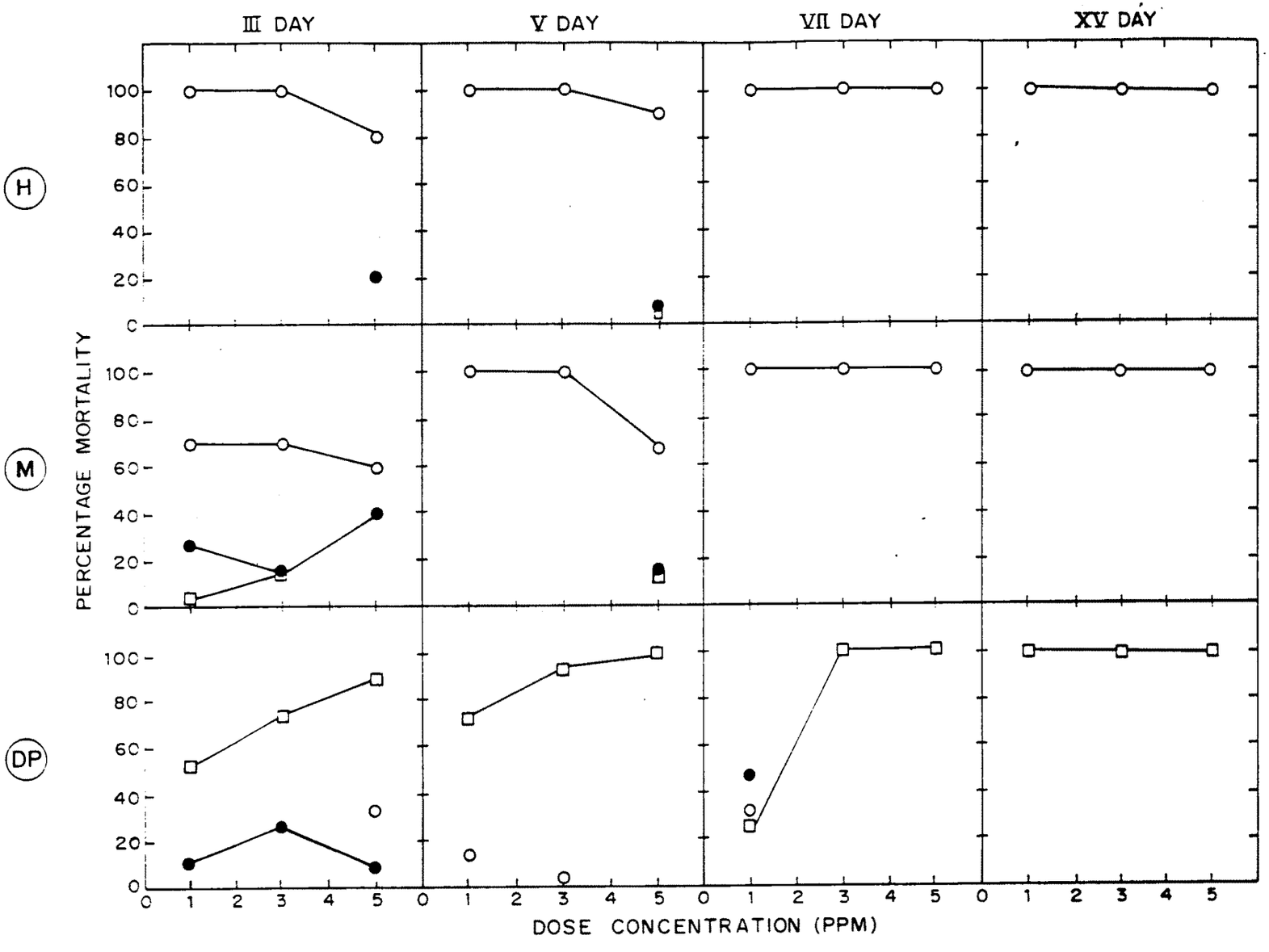

FIG.5 : BIOLOGICAL ACTIVITY (DOSE-MORTALITY RESPONSE) OF IGR'S AT DIFFERENT TIME INTERVALS ON A. AEGYPTI PUPAE AT 38°C TEMPERATURE.

[H-HYDROPRENE ; M-METHOPRENE ; DP-DIMLIN (Tech.) ; □-PM-PUPAL MORTALITY ; ●-AM-ADULT MORTALITY ; ○-NA-NORMAL ADULT]

In the present study, results indicate that residues of 5 ppm S-Hydroprene at 29°, 34° and 38°c were not at all active after 3 days. However, at lower temperature of 20° and 25°, biological activity was observed in 3 day old 5 ppm residues. From this it could be inferred that lower temperatures confer some protection to the aging residues.

Results with S-Methoprene were also similar. At lower temperatures viz. 20° and 25°c after 5 and 7 days aging, biological activity was higher. At higher temperatures (34° and 38°) biological activity declined even in 3-4 day old residues.

In case of Diflubenzuron (Tech.) even 15 day aging at all different exposure temperatures viz. 20, 25, 34 and 38°c, distinct activity was elicited as measured by exposure of test pupae to different samples. An apparently anomalous observation was the disappearance of activity in 3 day old samples aged at 29°c.

Curiously, when dimlin (25 wp) formulation was kept likewise at different temperature regimes (20-34°c) no activity was observed. However, interestingly, at 38°c dimlin (25 wp) 1,3,5 ppm samples exhibited biological activity. This seems to indicate that in contrast to most bioactive chemicals, the formulated dimlin (25 wp) seems to become active at higher temperatures. As dimlin (tech) does not exhibit this properly, it may be concluded that experiments in the formulation somehow contribute to this manifestation of activity at higher temperature.

With the more sensitive IV instar larva stage persistence of bioactivity could be demonstrated for 15 days (1 ppm) to 1 month (> 1 ppm doses) old residues (aged at 25°c) of all IGR's.

In practical management operations, this demonstrates that, considering the brief pre adult duration of most mosquito species persistence of bioactivity for a week would usually be sufficient to destroy all stages from egg to pupae. Pupal stages never last beyond 5 days and all combined larval stages not > 1 month within two periods for both stages are shown to be covered by the IGRs, used in the present study. In other words, at appropriate doses, IGRs used in this investigation could afford complete control on pre-adult exposure, irrespective of stage of development.

In general it may be concluded that bioactivity was retained in aging residues of IGRs aged for a week or more, at the lower temperature ranges. Only the formulated diflubenzuron (dimlin 25 wp) manifested contrarily by exhibiting bioactivity at a higher temperature (38°c). For the less hardy IV instar stages, this period of persistence of bioactivity of residues aged at ambient temperature (28°±2c) extended from 15 days to 1 month at various doses.

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General Discussion and Conclusion

GENERAL DISCUSSION AND CONCLUSIONS

The exciting discovery of the blood borne juvenilising factor in the blood of insects by Wigglesworth in 1936 has shaped progress in the insect sciences including pest control measures since the last three decades. After the initial investigations in physiology and biochemistry the role of the JH hormone in insect development and metamorphosis, concomitant with the disillutionment with conventional synthetic organic insecticides, there was a definite movement towards exploration of the possibility of using these new bioactive molecules for controlling insects. Further support for this attempt came from the discovery of paper factor (Williams C.M. 1956), which was actually a JH mimicing molecule found in paper pulp made from the balsm fir; still later Bower et al (1966) was able to hit upon a chemical principle countering the effects of JH in a horticultural weed. Apart from the JH and anti-JH molecules, substantial work was also carried out on the molting hormone and its various natural and synthetic molecules, especially in Japan. The discovery and identification of chemical factors governing insect ecdysis eventually led to the proposition and discovery of anti-ecdysial substances which eventually took the shape of the modern anti chitin product called dimlin.

As far as the Juvenile Hormone is concerned most bioactivity comes from a wide cycle of acylic or aromatic compounds. Insect development is a highly precise programme in which the various chemical influences are rigidly timed and defined. Application of exogenous JH induces a wide ranging disruption of physiological and developmental events resulting in eventual population decimation. In a similar manner anti-JH principles such as the precocenes also cause physiological disruption resulting in formation of a precious or otherwise non-viable progeny.

Whereas, in principle, it appears that these disruptive properties of the newer molecules, which were variously labelled as Insect Growth Regulators (IGR) or Third Generation Pesticide could be harnessed for modernestic pest management, in practice it was found that there were certain handicaps which prevented their actual field deployment and success. First of all was the major handicap of lack of persistence and high lability of these bioactive molecules. Secondly, these compounds tended to be effective only at specified developmental stages. The immunity of other non-sensitive stages to these compounds made them technically unfit for incorporation in field protocols. Lack of immediate apparancy of consequences such as mortality of target insects cause consumer resistance towards such products and finally in some cases their cost was yet another discouragement.

Inspite of the above various short commings a few products e.g. Hydroprene and Methoprene, (synthetic analogues of insect juvenile hormone developed and promoted by Zoccon Corporation of USA) have found partial, commercial and field success. Thus, methoprene has been used for the control of swamp mosquitoes and hydroprene has been used routinely for controlling poultry pest. The newer series of chitin inhibitors such as dimlin are more generalised IGR's which do not suffer from the major handicap of being restricted only to a few selected insects or stages. As a consequence dimlin is now being slated for widespread use as a modern biorational pest control agent in diverse applications which include forestry, agriculture and public health areas.

With the countinuing revelations of health and environmental hazards of the conventional synthetic insecticides, efforts are naturally being made world wide for obtaining newer bioactive molecules without the undesirable properties of high toxicity, persistence etc.

The present investigation has been carried out using two new more active isomers of the popular JHAs hydroprene and methoprene. These isomers S-hydroprene and S-methoprene were obtained by us from Zoccon Corporation, USA. In addition, to compare and contrast results and effects, the equally popular IGR, dimlin was also examined. The yellow fever mosquito Aedes aegypti was chosen as the test insect on account of its convenient rearing and availability round the year as well as important vector status. Apart from methoprene, efforts are now being made to control this vector with some of the modern pesticide such as B. thuringiensis (Angus, 1971). It is important to note that both latter compounds are used for source reduction against the larval stages. We have also chosen to examine the effects of the more potent JHA's and dimlin against larval as well as pupal stages of A aegypti. In our design we have used the II, III, IV larval instars of the test insect as well as the acknowledgedly more hardy pupal stage. It may be remarked that the studies and information on pupal stage are comparatively scanty. In the present instance no information exists on the effects of these modern IGR's on the pupal stage of A aegypti. The data obtained in the present study of the new JHA's S-hydroprene and S-methoprene is also new in respect of both various larval and pupal stages. It may be remarked here that since JH titre is absent in pupa, effects and absence of exogenous application of JH on this stage are of interest. We have also chosen to study the effects of various test IGR's at various temperatures including ambient, as well as different exposures. Effects of different temperatures on sensitive IV instar larval stage and more hardy and pragmatic pupal stage were also examined the latter study being a first such attempt.

The effects examined included toxicity, effects on oviposition, development, fertility and fecundity. This provided an insight into both the spectrum of bioactivity as well as latency of the latter.

Studies on gross biochemical changes obtained in relation to different exposures and temperatures with the new IGR's provide preliminary data base; the first in case of pupa for further investigation on physiological and biological implications of the activity of these compounds.

Studies with aged residues of these compounds, especially the most hardy stage of pupa, were designed to establish the potential in terms of actual field deployment of these test IGR's, by evaluating their potency on the target insect at different stages, temperatures, exposure and persistence.

Results obtained in the present investigation are now recapitulated in some detail in order to arrive at meaningful conclusions and inferences.

Continuous exposure of II and III instar mosquito larva to the test JHA's S-hydroprene and S-methoprene did not induce any significant effects including mortality up to $1x10^{-2}$ppm concentration (Chapter I : Table - I,II IV and V). However, these two JHA's were equally active on IV instar from $1x10^{-6}$ ppm onwards (Chapter I : Table III and VI). On the other hand II instar larvae exhibited mortality from $1x10^{-6}$ ppm dose onwards (Chapter I : Table - VII), when dimlin technical was used. However, with dimlin (25 wp), larval mortality began at $1x10^{-5}$ ppm dose (Chapter I : Table X). In case of III instars, continuous exposure to both test dimlin compounds resulted in larval mortality at $1x10^{-6}$ ppm doses (Chapter I : Table VIII and XI). Both formulations of dimlin were active on IV instars from $1x10^{-4}$ ppm onwards (Chapter I : Table IX and XII).

IC_{50} values for various chemicals were determined on continous exposure with various larval instars of Aedes aegypti and are given in Chapter I (Table XIII).

IC_{50} values of S-hydroprene and S-methoprene with II instars are more or less equal (1.322×10^{-1} ppm and 1.371×10^{-1} ppm). However with III instar it became 2.220 ppm for S-hydroprene and 1 ppm for S-methoprene. The IV instar larva with S-hydroprene the IC_{50} value was 1.06×10^{-5} ppm and for S-methoprene 9.94×10^{-5} ppm.

On the other hand IC50 for dimlin technical and 25 wp formulation against II instars were 3.477×10^{-6} and 6.04×10^{-4} ppm respectively. When III instar were exposed to dimlin (T) the IC50 value was 1×10^{-6}. For dimlin (25 wp) it was 4.70×10^{-6} ppm. However with IV instars, the IC50 values were 1.597×10^{-4} and 1.7×10^{-2} ppm for dimlin (T) and WP respectively.

Larval Growth Index (LGI) and Total Development Growth Index (TDGI) of larval instars were also studied and are tabulated in Chapter I. (Table I - IV).

It is known that JHAs influence development and lead to various morphogenetic and physiological anamolies. These may also cause reproductive failure and inhibit embryonic development affecting fecundity and fertility as well.

When IV instar larva were exposed to IC50 concentration of the 4 test chemicals, they did not induce any significant biological effects in subsequent F1 and F2 generations (Chapter II Table I).

On the other hand adults exposed to all test chemicals did induce significant biological effects on oviposition, hatching in the following F1 generation (Chapter II - Table II). It was also observed that larva hatching from eggs of treated adults failed to survive.

Comparison of biological activities of different test chemicals at different temperatures (Chapter III : Table III) reveals that the IC50 value of S-hydroprene at 25°c was 0.0092 ppm and that of S-methoprene 0.0982 ppm. IC50 value of dimlin technical was < 0.000001 ppm and that of dimlin (25 wp) 0.0000123 ppm. However, at 29°c (ambient temperature) IC50 of S-hydroprene was 0.0000106 ppm and that of S-methoprene 0.0000994 ppm. IC50 value of dimlin (T) was 0.0001597 ppm and that of wettable powder (25 wp) formulation was 0.017 ppm. At 34°c temperature, however, S-hydroprene did not exhibit any biological activity even at 1 ppm dose, but S-methoprene was highly active (IC50=0.000929 ppm). Both dimlin compounds exhibited same activity at the highest temperature tested (IC_{50} < 0.000001 ppm).

In case of pupa, comparasion of biological activity of different test chemicals at different temperatures (Chapter III - Table IV) reveal that IC50 values at 20°c temperature of S-hydroprene was 2.914 ppm followed by 0.173 ppm for S-methoprene and 0.972 ppm for dimlin (T). 25°c temperature the IC50 values were 4.91 ppm S-hydroprene, 1.756 for S-methoprene and 0.705 ppm for dimlin (T). However at ambient temperature (29°c) these were 6.977, 6.096 and 0.777 ppm for S-hydroprene, S-methoprene and dimlin (T) respectively. At 34° c the IC50 values were 5.178 ppm for S-hydroprene, 4.519 for S-methoprene and 0.115 ppm for dimlin (T). Finally at the highest temperature (38°c) the values were 1.741, 1.322 and 0.030 ppm for S-hydroprene, S-methoprene and dimlin (T) respectively.

With respect to biochemical parameters, protein profile of pupa at IC 50 dose at 20°c, 29°c and 38° are given in Table I-III (Chapter IV). Similarly glycogen titre of pupa at IC50 doses of these temperature levels are presented in Chapter IV (Table IV-VI).

Biological activity of different aged residues of different IGRs on Aedes aegypti pupa at 20°, 29°, 34° and 38°c were studied and are tabulated in Chapter V. (Table I-V).

In deriving conclusions and inferences from the foregoing, it is important to restate the underlying theme of the present investigations. Primary objectives of the present work were two fold. One, to evaluate and establish (increased) bioactivity of the two new JH isomers from Zoecon Corporation (S-hydroprene and S-methoprene). The second aim was more comprehensive, in seeking to assess potential of these, and the new IGR moiety, Diflubenzuron in terms of projected field deployments in a broad spectrum of environmental and biological correlates. Thus, we chose different temperatures, different exposures and bioaction of these on different instars. The latter also included the pupal stage, allegedly and admittedly the most resistant/hardy stage of all. By analysing the data obtained, it is possible to develop conclusions about potency as well as suitable combinations of chemicals, concentrations, exposures and target stage susceptibilities for practical control strategies. These are now enlarged upon as -

Conclusions and Inferences :

(1) The two new JHA isomers tested viz. S-hydroprene and S-methoprene despite being more active than the parent compounds, hydroprene and methoprene, were not significantly effective on the earlier larval stage of the test mosquito species. These included the II and III instars of Aedes aegypti. The IV instar was, however, highly susceptible to their action even at low dosages. The test isomers found to be two times more active than parent compound.

(2) Data obtained from experiments involving different (continuous and discontinuous) exposures reveal that JH activity apparently fails to persist sufficiently. In other words JHA applied to early instars is unable to influence developmental events, and activity of JHA's does not persist long enough to affect the susceptible IV instar stage. This clearly places a restriction on deployment of JHA's since in any given target field population, for them to effectively control mosquitoes, it would be incumbent to have the susceptible IV instar stages only. The earlier instars would escape deleterious action, and by the time they reach the sensitive IV instar stage, the chemical would have lost its activity.

(3) On the other hand the IGR dimlin was effective against all larval instars, thus giving it a decided advantage over JHA's.

Surprisingly dimlin technical was found to be more active than formulated product viz. 25 wp irrespective of dose or exposure.

(4) Exposure of selected stages to IC_{50} concentration of test chemicals established that treatment of IV instar larval stage failed to induce any significant biological effects

in the following generations. However, treatment of adults affected oviposition, fecundity and fertility in subsequent generations also.

(5) In temperature studies it was brought out clearly that exposure at extreme temperatures induced greater intensity of effects. The additive action of chemical and thermal stress was the obvious cause for this.

(6) Studies with the pupa, apart from presenting new data on this instar again demonstrated heightened efficacy at either extremes of temperatures in case of JHA's, with dimlin only higher temperatures produced higher bioactivity.

(7) All test chemicals affected the selected biochemical parameters levels, but the most potent induction in this respect was by S-methoprene. Different levels of exposures and temperatures also affected the biochemistry of the target stage studies. It may be recalled that these studies were carried on the pupal stage, the data again being the first obtained for such a study.

(8) Persistence of test chemicals could be established for a period of 1 month when higher doses of 3.85 ppm were used against IV instar larvae. Even in case of the more sturdy pupal stage, residues of these doses aged up to 15 days were found to be active. It was also found that barring the formulated dimlin product all other IGR's exhibited greater persistence of activity on pupae at lower temperature. Dimlin (25 wp) on other hand, exhibited high persistence at high temperature.

In summation the present work demonstrates that JHA isomers of S-hydroprene and S-methoprene are definitely more active but continue to exhibit the same limitation of target stage sensitivity characteristic of this class of compounds.

It was also demonstrated that among JHA's S-methoprene and of the diflubenzuron, the technical compounds were active on the hardy pupal stage. S-hydroprene and Dimlin wettable powder were inactive on the pupa. This is an interesting observation since compounds effective on pupa also would obviously be more potent and feasible as pest control agents in actual field situations with mixed stages in the target populations. It may also be remarked here that considering the ineffectiveness of JHA's on earlier mosquito larval instars, S-methoprene exhibiting extended action on the pupa apart from the IV instar larva is obviously a better proposition in practical terms than S-hydroprene. Dimlin by virtue of its bioactivity on all stages including pupa must be adjudged better than the JHA's in this respect. The inactivity of wettable powder formulation on pupa is a interesting puzzle which merits further investigations. The present work also establishes indubitably that all the IGR's examined retained their activity over a broad spectrum of temperature rendering them fit for deployment in nearly all climates ranging from the cold to the tropical. Whereas, persistence at IC_{50} or slightly greater values is not much, the same at higher doses is nevertheless sufficient to affect later stages also, and thus ensure effective control by atleast the IGR's which are active on pupal stage as well. Finally, induction of biological effects on F1 and F2 generations by treatment of adults is also a plus point which, however, needs further refinement and elucidation in terms of activity and consequences. Effects on selected biochemical parameters also supplement the conclusion that IGR's as pest control agents are likely to be more effective in terms of wide ranging biological effects as compared to the conventional insecticides which are limited to toxic actions only. The present work reemphasizes the need for generation of more fundamental data on the diverse properties and bioactivities of these new molecules, both the JHA's and diflubenzuron obviously well set to take over as the newer kind of mosquito control agents.

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