CHAPTER- I

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 artifical 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 poisonous 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 biomagnification 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 continous and intensive use of certain insecticides against various insect pests has resulted in the development of races or 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 indiscriminate eradication propounded & practised

earlier. This biological approach obviously includes conservation of species as an inherent proposition.

Insecticides that indiscriminately 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 et al., 1976) are of special mention. The commercial synthesis/production of phermones 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 palnt 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 semiochemicals and the plant nutrients (Thorsteinson, 1953,. Sugiyama & Matsumato, 1959,. Waldbeuer, 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 these 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.

What are mucosubstances?

The term "mucus" is denoted to a slimy, viscid, tenaceous substance of animal body. This term was freely used in Latin and medical texts of the 18 th century. It was Eichwad (1865) who for the first time provided the chemical evidence for the presence of carbohydrate in mucins. According to him mucin is " a conjugated single compound of a moiety released under certain conditions as a sugar". Hammarstein (1895) demonstrated the acidic nature of the mucin from submaxillary gland. He referred occassionally to mucins as "Glikoproteid", a name still favoured in the German literature. He also introduced the term "mucoid" for a number of carbohydrate containing proteins.

The term "mucopolysaccharide" has been originally coined by Meyer (1938) to describe hexosamine - containing polysacclarides of animal origin, occurring either in pure form or conjugated with proteins through a salt linkage. Meyer (1945, 1953) modified his scheme twice. In 1953, he introduced the terms "mucoproteins" and "mucoids" into original scheme.

Due to confusion regarding the terms mucins, mucoids, mucopolysaccharides, mucoproteins, glycoproteins etc., it is indeed difficult to distinguish them by definitions. But Dorfman (1963) has given definitions of each of these terms, which are very well accepted now (Curran, 1964). Hunt (1970)

commented on earlier definitions of glycoproteins in which protein part predominates and of mucopolysaccharides in which the polysaccharides predominate. He further described that the terms are rather unsatisfactory in that they fail to refer directly to the protein part of the complex, while prefix may be held to imply mucinous, viscous, slimy proteins which are not necessarily characteristic of the material in natural state. The term mucopolysaccharide is now well established in the biochemical literature.

Spicer et al. (1965) classified carbohydrate rich tissue components and suggested the word "mucosubstance" a general term for histochemical reference to any carbohydrate rich component. In their classification, they named the mucosubstances according to site (e.g. epithelial mucosabstances, connective tissue mucosubstances etc.), according to the presence of neutral & acidic groups such as neutral mucosubstances, sialomucins, sulfomucins etc. and according to the following histochemical reactions such as a) affinity for basic dyes such as azure A, (b) affinity for alcian blue, c) lability with respect to testicular hyaluronidase and d) lability with respect to Vibrio cholerae neurominidase.

At present the terms such as neutral mucosubstances, sulfomucins, sialomucins etc. are commonly used in the histochemical literature.

A general survey of Carbohydrates in insects :

Carbohydrates are important in insects, since they function as a major energy source and are utilized as a major exoskeleton compentent. There are many reviews available on the biochemistry of these materials (Wyatt, 1967, Chefurka, 1965;a, Sactor, 1965) and recently certain aspects of their physiological chemistry has been discussed (Steele, 1981; Friedman, 1985; Candy, 1985). However, most of these studies are related to the carbohydrate metabolism of fat body and haemolymph (Wyatt, 1967, Friedman, 1970,1978; Florkin & Jeuniaux, 1974; Steele, 1981).

When compared with vertebrates, the total carbohydrate content of insect haemolymph is considered to be high. levels generally are greater than 0.5% but may reach 8.1% (Wyattt, 1967). The most characteristic carbohydrate found in insect haemolymph is trehalose, a non-reducing disaccharide. However, in certain insects it may be absent or present in very low concentrations (Evans and Dethier, 1957; Wimer, 1969; Bedford, 1977). In honeybees it may represnet as much as 95% of the total carbohydrates during postembryonic development (Tsao & Shuel, 1973). It may increase in concentration during larval development (Boctor, 1974). However, its value may change in different insects(Wyatt, 1967). Glucose is present in the haemolymph of many insects, but generally at lower levels & like trehalose its concentration also varies among and within

species. Glycogen may be found in haemolymph but in very small amounts (Wyatt, 1967; Chippendale, 1973 a). Other sugars that are reported in insect haemolymph include arabinose, cellobiose, fructose, fucose, galactose, maltose, mannose, ribose and sucrose (Wyatt, 1967; Florkin & Jeuniaux, 1974; Phillipe et al., 1976). In many cases the composition of the haemolymph sugars is related to the diet on which the insects feed (Hansen,1964; Maurizio, 1965). In addition to these sugars other carbohydrates or derivatvies have been reported to occur in the haemolymph. These include hexosamines in Blaberus craniifer (Phillipe et al., 1976) and other insects (Florkin & Jeuniaux, 1974), β-glucosyl-O-tyrosine in silkworm pupae, and Leucania separata and Drosophila busckii larvae (Chen et al., 1978, Isobe et al., 1981) glucornic acid and inositol in Anopheles stephensi (Mack et al., 1979 b) and Scyllo - inositol in Schistocerca gregaria (Candy, 1967).

Of the several carbohydrates, the major compounds in which hexose residues are stored in insects are presently known to be three : trehalose, glycogen & chitin. There are indications, however, that less clearly indefinable glycoproteins may play important roles as sources of hexose in Periplaneta americana (Lipke et al., 1965 a) and that polysaccharide other than glycogen may occur in some insects, Tribolium confusum (Villeneuve and Lemonde, 1965). The metabolism of these various carbohydrates in general is described below.

Glycogen :

Glycogen forms an important carbohydrate reserve in insect. As the major polymeric storage form of glucose in animals, glycogen has been shown by histochemical methods to be present in a mariyad 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. Glycogen isolated from the boll weevil, Anthonomus grandis larvae has been shown to have the same chemical configuration similar to that isolated from mammals (Betz et.al., 1968). But childress and Sacktor (1969) have shown that glycogen isolated from Phormia regina flight muscle behaves differently, this is probably due to a different mode of isolation technique. Lindh (1967) working with the pupae of Calliphora erythrocephala, has shown that glycogen is covalently linked with proteinaceous material.

The amount of glycogen has been shown to vary considerably through development, ranging from as little as 0.01% wet weight to values of more than 2% (Kilby, 1963; Neettles and Betz 1965). Its distribution in adult Drosophila melanogaster was examined by Wigglesworth (1949), who found the bulk of the glycogen in the abdominal fat body as large peripheral intracellular deposits. Other sites, in order of decreasing importance, were the halters, flight muscles, proventriculus and the midgut cells. In general, glycogen is stored in large amounts in the flight muscle of those insects which use it as an immediate source of glucose units for flight energy, and its also stored in fat body, from whence glucose is provided to other tissues after transformation into the circulating blood sugar, trehalose. The amount of glycogen

in insect fat bodies can vary widely, depending upon the stage in the life history, on past nutritional level and on the demands made by energy requirements (Clegg & Evans, 1961; Childress et.al., 1970; Rowan and Newsholme, 1979; Downer & Perker, 1979; Van Marrewijk, et.al., 1980).

The synthesis of glycogen appears to follow the same pattern described to other animals. It appears probable that both the phosphorylase and the uridine diphosphate glucose (UDPG) routes for glycogen synthesis are operative in the fat body. The synthesis of glycogen proceeds by the addition of glucose, as UDP-glucose, to a glycogen primer, the reaction catalysed by glycogen synthetase. Trivelloni (1960) has shown that incubation of fat body preparations from Schistoceria cancellata with labelled UDP.glucose - ^{14}C resulted in the transfer of a part of the radioactivity to the glycogen, and when this was treated with β-amylase, all the radioactivity was liberated as maltose. There was thus enzymatic transfer of glucose residues from the UDPG to the ends of the glycogen primer chain by the formation (1→4) Linkages.

During primary degradative pathways glycogen in tissues is converted to free or phosphorylated glucose before being further metabolized by glycolytic or other pathways. The enzyme mainly responsible for the primary breakdown of glycogen is glycogen phosphorylase. A study of phosphorylase in insects has shown it to be present in midgut, fat body and flight muscles of various species, and to exhibit, in general, properties similar to those of the vertebrate muscle enzyme. There exists a large body of literature on this enzyme (Sacktor, 1975; Childress & Sacktor,1970; Fischer et.al.,1971; Hansford & Sacktor, 1970; Sacktor et.al., 1974; Rowan & Newsholme, 1979; Marrewijk et.al., 1980; Mcclure and Steele,1981).

Trehalose :

Trehalose, a non-reducing disaccharide, is known to occur in the haemolymph, and to a limited extent, in the fat body of a variety of insects (Wyatt & Kalf 1959; Howden & Kilby, 1956; Evans & Dethier,1957; Crompton & Birt, 1967). The concentration of trehalose varies from zero to seven percent among the different groups of insects (Mochnaka & Petryszyn, 1959; Ehrhardt,1962; Crompton & Birt, 1967; Friedman, 1969).

The obvious function of trehalose is that of energy source used by the tissues bathed in the open haemolymph system. It gives as much as twice the amount of energy as does glucose and the enormous amount of it being utilized by flight muscle (Weis-Fogh, 1964).

Treherne (1958 a,b) has shown that 14_C-glucose introduced into the gut of the locust is rapidly absorbed and is almost completely converted into trehalose which accumulates in the blood. The absorption was largely confined to the caeca, the proventriculus being less active and there was no significant uptake from the hindgut. The absorption occurs through a process of diffusion across the gut wall. It has been shown that the fat body is the main site of trehalose biosynthesis from glucose (Candy and Kilby, 1959; 1961; Clegg and Evans,1961).

The pathway involved in the synthesis of trehalose was first studied by Candy & Kilby in 1961 with an extract of adult Schistocerca gregaria fat body. The intermediates in trehalose formation are glucose-6-phosphate and UDPG. Glucose first converted into glucose-6-phosphate which then reacts

with UDPG to produce trehalose phosphate. A specific phosphatase then hydrolyses this to trehalose. The fat body also contains the enzymes required for the regeneration of the UDPG.

The only means whereby trehalose is known to be degraded in insects is through a hydrolytic cleavage resulting in the production of two moles of glucose. The reaction is catalyzed by the enzyme trehalase, which has been demonstrated in a number of species. The enzyme has been identified as a soluble protein from gut tissue (Gussin & Wyatt,1965, Gilby et.al., 1967), blood (Friedman,1961) and flight muscle (Hansen,1966a; Stevenson,1968).

Chitin :

Chitin is a structural carbohydrate in the form of polysaccharide and is found mainly in the endo- and exocuticle of insects. The amounts of chitin in cuticle range 10% to more than 60% (Hackman, 1964). It is generally found as a chitin-protein complex (Blackwell and Weih, 1980). Three forms of chitin i.e. α ,β and γ are known (Rudall,1963). The unit cell of α-chitin consists of two chains, each composed of two residues, β-Chitin has a unit cell consisting of a single disaccharide (Blackwell,1969), but the crystal structure of γ-chitin is not yet proved. β-and γ-chitin are present in limited amounts in insects while β-chitin known only from cocoons of a few beetle species, and γ-citin similarly from a few eocoons and also from the peritrophic membran of a number of species (Rudall & Kenchington,1973). Both of these chitins soften & swell in water. α-chitin is highly resistant to swelling in water, organic solvent penetration, and alkali and dilute acid hydrolysis.

The synthetic reactions leading to the production of chitin follow, in general, those of glycogen synthesis, in that the ultimate precursor is a nucleoside diphosphate sugar. In the case of chitin, the sugar moiety is N-acetyl glucoseamine, and the most important synthetic reaction in the sequence is probably that involving the amination of fructose-6-phosphate, since it is the crossover point between reactions leading to glycogen or trehalose on the one hand, and chitin on the other. Various reactions leading to the synthesis have been studied extensively in insects (Candy & Kilby, 1962; Jaworski et.al.,1963; Krueger and Jaworski, 1966; Benson,1968; Benson & Friedman,1970; Surholt,1975a, Enghofer et.al., 1978; Vardanis,1979).

The breakdown of chitin generally occurs at the time of the molt, when the endocuticle is being resorbed and used both for the production of new cutide and to provide energy for attendant metabolic processes. The enzyme responsible for the degradation of chitin is chitinase. The degradation occurs in two steps (Waterhouse & Mckelkar,1961; Kimura,1973; Winicur & Mitchell,1974; Bade,1975; Spindler,1977; Zielkwoski & Spindles,1978; Bade & Stinson, 1978, 1979). It has been shown in Periplaneta americana that the concentration of the enzyme was found to be highest in the haemolymph, although total activity was greatest in the cuticle. However, there was significant activity in the gut (Irzykiewicz, 1963,1967).

Other compounds :

Other compounds which may contribute hexosyl residues for various functions include glycoproteins, polysaccharides which

do not appear to be glycogen, and mono- and disaccharides, which in certain cases exceed trehalose levels in blood.

Glycoprotein :

Glycoprotein molecules consist of one or a mixture of carbohydrate species bound as a short chains in covalent linkage with protein & have been histochemically established by various methods in a number of insect preparations. However, their functions are not at all clear among the insects since they show varied locations in different tissues (Sreng,1979; Thomas & Gournanton, 1980). The only significant analysis of plasma glycoprotein in insects has been made by Lipke et.al. (1965a) on P.americana nymphs at various stages of development. The carbohydrate compounds described included not only glucose, mannose, galactose, glucosamine and galactosemine (probably as N-acetyl derivatives), but the pentoses xylose and arabinose. These sugars have also been found into the cuticle.

Recently a number of studies have been carried on certain purified insect glycoproteins in an effort to specify the nature of the carbohydrate residues (Sinohara,1979; Kunkel et.al.,1980; Butters & Hughes,1981).

Polysaccharides :

The occurrence of polysaccharides other than glycogen and chitin in the haemolymph and tissues of insects is presumptive evidence that enzymes are present to handle them. Presence of the amylose-like polysaccharide has been shown in Tribolium by Villeneuve & Lemonde (1965). Acid mucopolysaccharides have been demostrated by histochemical methods in a number of insect tissues,

but their functions remain unknown and only a few attempts have been made to characterize them or to examine them throughout development. Among these studies is that of Hoglund (1976), who has shown that changes in the ratio of chondroitin sulfate to heparin (or, perhaps, keratin) sulfate occur as Calliphora erythrocephala matures, and that there are specific larval acid mucopolysaccharides which disappear after pupation (Ashhurst,1971a).

In several species hyaluronic acid has been found in various glands, e.g. salivary glands (Vadgama & Kamat,1971) and dermal glands (Baldwin & Salthouse,1959). It also occurs in the connective tissue of brain, ganglia & imaginal discs of M.domestica (Mustafa & Kamat,1970) and in the Malpighian tubules and the spittle of cercopoid larval (Marshall,1966 a, b).. Sulfated acid mucopoly saccharides occur in the connective tissue surrounding the ejaculatory duet and in the ganglia of the locust, Locusta migratoria (Ashhurst & Costin, 1971, a, b). Biochemical characterization of some acid mucopolysacchhides from the peritrophic membrane and from the midgut extracts has been reported (Nisizawa et.al.,1963; Estes & Faust,1964).

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

Captions to Figures

Fig.1 : T.S. of oesophagus of untreated cockroach showing cuticular hairs (CH), epithelium (Ep) with positive PAS reaction X 450

Fig.2 : T.S.of oesophagus of untreated cockroach stained with AB.pH 2.5 -PAS to show blue purple staining in the epitheluim (Ep) and Pink staining in the muscular layer (M), X 450

Fig.3 : T.S. of oesophagus of normal grasshopper stained with PAS to show an positive reaction in the epithelaium (Ep) and musculature (M), X 450

Fig.4 : T.S. of oesophagus of treated cockroach indicating reduced staining intensity of periodate in both epithelium (Ep) and musculature (M),X 450

Fig.5 : T.S. of oesophagus of treated grasshopper stained with AB-PAS sequential technique. Note reduced staining in the epithelum (Ep) and musculature and in the peritoneum (P), X450

Fig.6 : T.S. of crop of normal cockroach stained with PAS Note intense staining in the musculature (M), X 450

Captions to Figures

Fig.7 : T.S.of crop of untreated grasshopper stained with AB pH 1.0 - PAS. Note +ve reaction of periodate & alcianophilia in the epitheluim (Ep) and musculature (M), X 450.

Fig.8 : T.S. of gizzard of normal cockroach stained with AB pH 2.5-PAS, Note intense staining in the epithelium (Ep) musculature shows pink staining reaction, X 300

Fig.9 : T.S. of gizzard of cockroach after treatment of neem oil. Both epithelum (Ep) and musculature(M) show weak staining with AF-AB pH 2.5, X 300.

Fig.10 : T.S.of mesenteron of cockroach stained with PAS. Note staining reaction in the epithelium (Ep) & Muscle layer (M), X 450.

Fig.11 : T.S. of mesenteron of normal grasshppper stained with AB 1.0 - PAS, X 450

Fig.12 : T.S.of mesenteron of treated cockroach. Note slight staining intensity in the peritrophio membrane (PR) and epithelium (Ep) and weak staining in the musculature x 450

Captions to Figures

Fig.13 : T.S.of mesenteron of treated grasshopper stained with AB pH 1.0 - PAS. Reduced staining intensity in the musculature (M), X 450

Fig.14 : T.S. of ileum of normal cockroach stained with PAS. Note staining in the intima (CI), Epithelium (Ep) and musculature (M), X 450.

Fig.15 : T.S. of ileum of normal cockroach stained with AB pH 2.5 - PAS. Note positive reactions at different sites, X 450.

Fig.16 : T.S.of ileum of treated grasshopper stained with AB pH 2.5 - PAS. Note no reduction in staining reaction, X 450.

Fig.17 : T.S. of colon of normal cockroach stained with AB pH 1.0 - PAS, X 450.

Fig.18 : T.S. of colon of normal grasshopper stained with PAS. Note the staining in the cuticular intima (CI) x 450.

Captions to Figures

Fig.19 : T.S.of colon of treated grasshopper stained with PAS,note reduction in staining, X 450.

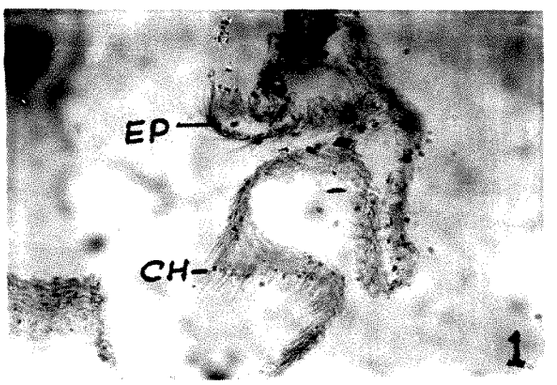
Fig.20 : T.S. of colon of treated grasshopper stained with AB pH 2.5 - PAS, X 450

Fig.21 : T.S. of rectum of normal cockroach stained with PAS, Note intense staining at different sites, X 450.

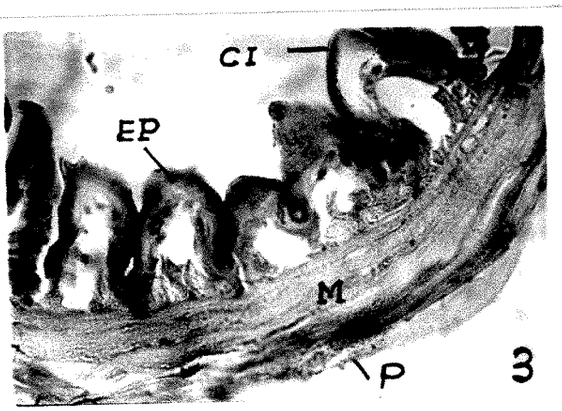
Fig.22 : T.S. of rectum of normal grasshopper stained with AB pH 2.5 -PAS. Note intense staining in the epitheluim (Ep) of rectal pads, X450.

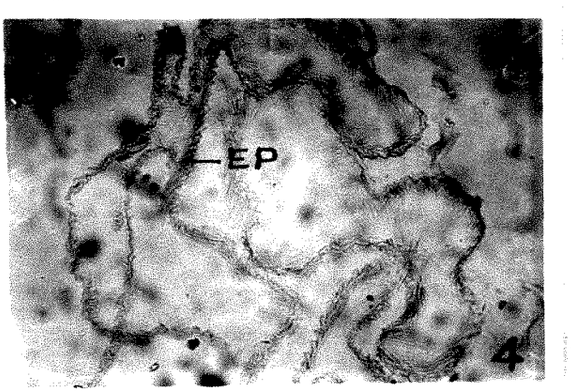
Fig.23 : T.S. of rectum of cockroach stained with PAS. after treatment with neem oil. Note no change in the staining intensity, X 450.

Fig.24 : T.S. of rectum of treated grasshopper stained with AB - PAS. Same reactions as above, X 450.

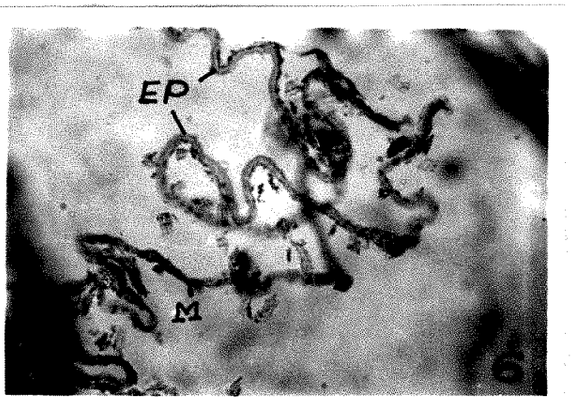

EP
CH
1

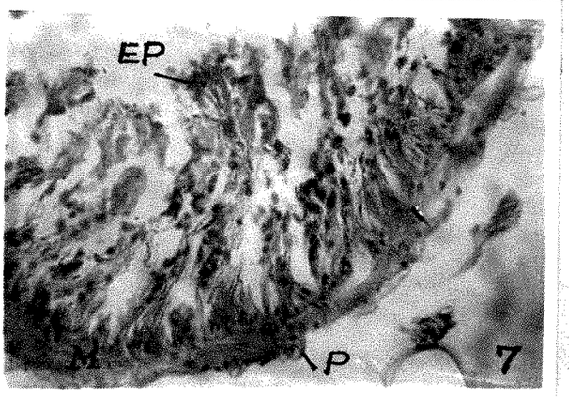

M
EP

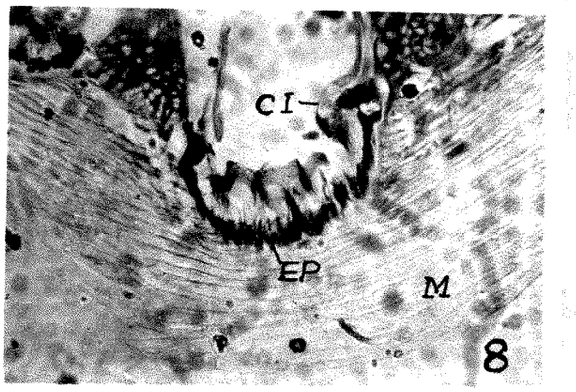

CI
EP
M
P
3

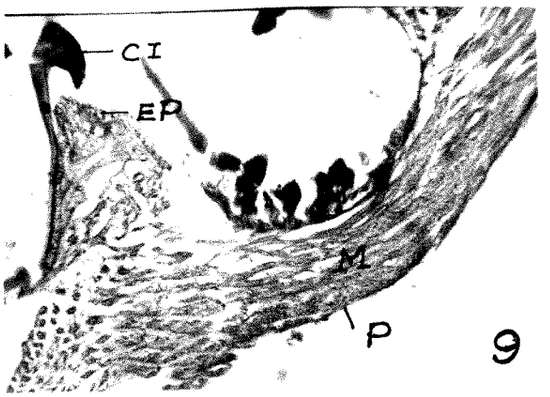

EP
4

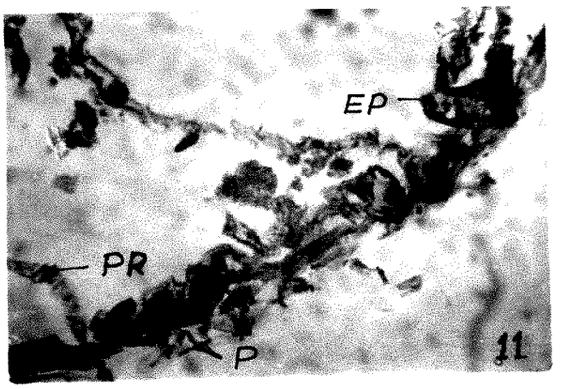

CI
P
5


EP
M
6

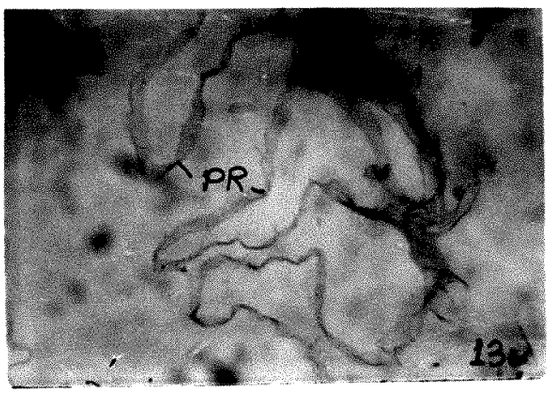

EP
M
P
7


CI
EP
M
8

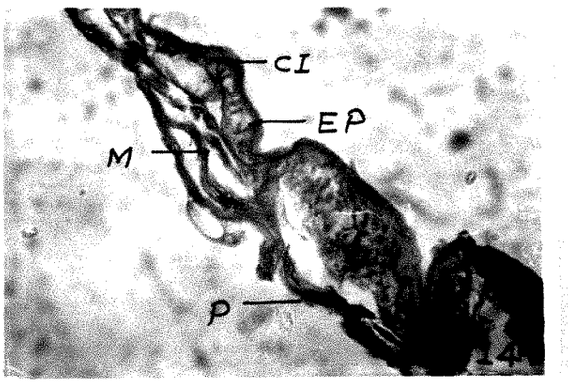

CI
EP
M
P
9


EP
PR
P
11


PR

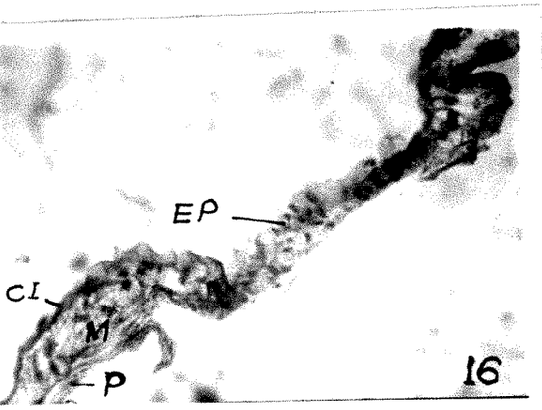


13

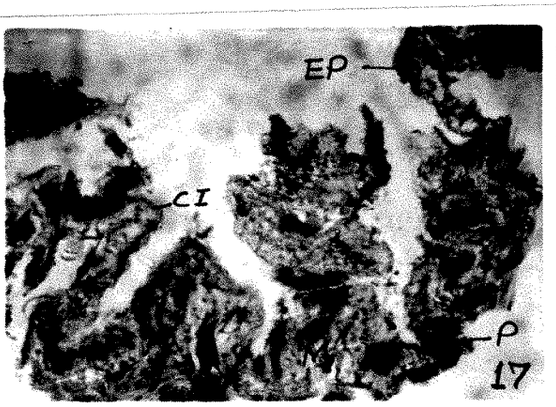


14

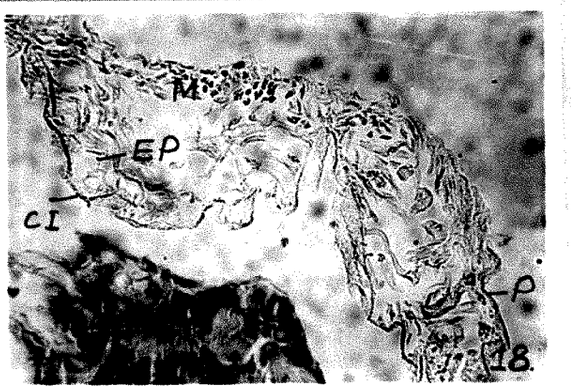
15



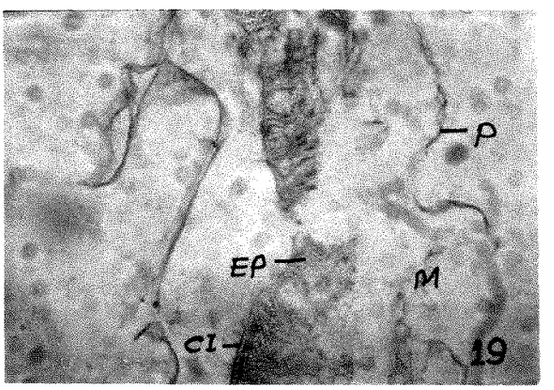
16

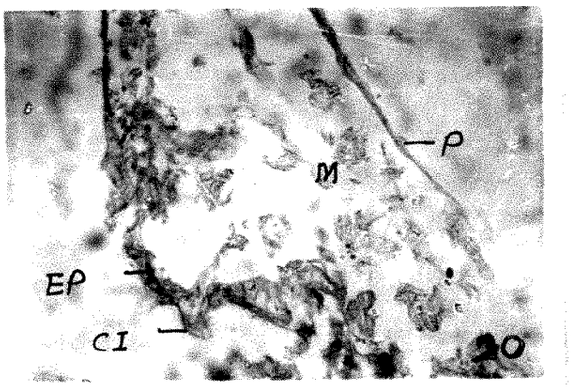


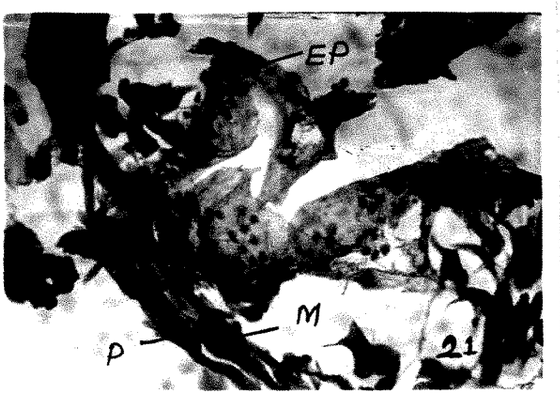
17

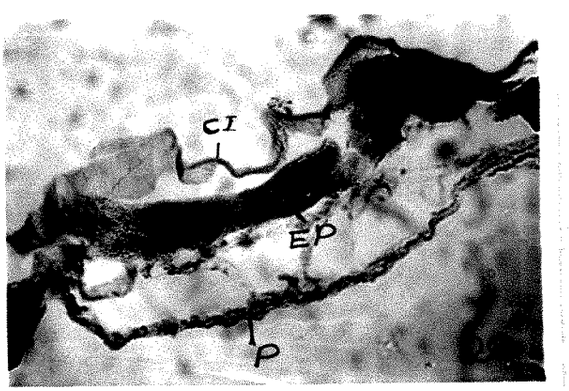


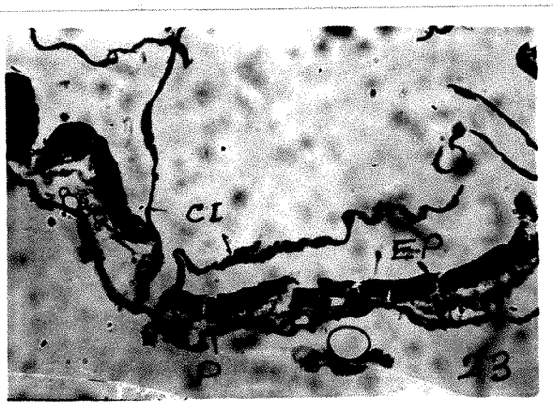
18

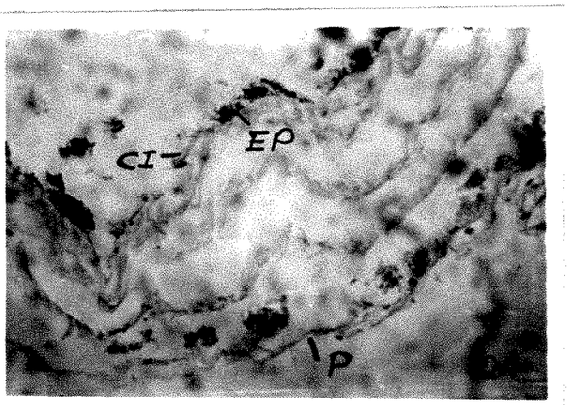

P
EP
M
CI
19


P
M
EP
CI
20


EP
M
P
21


CI
EP
P


CI
EP
P
23


CI
EP
P

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 properties 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, maliantroil 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 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 anti-feedent 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; Dörn 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 nematicidel 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 distributional pattern of certain chemical moieties like mucopolysaccharides in the alimentary

tract of insects. Perusal of the data available indicates that no efforts have been made to study the mucopolysaccharides from the different regions of the alimentary tract of the insects. Studies in connection with certain acid mucosubstances have been reported from certain organs and tissues only (Ashhurst & Richards, 1964; Baldwin & Salthouse,1959; Estes & Faust,1964; Mustafa and Kamat,1970; Vadgama & Kamat,1971; Ashhurst & Costin, 1971a, b). Therefore, the present study has been taken up with a view to investigate different mucopolysaccharides as well as to see the effect, if any, of neem oil on these chemical moieties from the different parts of the alimentary tract of two insect species of economic importance.

During the course of present investigation, two insect pests with entirely different feeding habits and habitats were employed, one being the common pest of household commodities, the cockroach, Periplaneta americana and the other agricultural crop pest Schistocerca gregaria. The reason behind selection of these insects is that (i) their easy availability at all seasons, (ii) their culture can be easily maintained in the laboratory and (iii) the adults are enough large so that they can be easily handled and the required organs for the study can be easily separated.

Thus the main aim of the present investigation is (i) to study the distribution of different mucopolysaccharides in the different regions of the alimentary tract by employing the well-known histochemical methods, (ii) to study the effect of neem oil on the distribution of mucopolysaccharides in the different regions of gut of both the insects, (iii) to compare the results obtained

with that of the available literature and to discuss the same from the view point of the insecticidal effect of neem as a potent bioactive principle.