

## INTRODUCTION

The lasting changes in the distribution, diversity or number of given taxa of animals or plants will have profound effect on organisms of surrounding ecosystem. The dependency of insects on plants for food and shelter creates drastic ecological changes leading to the production of certain secondary chemicals in plants. These chemicals are capable of producing various adverse effects on insects, in defence, growth regulation, feeding inhibition and reproductive regulation. Early investigations lead to the conclusion that these secondary metabolites are used by plants for various purposes. Scientists and biologists were amazed by the effects of these compounds and started identifying and characterizing them. The identified and characterized phytochemicals include ecdysteroids, their analogues, juvenile hormone analogues, anti-hormones and anti-gonadal compounds.

The discovery of anti-juvenile hormone from plants by Bowers *et al* (1976) was therefore of considerable interest. The extract from a common compositae plant *Ageratum houstonianum* contains anti-juvenile hormones which are identified as Precocenes. These compounds inhibit allatal functions leading to morphological and physiological disorders (Rembold, 1985). Extract from another plant *Acorus calamus* contains two

structurally related chemicals with anti-JH activity (Saxena *et al* 1977). Progress was thus made towards the development of various compounds which could be used to control insect pests..

The history of pest control in the modern age dates back to the second half of this century when the colorado potato beetle was identified as a major pest in the United States of America. Application of paris green to check this pest opened up a new area of scientific research on the use of biocides.

As far, pesticides are mainly of three kinds. One form which contains organic chemicals such as chlorinated hydrocarbons, organophosphorus compounds etc. The organophosphorus compounds are esters of organic salts of phosphoric acid or its derivatives. Many of these compounds are effective against phytophagous insects(Eto , 1974). These compounds produce an indirect effect on the pest. These compounds are usually absorbed by plants and are distributed to various regions where they are stored. When the pests consume these plant regions, the chemicals act as stomach poisons and kill the pest.

Carbomates are another class of synthetic insecticides. The properties of these compounds are worked out by Kuh and Dorough(1976). Thus several organic compounds are used as pesticides. All these compounds are very stable and are



non-degradable. These materials leave residues, which are when accumulated in vertebrates, especially in man (Biomagnification) (Kumar ;1984). As an alternative, a third generation of pesticides are developed from plants. Some of these substances are pyrethrum , rotenone and nicotine. These substances have the ability to penetrate the cuticle of the insects and are therefor very effective. These substances can either paralyse the insects or regulate their oxygen consumption.

In the mid sixties and early seventies scientists of western world were surprised to know the vast potentialities of neem tree in pest control. The properties of neem tree, *Azadirachta indica* or *Melia azadirachta*, as insect repellent and antifeedent were known in India for centuries. The common species of neem found in India are *Azadirachta indica* or *Melia azadirachta* (Persian lilac), native of West Asia and *Melia compositae* (Malabar neem tree).

Great deal of scientific effort has been made to isolate and characterize the potent fractions from the leaves, seeds and bark of the neem tree. These fractions have antifeedent, growth regulatory, repellent, phagodeterent and reproductive inhibitory effects on a wide range of insect pests.

The neem extract , especially the kernal extract contains small amount of active compounds other than azadirachtin (Warthen ; 1979; Kraus *et al*, 1981; Rao *et al*, 1984) . The crude methanolic or acetone extract of neem seed kernel contains compounds besides azadirachtin, exert synergistic or antagonistic effects evoking stronger growth regulatroy effects (Schmultterer *et al*, 1983) . Any form of neem extract, whether kernel, oil, leaf powder or leaf extract produces antifeedent, growth regulatory, sterilizing or mophogenetic effects on several pests species of agricultural, medical as well as veterinary improtance (Fagoone ,1987). Powdered or crushed seeds or leaves are very effective against stored product pests (Abdul Kareem, 1981; Adhikary 1981, Karnavar and Dlamini, 1987) Chellayan and Karnavar, 1990 a).

Some commercial formulations from neem,depending on the mode of extraction and solvent used,are given different names such as AZT-VR, AZT-VR-NR etc (Feeurhake *et al*, 1982), Neem rich I and II (Sharma *et al*, 1984); Fraction G, AI1 to AI 14 (Sharma *et al*, 1984) etc. All these fractions have the capacity to interfere the ecdysone or JH controlled systems (Leuschner 1972) .Insect growth regulatory effect ( IGR) of some of the ingredients of neem kernel extract (NKE)namely 22-23-dihydro-23B-methoxy azadirachtin , 3-Tigloyl

azadirachtol and 1-Tigloyl -3-acetyl -11 - methoxy azadirachtin are also identified.

All these neem derivatives produce specific effects on various insects. Spraying 3% neem oil on rice plants prevents the attack of *Nilaparavata lugens* (Heyde *et al*, 1984). Some lepidopterous insects are repelled by neem products. Egg laying is totally inhibited on treated plant parts. The same effect was observed in cabbage web worm, *Crosidolomia binotalis* and the Afro-Asian cotton boll worm, *Helicoverpa armigra*. The dipterous insect, *Lucilia sericata* also shows the same effect. (Schmutterer, 1990) One of the most potent fractions isolated and purified from the seed kernel extract is azadirachtin. Azadirachtin contains four compounds which are identical in their chemical nature and show similar biological activities (Rembold,*et al*, 1984). The structural similarity of azadirachtin with that of moulting hormone was identified (Nakanishi 1975) and its strong antifeedent and growth regulatory properties were worked out (Butterworth *et al*,1971, Rembold *et al*, 1984; Schmutterer, 1988). The properties of azadirachtin such as growth inhibitory, moulting inhibitory and sterilizing were confirmed by Warthen(1979) in several major insect pests.

The inhibitory effect of azadirachtin on ecdysis was observed in insects which do not have strong feeding inhibition and in those insects which are strongly deterred by the compound (Mordue *et al*,1985). The common phenomena observed in azadirachtin-treated insects are prolonged instars and moult abnormalities . It is well known that moulting and metamorphosis are controlled by the brain, corpora cardiaca, corpora allata system. There is a possibility of the interference of this compound on the functions of the nervous system also (Mordue *et al*, 1985). Based on these informations, Rembold (1985) has suggested a massive blockade of factors located in the central nervous system. Kauser and koolman (1983) found out an anti-ecdysteroid effect of azadirachtin by blocking the protein receptor sites of *B*-ecdysterone. The antifeedent effect of azadirachtin was due to this inhibitory effect on central nervous system thereby inhibiting the sensory centres of the insects (Schoonhoven,1981; Sieber *et al*, 1983; Schluter *et al*,1984).

The diverse effects of azadirachtin in insect system depend on other active principles present in the neem extract. Research on this track led to the isolation of some more potent ingredients like salanin, salannol, salannoiacetate, 3-deacetyl salanin ,azadiraion, 14-eporryazaradion, gedunin, nimbin, deacetylnimbinen etc (Jones *et al*,1984) from seed kernal

extract by using NMR, mass spectrometry or X-ray crystallography. Majority of these fractions exhibited antifeedent activity (Lavie *et al*, 1976 Stéels *et al*, 1976, Schwinger *et al*, 1984, Kubo *et al*, 1986).

Though many investigations have shown clearly the effects of neem products on insects species, very few of them are directed towards the possible effect of these compounds on the intermediary metabolism and hormonal regulation of insects. It is of considerable interest to explore the biochemical changes brought about by these compounds in the cricket, *Teleogryllus mitratus*. Under these studies, the levels of triglycerides, calcium and the activities of enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were investigated. Similarly the ecdysteroid titre and morphogenesis under the influence of these neem compounds were also studied.

In higher organisms, estrogen constitutes a group of hormones, which are responsible for the development and maintenance of the reproductive structures. Almost all vertebrates, from lower forms like cyclostomes to higher vertebrates like mammals, exhibit responses to steroids. It assists in the differentiation of mammary gland tissues (Lyons *et al*, 1958, Hilf *et al*, 1967), phosphoprotein synthesis in the liver, (Greengard *et al*, 1965)

the synthesis and deposition of fat in adipose tissues (Gassner *et al*, 1958) and the uterine cell differentiation (O' Malley, 1969, Oka and Schimke, 1969a).

In vertebrates, the estrogenic compounds have well known functions like the biosynthesis of proteins, lipids, glycogen, and nucleic acids. ( Mueller *et al*, 1961, Aizawa and Mueller 1961, Hamilton 1963). It is also reported that glucose uptake and oxidation are stimulated by the presence of this compound (Nicolette and Gorski, 1964). The accumulation of fat in the subcutaneous tissues is also reported (Guyton, 1986). Stimulation of protein synthesis in liver and other organs, inhibition of lipid accumulation in the liver, increased phosphoglyceride turnover in the uterus and reduction of cholesterol formation in vertebrates are some other effects of these compounds (Smith *et al*, 1983). In ovariectomised females, administration of estradiol 17-*B* leads to an increase in the circulatory glycerides, but cholesterol level was not affected (Thompson *et al*, 1983). Haslam (1988) pointed out that estrogen can produce nitrogenic effects locally or systemically and these effects are dependent on the age and developmental stage of the target tissue. In immature rat ovaries, it was reported that estrogen stimulates the cell division and DNA synthesis (Rao *et al*, 1978, Richards, 1980). The DNA polymerase activity was also



stimulated (Usuki and Shioda, 1986). In ovariectomised *Trichosurus vulpecula*, administration of estradiol induces an overall weight increase, RNA : DNA and DNA : Protein ratio (Curlewis and Stone, 1987). Estradiol administration in an apodan *Gegenophis carnosus*, stimulated the activities of adenosine triphosphatase, cytochrome oxidase,  $\alpha$ -Glycerophosphate dehydrogenase, Malate dehydrogenase and inhibited Succinate dehydrogenase and Lactate dehydrogenase (Sudharam, 1990).

Studies on the effect of the other steroid, progesterone are very few and most of these studies are on the chick oviduct (O' Malley *et al*, 1969). In adult female rats, an increase in the body weight and food consumption was observed (Wade and Gray, 1979). In the uterus of higher organisms like mammals, the estrogen stimulated- uterine peroxidase was inhibited by progesterone ( De Sombre and Lyttle, 1979). In rabbit endometrium enhancement of specific protein accumulation occurred by increasing specific mRNA by progesterone administration (Loosfelt *et al*, 1981). In Lizards, the natural and gonadotropin induced ovarian growth was inhibited by progesterone treatment. In turtles, progesterone diminishes estrogen-induced vitellogenesis (Callard *et al*, 1976). In reptiles the egg retention is controlled by progesterone in

oviparous species (Roth *et al*, 1973). In turtle, *Chrysemya picta*, progesterone inhibited ovulation (Klicka and Malimoud, 1977).

In insects and most other arthropods, steroids are not biosynthesized. The experimental evidence is provided by *Dermestes valpines* (Sedee, 1961) in *Caliphora erythrocephala*, (Kaplanis *et al* 1963); in *Musca domestica*, *Thermobia domestica*, (Schaefer *et al*, 1965); and in *Neodiprion pratti* (Gillbert, 1967). The possible way of accuring steroids in insects is the dietary source. (Clayton, 1964). The utilization of these steroids by metabolizing them in insect systems was intensively studied (Robbins *et al*, 1971). Other reviews by Thompson *et al* (1973) Savoboda *et al* (1975) also pointed out similar observations.. The major difference between insects and other organisms is their inability to synthesize steroids (Clayton, 1964, Nes and Mekean, 1977). This deficiency represents an important biochemical difference between insects and other organisms.

The presence of vertebrate type of steroid was reported in *Homarus americanus* in late 40's (Donahue, 1957). Later, using advanced techniques like gas chromatography and mass spectrometry, the vertebrate type steroids were identified in invertebrates, especially in insects. Several workers were

able to identify steroids by using these techniques in various insects (Lehoux, 1970; Onishi *et al*, 1985).

Another prominent steroid present in insect is the ecdysteroid. The discovery of ecdysteroids and their potency to regulate the growth, metamorphosis and differentiation of the reproductive system opened a new vista of scientific research. First of its kind, the ecdysone was identified by chemical method by Karlson *et al* (1963). Later several workers showed ample evidences about the occurrence and possible functions of ecdysteroids in insects like *Leucophaea maderae* (Matz *et al*, 1975), *Locusta migratoria* (Lagueux, 1977) and *Aedes aegypti* (Schlaeger *et al*, 1974). In some insects, the embryogenesis was assisted by ecdysteroids (Bulliere *et al*, 1976). In some other insects ecdysteroids are present in newly laid eggs (Mizuno *et al*, 1975, Ohmishi *et al*, 1977 a).

Thus it is evident that steroids play an important role in the life of insects. It is of considerable interest to study the possible influence of typical vertebrate steroids like estrogen and progesterone in insects. *In vivo* experiments were conducted and levels of protein and activity of transaminases were measured.

Special cell groups in vertebrate body which are able to produce hormones are of considerable research

interest. The Islets of Langerhans are such a cluster of cells. The specialized  $\beta$ - cells of this region produce insulin (Falkmer and Patent 1972; Epple and Lewis 1973). In mammals and other vertebrates lack of insulin secretion from these cells reduces the normal life span (Bentley,1976). In all vertebrates it has been identified that the consumption of food materials, its transformation, storage and utilization are regulated by insulin, thus revealing the central role of this hormone in intermediary metabolism (Bentley, 1976).

In higher classes of animals like mammals and other vertebrates,insulin could be considered to be a general metabolic hormone. In vertebrates, insulin facilitates gluconeogenesis, conversion of amino acid, triglycerides to fatty acids, increased lipogenesis and increased amino acid uptake by body tissues and liver (Bentley, 1976). The increase in the RNA content by the action of insulin leads to the promotion of protein synthesis partly by a direct action on ribosomes.

In fishes, the most obvious response exhibited by insulin is hypoglycemia (Ince, 1983). Several other examples could also be cited for such an effect. *Esox lucius* (Thorpe and Ince, 1974), *Anguilla anguilla* (Lewander et al,1976) and several other marine fishes (Root et al,1981) are some other examples exhibiting similar response.

Insulin has profound influence on the regulation of protein metabolism in fishes (Muret *et al*, 1981). It was proved in carps that the administration of insulin reduces the protein turn over (Castilla and Murat, 1976). In eels, the primary function of insulin is to control the lipid rather than the carbohydrate metabolism (Duve *et al*, 1979). Thus it is evident from all these studies that the insulin has a distinct regulatory effect in the intermediary metabolism of vertebrates.

Presence of insulin in arthropods, especially in insects is also reported. *Apis mellifera* contains insulin-like peptides (Ishay *et al*, 1976). The brain, corpora cardiaca, corpora allata of *Bombyx mori* contain peptides similar to insulin. In the fruit fly, *Drosophila melanogaster*, the haemolymph and the whole body extract contain insulin like peptides (Seecof *et al*, 1974). All these identifications of vertebrate peptides in insects are based on the principles of RIA and immuno histochemistry techniques.

By using bio-assay, radioimmunological and immunocytochemical techniques, a number of laboratories around the world have shown that vertebrate peptide-like substances are present in the neuroendocrine or gastrointestinal tissues of very primitive organisms. The function of the vertebrate like

peptides in insects is not clearly known (Gilbert and Kerkut, 1986).

In the past few years many peptide hormones were identified in the central as well as peripheral nervous systems. Several 'neuro active' peptides about twenty are found in various regions of vertebrate nervous system. The exact functions of these peptides are not clearly known. These substances probably function as neurotransmitters or neuromodulators. In insect neurosecretory system also these peptides may have similar functions (Reddy *et al*, 1994).

In the light of the above information it is of considerable importance to study the influence of the peptide-insulin on insect metabolism. Therefore, *in vivo* study was undertaken in *Teleogryllus mitratus* after administration of vertebrate (Human) Insulin. The levels of protein, AST, ALT, Trehalase, and Glycogen levels are investigated.

In all groups of vertebrates, thyroid hormones produce marked influence on the metabolism of carbohydrates, mobilization of glucose, and important synthetic processes etc. (Eberharlt *et al*, 1980). The effect of thyroid hormones on protein metabolism is also well studied (Eberharlt *et al*, 1980). The synthesis and breakdown of protein molecules of hepatic and muscle regions are thoroughly investigated (Goldberg *et al*, 1979). Many workers were able to demonstrate an increase

or decrease in the rate of protein synthesis in hypo as well as the hyper thyroidism ( Goldberg *et al*, 1979).

The possible effects of thyroid hormones in the synthesis, mobilization and metabolism of lipids, are also reported (Eberhardt *et al*, 1980). In lower vertebrates, the effect of thyroxine on the metabolic regulation is contradictory (Plisetskaya *et al*, 1983; Matty *et al*, 1985). The prime effect of thyroid hormones in the lower vertebrates, like fishes, is the regulation of carbohydrate metabolism (Letherland and Sonstegard 1980; Matty *et al* 1982). It is reported that thyroid hormones enhance the protein synthesis in fishes (Narayan Singh and Eales, 1975). In amphibians, these hormones produce a proteogenic effect (Tata, 1971; Weber, 1974). In lower vertebrates the action of thyroid hormones is some what different from that of higher vertebrates like mammals. In these animals, the synthesis of protein is triggered by thyroxine by binding to specific receptors in the nucleus. (Van der Kraak and Eales, 1980; Darling *et al*, 1982). Several other workers have also focused their attention on the thyroid hormone receptors in the nucleus. (Tata, 1974, 1975; Oppenheimer *et al*, 1976)

Thyroxine has an important regulatory effect on metabolism . The intermediary metabolism of birds are regulated by the administration of thyroxine (Mellan and Wentworth, 1962). The synthesis of lipids are lowered by

thyroxine in birds (Takashima *et al*, 1972). Mammals also show the same effect (Tata 1964). The general effect of thyroid hormone is thus an anabolic type and promotes growth and differentiation.

In insects and other lower animals, peptide hormones are identified, some of them are closely related to thyroid hormones. However, the characterization and the effect of thyroxine are not very well studied. Certain reports are available on the effect of thyroid hormones on the growth and development in insects. ( Reddy, 1994). In insects like *Bombyx mori*, it was proved that treatment of thyroxine leads to the enhancement of cocoon characters (Thayagarzaja *et al*, 1991). In tasar silkworm, *Antheraea mylitta* Drury, treatment with L-thyroxine leads to the enhancement of protein and nucleic acid turnover in the male and female reproductive structures (Reddy *et al*, 1994). Some other workers are able to demonstrate the effect of this hormone in protein turnover and enhancement of ecdysteroid levels ( Kiguchi and Agui, 1981).

The present study was undertaken with a view to elucidate the influence of thyroxine on the intermediary metabolism of *Teleogryllus mitratus*. For this, estimation of important biochemical constituents like protein, glycogen and key enzymes of intermediary metabolism viz, transaminases (ALT & AST)



trehalase etc. were assayed in normal and thyroxine treated  
*mitratus*.