<u>CHAPTER I</u>

I N T R O D U C T I O N

INTRODUCTION

Scientific revolution has entailed the development of the agriculture with the introduction of certain specific monoculture techniques. Such techniques were utilised by man for growing specific plants that were useful to him in terms of food and other products. All the cultivated plants of the present day are the products of artificial selection and careful breeding practised by man on wild life from very ancient times. This artificial method of plantation resulted in equally unnatural establishment of various insects on plants maintained in this manner. This association between plants and insects came to be looked upon as pestilineal infestation in course of time and then resulted in various measures being taken by planters to protect the useful plants from such insects which came to acquire the status of pests. One of the measures adopted by mankind was the use of available chemicals - the first generation of pesticides incorporated largely as inorganic poisons such as copper sulfate, the arsenicals, petroleum oils etc.

Man's desire to control his environment has created many useful chemicals. The first synthetic organic insecticides that appeared for public use were dinitrocompounds and thiocyanates (Murphy & Peet, 1932). Perhaps the most significant discovery leading to the proliferation of new synthetic insecticides was that of DDT. This ushered in the era of second generation pesticides. The use of DDT revolutionized the control of insect pests. Other chlorinated hydrocarbon insecticides such as BHC, aldrin, dialdrin, chlordane etc. followed immediately thereafter. The second massive introduction of new insecticides was organophosphorus compounds whose use for insect control is now unmatched by any other group of insecticides. The carbamates and more recently the synthetic pyrethroids all belong to this classification (Brown, 1951). The age of second generation pesticides is by no means over.

With the advent of new discoveries, the most recent generation of pesticides have been introduced. These include mostly nontoxic bioregulators such as the Insect Hormones (Williams, 1967) and their antagonists (Bowers *et al.*, 1976). These are more subtle chemical compounds which secure pest management by influencing or interfering with some aspect of behaviour, physiology or development of target insects. Highly laudable efforts are being made in developing more of similar chemicals (Staal, 1972; Sharma *et al.*,1977) however, the second generation pesticides still continue to be the largest and most dominent types of chemicals in use even now.

Inspite of their undoubted pest control efficacy, the conventional synthetic insecticides have several disadvantages that have brought into light in recent years (Edwards, 1973; Pimental *et al.*,1980). The biocidal agricultural chemicals, the largest group of poisenous substances, are related mainly to their intrinsic high toxicity, some of them, for example, the chlorinated hydrocarbons have an affinity for fatty tissues which results in accumulation there and then in turn leads to bio-magnification in the ecological food chain and pyramids (Smith and Van den Bosch, 1967). The persistence of residues of insecticides on treated produce is a factor of importance not only in determining the degree of residual protection from insect attack but also in producing possible deleterous effects upon human being and animals consuming the treated substances. The problems of food contamination are most acute with organic insecticides soluble in oils, fats, waxes of plants and animals.

Another important problem of target pest organisms is their inductance of resistance (Brown, 1961, 1971; Metcalf and Luckmann, 1975; Metcalf, 1980). The continuous and intensive use of certain insecticides against various insect pests has resulted in the development of races of strains sufficiently resistant to the action of the insecticide as to necessiate a complete change in control measures. Therefore, the concept of Integrated Pest Management is now a new approach to pest control (Beirne, 1969; Metcalf and Luckmann, 1975). Its aim is to achieve pest control by combining many different approaches which include biological, chemical, physical and economic methods (Metcalf, 1980; Flint and Van der Bosch, 1981). The concept of pest management further advocates reduction of pest populations below the economic injury threshold instead of indiseriminate eradication propounded & practised earlier. This biological approach obviously includes conservation of species as an inherent proposition.

Insecticides that indiseriminately destroy both harmful as well as non target species are necessarily least desirable according to this revised philosophy. Besides this, another disadvantage of these chemicals is the phenomenon of secondary pest resurgence which results in emergence of previously unimportant or minor pests as major menaces in a given ecosystem

(Smith and Van den Bosch, 1967). Such occurrence of new pests results in the necessity of evolving entirely new pest control protocols using biorational pesticides (Menn and Henrick, 1981).

With this philosophy of new pest management protocols for different pests and crop ecosystems, widespread efforts have been made to obtain newer chemicals with desired properties of lesser toxicity, higher specificity and a physiological and behavioral modes of action. In this connection, the discovery and development of the juvenile hormones (wigglesworth, 1935, 1939; Williams, 1969; Staal, 1972) and later the antijuvenile hormones (Bowers el at., 1976) are of special mention. The commercial synthesis/production of pheromones of different economically important pests are equally important examples. A least attention has been paid to yet another group of chemicals which form the basis of many insect plant interaction. (Jacobson, 1960; Gary,1962). These chemicals have been variously called secondary plant chemicals (Beck, 1965; Kennedy, 1972; Sharma, 1979), allelochemics (Whittakar, 1970), biorational pesticides (Menn and Henrick, 1981) etc. These chemicals appear as secondary products of plant metabolism with or without specific functional roles in plant physiology. But it has been shown that they influence greatly the behaviour of insects especially in host plant resistance or susceptibility to insect attack (Painter,1951; Thorsteinson, 1960; Beck, 1965; Fraenkel, 1969).

Around the middle of the present century, different concepts were formulated in connection with the secondary plant chemicals and insect plant interaction or host specificity. Painter (1951) proposed a view of twin principles of non-preference and antibiosis. The secondary chemicals or odd substances (semiochemicals)were brought into focus by Lipke and Fraenkel (1956). Further, Kennedy (1953) put forth more emphasis on the nutrients and suggested the discrimination theory of selection in which both the nutrients as well as the secondary chemicals were given importance. There is a wealth of data available indicating mutual complementary roles played by the semio-chemicals and the plant nutrients (Thorsteinson, 1953; Sagiyama & Matsumato, 1959; Waldbauer, 1962, 1963; Wensler, 1962; Nayar & Thorsteinson, 1963; Mitler & Dadd, 1964; Beck, 1965).

It would appear from the foregoing that the chemicals exist in plants which exercise a vital influence on various aspects of the biology and life cycle of various insects. It becomes obvious, therefore, that a different approach would be hunt for such chemicals in different wild or naturally occuring plants which would determine the chemical basis of resistance in agronomically developed varieties of important crop plants. At present there has been a plethora of such biologically active chemicals. Selection of plants for this surpose may be random or based on indications from history or early science. Not only this but also the abundance of a plant is another important factor of quick investigation for this purpose. The famous Indian Neem tree is a case which examplifies both the above cases.

India being a tropical country, it has a wealth of plants which can be investigated. However, there have been scattered and sporadic efforts to utilize this natural and renewable resource for obtaining pest control agents. Plants with applied importance can be utilized for exploitation of bioactive principles only if they are found in abundance or atleast amenable to succeseful & economic cultivation. In this context, the recent emergence of the neem into limelight has been because of the twin factors of its high abundance as well as being a great source of allelochemicals (Schmutters _et al_; 1981).

There are several other varieties of plants which are known to have the sources of potent allelochemics. Some of these plants grow naturally abundance while others are cultivated specially. Many of these yield products especially the oils generally obtained from seeds. Many such seeds have been identified as sources of useful substances of economic value. From this point of view, chemical analysis of many tree seeds, wood and leaves has been done. Those trees from which both edible and nonedible oils can be obtained have been the subject of some scientific investigation, since little information exists on the pest control potential of chemical constituents of tree parts including seeds. It would be of enormous values if a useful & economically viable bioactive principle could be identified & obtained from such seeds/tree oils.

As a result of various investigations, there exists a fair amount of data with reference to the yield, oil contents, some physical properties and chemical constituents of the oils of different plants. However, it seems that many of the tree oils remain both underutilized and under exploited particularly in relation to pesticidal polential. Among all those plants known for their medicinal and economic value, the commonly popular Azadirachta indica or the Indian Neem for all its professed or demonstrated properties and principles of utilitarian value in agriculture, public health and pharmaceuties it still remains underutilized as compared to its total availability. With this fact in mind, trees like Neem which are growing fairly wildly, its oil has been chosen for evaluating its pest control potential. An important consideration in making the selection of this oil for the present investigation was its commercial availability.

Survey of Amino acids and Proteins in Insects :

Insect ontogeny consists of both embryonic & postembryonic development. One outstanding feature of postembryonic development is the striking change of forms occuring at successive ontogenic periods, especially in holometabolous insects a pupal stage is interposed between the larva and the adult. The phenomenon of metamorphosis is known to be complex. Despite such a complexity there is apparently difference between the development of an insect and that of other animals.

The metamorphic events culminate into the formation of an imago. Adult plerygote insect does not molt but it generally undergoes some further development after emergence. Most of the organs, particularly the reproductive ones attain their full functional status. Alongwith this, certain age-related physiological and biochemical changes occur. The overall metabolic pathways are however largely the same in insects as in other animals (Chen, 1985).

Insects are characterized by high levels of free amino acids and there exists a large body of literature on both free amino acids and protins among them. However, most of the information comes from the study of haemolymph and very little is known about their distribution & metabolism in tissues. The total concentration of free amino acids in the haemolymph is generally lower than 700 mg/ 100 ml in exopterygotes and higher than 700 mg/ 100 ml in endopterygotes. As compared to the blood of human beings, their total quantities are about 100-300 times higher in insect haemolymph (Chen, 1985).

Amino acids and their derivatives have a number of different functions in insects. Perhaps the most important of these is that of protein synthesis, for which all 20 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 and Inokuchi, 1978). The amino acids required for protein synthesis are derived from food proteins & from turnover of cell proteins.

There is sufficient evidence indicating that amino acids fulfill additional metabolic functions. In aquatic insects the they may play an important role in osmoregulation. Beadle & Shaw (1950) demonstrated that the amino acid content in the larva of *Sialis lutaria* varies inversely with the salt concentration in the surrounding medium. Various amino acids are known to function as neuro-muscular transmitters (Chen and Widmer, 1968; Neal, 1975; Lunt, 1975). Detoxification of certain metabolites can be effected through their interaction with amino acids (Chen, 1971). Several amino acids participate in the synthesis of phospholipids. Phosphate and glycero-phosphate esters of serine, threonine and ethanolamine are of common occurrence in various insects. Degradation of amino acids for energy production has been particularly well studied for insect flight (Kirstew et al; 1969, Mayer & Candy, 1969 b; Hargrove, 1976; Mordue & Kort, 1978; MeCabe & Bursell, 1975; Bursell, 1977, 1981; Weeda et al., 1980). Some amino acids are particularly involved in certain morphogenetic processes. Tryptophan, for example, has a key role in the formation of eye pigment (Cochran,1975) and that of tyrosine in the sclerotization of the cuticle (Perunet, 1980, Chen, 1985).

One of the important features of amino acids in insects is that they exhibit pattern specificity. The composition of free amino acid is species-specific both quantitatively & qualitatively. However, quantitative differences are in general more pronounced than qualitative ones. Chen (1962) has made a survey of 20 species belonging to seven orders and he found that some of the amino acids viz, cysteine, hydroxyproline, methionine sulfoxide, ornithine and aminobutyrate are of much less common occurrence than other components. Some amino acid derivatives are characteristic of a single genus or a single order (Chen, 1971, Bodnaryk, 1978, Kramer, et al, 1980).

The composition of free amino acids is not only species-specific but also exhibit tissue- and sex-specificity. The pattern of free amino acids studied from the two tissues of mature Drosophila larva. indicate that the glutamate content of salivary glands is 10 times higher than that in the haemolymph (Chen, 1966). Such differences are mainly related to the metabolism of the tissue concerned. In a similar way, the adult females of Culex pipiens contain about 5 fold more methionine sulfoxide than the males and the reverse is true for β-alanine (Chen, 1958 b). Various sex-specific amino acids found in the male accessory gland of Drosophila have been shown to modify the physiological properties of the female after mating (Chen, 1978).

It has been shown that the nature of the diet and starvation exert a significant alteration in the free amino acid composition in insects. The developing insect larva, in contrast to the egg and pupa, depends on a continuous supply of food for energy production and growth, The same is true for the adult. With some exception, adult females need exogenous nitrogen sources for egg production, whereas in males probably different proteins are synthesized in connection with spermatogenosis & secretion of the accessory glands. It therefore seems reasonable that the above mechanisms in both the sexes are directly related to both amino acids & proteins in the diet.

There is a large increase in all amino acids in the early third instar larvae of Drosophila when fed with casein, but if they are fed with sucrose, most amino acids exhibited a very low level with the exception of alanine (Chen, & Hadorn, 1955). Burnet & Sang (1968) have also shown in Drosophila larvae that the composition of amino acids can be altered by different diets. Similar nutritional efforts on the level of free amino acids have been reported in many insects by a number of workers in the field (Auclair, 1959; Irrevere & Levenbook, 1960; Maltais & Auclair, 1962; Strong, 1964; Singh, 1965).

The effect of starvation on free amino acids is variable in different insects. The concentration of most of the amino acids decreases while that of some increases during starvation. Chen & Hadorn (1955) have demonstrated a rapid fall of most amino acids which then remain at a low level for several days in the starved larvae of Drosophila. During the period of prolonged starvation, there is a large accumulation of ninhydrin-reacting components, probably as a result of tissue autolysis (Strong, 1964). A 2-fold increase in amino nitrogen in starved Popillia japonica larvae was noted by Dogra & Gillot (1971) whereas a constant level of free amino acids was evident in virgin females of Melanoplus sangunipes starved for three days or during subsequent feeding. Recently Bosquet (1977) reported that in Philosamia cynthia the concentration of lysine, histidine, arginine and glycine in the haemolymph increase after 48 h starvation whereas that of ornithine declines to one third of its initial value. The total concentation shows a slight increase if ornithine is excluded.

There is ample evidence indicating an overall variations in the amino acid pool during embryonic and post-embryonic development, especially in relation to larval growth and metamorphosis (Hadorn and Stumm - Zollinger, 1953, Chen and Hadorn, 1954; Chen 1960, 1966, 1971; Dinamarca and Leeven book, 1966; Crompton and Birt,1967; Williams and Birt, 1972; Firling, 1977).

The major activity in adult insects is ~~xxpxxx~~ reproduction. In the females this involves predominantly yolk synthesis in relation to egg production. In *Drosophila melanogaster* it has been shown that during first week of adult life of males and virgin and mated females the concentration of alanine, leucine, isoleucine, glycine, glutamine, threonine, glutamate and histidine etc. is higher in females than in males. The size and the weight of the body also accounts for such differences in both the sexes (Chen, 1972). However, the following lines of evidence suggest the relation between the high amino acid content and egg production in that -

a) The increase is most rapid during the first two days of adult life and reaches a higher level in mated than in unmated females. Oviposition in mated *Drosophila* is known to start on the second day after eclosion & stays high as compared to virgin females. b) Several above mentioned amino acids are found to be abundant in Drosophila eggs (Chen et al; 1967), and c) At the beginning of adult life protease activity in the midgut increases much more rapidly in females than in males indicating that there is a need of amino acids for vitellogenesis (Waldner - Stiefepmeier, 1967).

After emergence, the corresponding periods of adult life in males are reflected by the slight increase & decline in the amino acids. Nowosielski & Patton (1965) noted in the house cricket, Acheta domesticus that total amino acids decreases by 20% during first 10 days following eclosion.

A distinct sex-specific difference in the free amino acid compostion of Culex indicated that male mosquitoes aged 4-6 days contain about seven times more β-alanine than the females while the reverse is true for methionine sulfoxide whose content is about five times higher in females than in males (Chen, 1958 b, 1963). In Drosophila Kaplan et al (1958) reported that female flies aged 2-3 days contain twice the amount of methioine as in males . In both Aedes (Dimond et al; 1956, Lea et al; 1958) and the boll weevil, Anthonomus grandis (Vanderzant, 1963), it has been shown that methionine is an important component in the synthetic medium and has the ability to promote egg production. In this connection a strong evidence has been provided by Geiger (1961) in *Culex pipiens* & *C. fatigans*. Recently Tobe (1978) found that there is unusually high turnover rate of the aminoacid and peptide pool during each reproductive cycle in the tsetse fly, *Glossina austeni*.

Age-related changes in free amino acids have also been followed in a number of insect species. Chen (1972) has noted in male Drosophila that senescence is characterized by large accumulation of taurine, α-alanine & β-alanine. However, the total amino acid level remained stable within a 30- day period. A decline in the turnover rates of lysine, glycine and α-alanine with a parallel reduction of their incorporation rates into the protein has noted in ageing insects by Bauman & Chen (1968) and Bauman (1969).

A peak of the concentration of amino acids occurs at 30 days in ageing females of *Aedes aegypti* (Stidham & Liles, 1969), but most amino acids maintained a rather constant level (Thayer and Terzian, 1962, 1970).

Some specific amino acids :

There are several selected amino acids which are known to play some specific roles during insect life. It has been shown that tyrosine has a role in the hardening and tanning of the cuticle (Anderson, 1979). In a similar way, β-alanine is equally involved in the selerotization process during puparium formation & at adult eclosion. Mitchell & Collaborators (Mitchell et al; 1960, Simmons & Mitchell, 1962) have shown that in D.melanogaster tyrosine is stored as tyrosine-0-phosohate & this compound is hydrolysed prior to its incorporation into the puparium & thus serves as a reservior for selerotization (Lunan & Mitchell,1969). The presence of tyrosine-0- phosphate in adult Sarcophaga bullata immediately after eclosion has been reported by Seligmann et al (1969). As in Drosophila, it occurs in the haemolymph and is used for selerotization of the adult cuticle.

In majority of the insect species various phenolic compounds serve as the major tyrosine storage metabolite for selerotization (Chen, 1975; Chen et al; 1978; Ischizak & Umebachi, 1980; Kramer et al; 1980, Lu et al;1982).

β- alanine is one of the unusual amino acids which is normally not included into protein molecules. In spite of its occurrence in quite a number of species, its metabolism in insects is not known (Chen, 1962). More recent results suggest strongly that β-alanine is in some way involved in the selerotization and in a number of insects B-alanine occurs in hydrolysates of selerotized cuticles (Hackman & Goldberg, 1971). It is also related to the colouration and in the hardening of the cuticle (Lindsley and Grell, 1967; Jacobs, 1970, 1978; Hodgetts,1972; Hodgetts & Choi, 1974).

Proline and hydroxyproline are also among the specific amino acids. There is a wide occurrence of proline in insects but the presence of free hydroxyproline is of rare occurrence since it only occurs in a few special types of proteins. Further, proline is converted to hydroxyproline only after its incorporation into a polypeptide (Gianetto & Bouthillier,1954). The presence of a hydroxyproline containing secretary protein has been reported in the salivary gland of the midge, Acricotopus lucidus (Baudisch,1977). However, this protein was found to be collagenous in nature (Ashhurst & Bailey,1980). These workers have isolated collagen from the ejaculatory duct of adult Locusta migratoria and its chemical analysis indicated the presence of glycine, proline and hydroxyproline.

Substantial quantities of taurine are found in a number of insects but information on its metabolism is very limited. Taurine is metabolically very inactive and there is no detectable evidence of interconversion and other metabolites. Almost all taurine that is synthesized in the developing pupa is carried over to the adult. The total concentration of taurine varies during different developmental stages of different insects (Chen & Hanimann,1965; Levenbook and Dinamarca, 1966; Evans and Crossley, 1974). The largest amount of taurine is stored in the haemolymph. It has also been shown that over 90% of adult taurine is present in the thorax, suggesting its association with flight muscle. Bodnaryk (1981 a) has shown a close relationship between taurine and brain development in Mamestra.

Metabolism of tryptophan, an another specific amino acid, has been extensively worked out in insects since this amino acid serves as an ommochrome precursor (Linzen, 1974; Kayser,1979). Tryptophan levels vary in the adult flies. It is mainly related to the biosynthesis of pigments. Experimental evidence indicated that tryptophan metabolites are sequestered from the haemolymph of the adult female(Graf, 1957; Egelhaaf,1963; Kog & Osanai 1967). In the papilionid butterfly, Papilio xuthus, free tryptophan derived from protein break-down increases gradually in the early pupa & declines quite rapidly shortly before adult eclosion (Umebachi & Katayama, 1966). The decline is correlated with a rise in Kynurenine which is a major component of adult wing pigment (Umebachi & Yoshida, 1970).

Protein Metabolism :

The development of a multicellular organism like an insect is a complex process genetically controlled enclosing the synthesis of macromolecules and intermediate reactions. Since the major biochemcial process underlying morphogenesis is protein synthesis, it is not surprising that the number and patterns of tissue-specific proteins vary at different stages and become increasingly more complex with advances in development. Further, the morphological changes are a result of endocrine interactions influencing the protein pattern of many tissues (collins, 1969, 1974; Thomson & Mitchell, 1972; Tysell & Butterworth, 1978; Riddiford & Truman, 1978; Dean, 1978; Butterworth et al,1979 ; Sass and Kovacs, 1980; Tojo et al.,1981). During the larval period the growth is the predominant phenomena whereas during the pupal life differentiating processes of high complexity are in the foreground (Chen, 1960).

The growth period develops alongwith a storing of proteins, glycogen and lipids which are needed for the development of imaginal organs (Schmidt, 1967).

Another important aspect of protein metabolism during larval development is the syntehsis of haemolymph proteins (Leevenbook,1966) and in parallel with the morphological changes there are vast changes in the number and concentration of haemolymph proteins. In general the protein concentration in the haemolymph increases rapidly during the later half of the larval development, falls at metamorphosis and declines to zero in early adult life (Chen, 1956, 1958; Chen & Levenbook, 1966; Laufer 1960).

The haemolymph has specific functions in the metabolism, as a transport and storing system and as a regulator of enzymatic actions. The proteins of the haemolymph can be utilized directly as a source of material for new protein synthesis, especially by the tissues of the developing adult in the pupa or nymph (Leevenbook, 1966 b, Tobe & Loughton, 1969).

The haemolymph proteins can be sequestered by a number of tissues, mainly by the fat body where they are stored in the form of protein granules (Locke & Collins, 1966, 1967). It is now generally accepted that nearly all these proteins are synthesized in the fat body (wyatt, 1980). In addition, the fat body serves as the major site of intermediary metabolism (chen, 1972). It has been shown that the increase in the haemolymph protein concentration is accompanied by a fall in the synthetic capacity of the fat body (Ruegg, 1968), This is logical since following protein production, the fat body changes to a storage organ for several selected haemolymph proteins for use during adult development (Price,1972, 1973, Tojo *et al.*,1980). Although, the fat body and the haemolymph individually have been the subject of many biochemical studies (Keeley, 1978; Wyatt & Pan 1978), the release of several proteins into the haemolymph and the uptake of selected haemolymph proteins into the fat body has been demonstrated in few insect species (Shigematsu, 1958; Locke & Collins, 1968; Mun *et al.*, 1969; Chippendale & Kilby, 1969; Martin *et al.*, 1971).

The storage of proteins in the adult fat body is associated with reproductive stage in the life cycle. Hill *et al* (1968) showed that in the adult female desert locust, *Schistocerca gregaria* the level of protein & lipid in the fat body could be correlated with the stage of egg development. The rate at which the larval fat body disappears during development of the adult is related to the development of ovaries in *M.domestiea* (Adams & Nelson, 1969). These workers noted that the adult fat body shows a volume decrease at certain stages of oocyte development.

The total haemolymph concentration also decreases in many insects, especailly in cockroaches, during vitellogenesis and the maturation of the oocytes. It remains nearly constant in the males. However, the reporductive significance of proteins in males of different insects has been worked out (Wilde & Loof, 1975; Leopold, 1976; Chen 1971, 1978; Baumann, 1974; Bodnaryk, 1978; Chen,1980).

Electrophoretic studies have pointed out that some protein fractions are associated with the molting cycle (McCormick & Scott, 1966 a, b; Fox & Mills, 1969).

Scope of the present investigation :

The Indian Neem tree, Azadirachta indica is a unique in possessing a broad spectrum of biological activities. Recent studies in the area of agricultural chemicals have identified neem plant as a promising source of natural pesticides. For centuries before petroleum-based pesticides were available, farmers on the Indian subcontinent protected their crops with natural insect repellants found in the fruits and leaves of neem tree. Now scientists have discovered that neem derivatives repel 123 species of insects, including pests of stored grain. The insect-repellant qualities of oil extracted, from the neem seeds, and of cake made from its residue, are being studied in a programme to develop biological, pesticides that cause no biological damage.

Scientists at the Indian Agricultural Research Institute (IARI) pioneered studies of antifeedant propperties of neem seed-kernel suspension against the desert locust, migratory locust, and other pests. Pradhan (1962) later showed that both sprays and granular forms of neem seed kernel reduced insect feeding and disrupted growth. This discovery of the locust antifeedant effect of neem cake at IARI has eventually lead to the identification of the potent insect feeding inhibitor, azadirachtin. The effect of neem products on the survival as well as on feeding has been studied in Sitophilus zeamais by Karnavar and Dlamini (1987). Recently it has been shown that azadirachtin has a distinct growth inhibitory property (Gill & Lewis, 1971; Ruscoe, 1972; Rembold et al., 1980). More recently, Barnby and Klocke (1987) have studied the effect of azadiractin from the view point of both nutrition and development in the tobacco budworm, Heliothis virescens and they have discussed its significance in relation to the hormonal changes.

Several attempts have been made to separate or isolate different derivatives such as nimbin, salanin, meliantroil etc. from the principal compound azadirachtin (Lavie et al., 1967; Warthen, 1979). Fractions like nemidin, vemidin, neemol and nemicidin have also been purified by a group of workers (Srimannarayana et al.,1987) and all these compounds have been shown to be effective against a major vegetable pest Epilachna vagintioctopunctata. Scientists working at the laboratories of ~~all~~ the Regional Research Laboratory, Hyderabad and National Chemical Laboratory, Pune have isolated highly biologically active crop protective fractions from the neem kernel (Press report, 1987). These compounds, namely Neemrich-I and Neemrich-II have been shown to have the pest control value in warehouses and the antifeedent properties against pests like Spodoptera litura. Disturbance of epidermal and fat body tissue occurred in the larva of the mexican been beetle, Epilachna varivastis when fed with azadirachtin (Schleuter,1984). Further, it has been observed that the epidermal cells lost the power of moulting due to their degeneration. Effect of azadirachtin on the endocrine events with reference to the moulting cycle etc, has been reported in several insects (Zebitz, 1984; Garcia & Rembold, 1984; Dern et al., 1986; Koul et al., 1987; Chang & Chu,1987). Oviposition deterrant activity has been examined in neem extract (Joshi & Sitaramiah, 1979; Sharma et al., 1983). A similar effect of neem kernel suspension on the hatchability of eggs has been shown in the desert locust, Schistocerca gregaria by Singh and Singh (1987).

Nitrification retarding principles as well as nematicidal properties have been identified in neem by Devakumar (1986). Insecticidal property of neem has also been examined against some insects (Mitra,1970; Attri & Prasad,1980).

It would appear from the foregoing literature that the products of the neem have been exploited from different angles. However, there are hardly any reports so far about the insecticidal effect of neem with reference to the changes in certain chemical moieties like amino acids and proteins. The present work therefore, has been taken up with a view to find out the effect of neem extract on the pattern of amino acids and proteins of the haemolymph, fat body and the alimentary tract of two insect species of economic importance. The present study, however, forms a preliminary nucleus and would form a useful guideline for the exploration and incorporation of more such products for the bioevaluation to other many pests of agriculture and stored products.

For studying the insecticidal effect of neem oil, two insect pests with entirely different feeding habits and habitats were selected. One being common pest of household commodities, the cockroach, Periplaneta americana and the other phytophagous agricultural crop pest, [redacted] Schistocerca gregaria. The reason behind the selection of these insects is that (i) their easy availability at all seasons, (ii) their culture can be easily maintained under the laboratory conditions, and (iii) the adults are enough large so that they can be easily handled and the required organs/tissues for the study can easily be separated.

Thus, the main aim of the present investigation is :

i) to analyse the free amino acids from the various tissues and organs like haemolymph, fat body and different regions of the alimentary tract by chromatographic method.

ii) to study the pattern of proteins by employing disc electro phoresis on polyacrylamide gel as well as their quantitative estimation by biochemical assay method,

iii) to analyse both free amino acids and protein by above mentioned methods after the treatment of neem extract and

iv) to compare the results obtained with that of available literature and to discuss the same from the view point of the insecticidal effect of neem as a potent chemical.