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GENERAL INTRODUCTION

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CHAPTER ONE

GENERAL INTRODUCTION

Insects comprise the most extensive class in the Animal Kingdom. The number of species described exceeds the number of all other known animal species. Insects have invaded almost every habitable terrestrial environment. some live in fresh water for the whole or at least the earlier stages of their lives.The role of the insects in the world of living things is becoming more appreciated, not only because of the attention given to the species that acts as pests, but also because of the increasing realization that many species are extremely valuable to humanity (Borror *et al.*, 1992).

Insects are most successful and higly evolved animals as regards both their life habits and variety of ecological niches which they occupy. Insects become successful in the struggle of life due to number of reasons such as presence of rigid and impermeable exoskeleton, ability to fly, small body size and high reproductive potential. They are also successful animals due to their behaviour, physiology and biochemistry in changing conditions.Physiologists study insects partly to answer these questions, to find out how insects work as biological machines, and partly to discover better means of controlling them when they are pests. Physiologists may, in addition, study insects because they are convenient model system for exploring more general physiological problems (Mordue *et al.*, 1980).

Lepidoptera is largest and important order of insect consisting of more than 105,000 species. Beetles, moths and butterflies are the most prominent representatives of the order. They are exceeded in numbers of species only by the Coleoptera (Beetles). The number of species in the order is estimated to be almost 1, 50,000.

The moths are nocturnal while the butterflies are diurnal in habit. They are beautiful insects representing species - specific colour pattern of the wings. The adults feed upon nectar and juices of flowers, and fruits (over-ripe ones) while larvae exclusively feed upon a variety of plants and are destroying foliage, shoots of trees, crops vegetables, fruits, food grains, flour, clothing etc. and behave as the external feeders or internal borers. The lepidopteran larvae are, therefore, pests of various economic crops and timber. The adults are, on the contarary harmless except some species of fruit sucking moths, such as the citrus fruit sucking moth, Otheris sp. or caster semilooper, Achoea sp.(Tembhare,1977).

The distinguishing characteristics of the Lepidoptera are as below:

1. Two pairs of membranous wings (rarely absent) covered with overlapping scales.
2. Sucking type of mouth parts in adults while larvae or caterpillars are with chewing mouth parts.
3. The metamorphosis is complete.
4. Scales on wings and body.

The larvae of order lepidoptera are commonly known as caterpillars. These larvae have no resemblance with the adults into which they develop. Caterpillars are wormlike, usually cylindrical and vary much in form and appearance. They have three pairs of thoracic legs, two to five pairs of abdominal legs, termed prolegs, which are thick, fleshy, membranous and not segmented. Caterpillars feed, with the exception of the larvae of clothes moths and a few others, upon plants or their products. Almost every plant has some species of caterpillar feeding upon some part of it or its products. Hence as much as many of the most important insect pests are caterpillars, the order is of much economic importance.

Lepidopterous pupae are of the obtect type with presence of antennae, legs, wings and mouth parts firmly cemented to the body, together with the long slender sclerites which form the maxillae, located along the midventral surface of the body, identifies most pupae of the order (Little, 1974).

The moths are included in the Noctuidae family.They are primarily nocturnal and are attracted to light, invading our homes in large numbers. Their nocturnal habits and their eyes, glowing in the dark, suggested the name of typical genus, Noctua (Latin, owl).

1. Insect Pests:

The insects which feed on plants and their products are known as insect pests, where as the insect pests which cause damage to crop plants are called agriculture pests. The pest is said to be an economically importent pest if it causes 5 per cent or more loss to the crop. The amount of damage caused to a crop is called economic damage. There is hardly any crop which is not damaged by one or more insects at different stages of its growth. Depending on their feeding habits the pests have been placed in different categories, i.e. monophagous, oligophagous and polyphagous. Monophagous are those insects which are known to feed on only a single species of plants. Oligophagous are insects which confine their feeding activity to plants of one family. The polyphagous insects feed on a very large number of cultivated and wild plants. Insect pests are capable of feeding on almost all types of organic matter.

Family Noctudiae is regarded as the most destructives among the families of the Lepidoptera due to largest number of pests. (Metcalf and Flint,1962).The armyworm previously called *Pseudaletia separata* but now identified as *Mythimna separata* (Walker/Haworth/Climbing

cutworm/Rice earhead caterplllar) is one of the most destructive pest from family Noctudiae.

2. Life Cycle of *Mythimna separata:*

The pest *M. separata* commonly known as armyworm is rather widely distributed extending throughout Asia, Australia, USSR, Pacific Islands covering about 27 countries islands from humid tropical to temperate regions (communicated by commonwealth Institute of Entomology, London).

Serious out-breaks of the pest on rice, wheat, sugarcane, sorghum and millets have occurred in India, China, Japan, Australia, New Zealand, Fiji, Bangladesh and Thailand. In India alone, as many as 16 outbreaks of the pest have reported between 1924 and 1981.Different factors possibly associated with the outbreak are reviewed by Sharma and Davies (1983). Arrival of the migrant population followed by an imbalance caused between the natural enemies, and the host population appears to be the major cause. Further, the long distance migration of adult (Oku and Koyma, 1976), heavy rainfall followed by drought (Ghosh, 1924), flooding (Butani, 1955; Puttarudraiah and Usman, 1957) and changes in relative humidity and rainfall also appears to be the other causes for the outbreak (Chen, 1979)

The infestation appears epidemically both in kharif and rabi seasons in India (Kalode *et al.*, 1972) and it attacks sorghum, rice, wheat, maize, and sugarcane (Bindra and Rathore, 1965). In India it is a notorious pest on a cereal crops. Other alternative host plants include oats, barley, beans, turnips, rye and tobacco (Grist and Lever, 1969), Pearl millet and johnson grass (Bindra and Singh, 1973).

At night time larva feeds voraciously on the green foliage of host plant leaving behind only the bare midrib of the leaves. The grass blades

are eaten entirely. It hides in the whorl of the plant during day to avoid the high intensity of light and become very active for feeding with sunset.

Generally damage caused by the larvae is mainly by defoliation, but in respect of wheat , rice and millets the pest cuts the base of earhead of panicles leading to great losses in yield (Sarup *et al.*, 1969; Pophali *et al.*, 1980; Krishnaiah *et al.*, 1980. Krishnaiah and Rai, 1981) Devastation by larvae results in great economic loss to the paddy crops hence the pest has assumed the status of a major rice-pest in Madhya Pradesh, Orissa and Uttar Pradesh (Katiyar and Patel, 1969; Kulshrestha *et al.*,1970; Sathpathy, 1978) and a major wheat-pest in Northern India (Sarup *et al.*, 1969; Bindra and Singh, 1973; Verma and Khurana, 1973).

The adult moth is pale coloured with medium size having a wing span of 1.5 inches. It has beautifully designed patchy forewings which have a wavy border pattern on the fringe. The hind wings are practically white (Pradhan, 1964). The moths are very energetic having strong power of flying. Due to nocturnal habit they found very active during night and remain hidden during day time (Shukla and Upadhyay, 1989). They are attracted towards light a strongly so to sweets like honey or decaying fruits (Metcalf and Flint, 1962). The life span is short ranging between 6 to 10 days.

After mating female starts egg laying. The eggs laid by female are in clusters or in rows between overlapping leaf sheaths or on rolled leaves or under leaf bases. The leaf is generally folded lengthwise and fastened about the eggs with sticky secretion. Freshly laid eggs are small, spherical and greenish white in colour. The average eggs laid by female has been recorded to be 500 to 550.Young and pale green coloured larvae hatched after 4 to 5days of incubation of eggs (Nayar *et al*; 1976). The half grown larvae are very specific because of their looping habit of crowling

Newly hatched larvae i.e first instar larvae and second instar larvae feed on the epidermis of tender leaves and this feeding activity was later marked by elongated scars on the leaves. From third instar onward larvae feed by cutting the leaf from edge towards midrib. The swarm practically browses a field like cattle and when one field is completely finished, the swarm marches on in regular army formation to the adjoining field. It can so happen that the younger stages of the larvae are passed among wild grasses in uncultivated areas where nobody notices their presence and from these they may suddenly march into the cultivated fields like an invading army. Due to the night feeding habit, their presence is not suspected until most of the crop is fairly damaged (Pradhan, 1964). According to Patel (1980) the bright sunlight and excessive heat under crowded condition in the rice field also cause marching of larvae .

The full grown larvae are nearly 1.5 inches in length. The colour of larvae is greenish-brown colour, with longitudinal stripes on body.According to Metcalf and Flint (1962) the pattern of stripes as below: a narrow broken stripe down the center of the back, bordered by a wide; somewhat darker; mottled one reaching halfway to the side, as seen from the side there are three stripes of about equal width; next to the wide mottled one, on the upper side a pale orange; white bordered stripe, next a dark brown; light mottled one just reaching to the spiracles and just below the spiracles; a pale-orange, unmolted one edged with white.They are characterised by honey combed head with dark lines, where as each proleg has a dark band on its outer side and a dark tip on the inner side. The skin is smooth, the spiracles are black and the mandibles are devoid of denticles.

The growing larval period is of 22 to 27 days, passing through six successive larval instars (Khanna, 1972). After complete development

larvae leave the plant. Then they enter the soil and construct an earthen chamber cell and pupate therein. In case water standing, they pupate naked in their fross at the base of clum (Khanna, 1972). Hence, one may at times find that the whole army of larvae suddenly disappears and the farmer may feel that the trouble has come under control till the next generation suddenly appears once again (Pradhan, 1964).

Pupa is dark brown and about 0.5 inch in length. Pupal period ranges between 9 to 11 days after which the moths emerges and give rise to new brood. There are about as five generations of armyworm observed in a year. They are characterized by presence of antennae, mouthparts, legs and wings. In India there are varying numbers of generations in the different regions of the country.

3. A Brief Survey of Investigation on Armyworm:

Mythimna separata is important pest damaging crops like rice, wheat, sugarcane and sorghum.Due to their economical importance many of investigators worked on life history,behaviour, control etc.of the armyworm. The brief survey of the investigation on armyworm is as below According to Laurent (1915) stated that armyworm off times becomes a plague when wet season followed by dry one. Drake and Harris (1927, 1940) were of opinion that the low laying grasslands act the breeding site for armyworm.

The evidence of unusual outbreaks of armyworm, *M. separata* in India were reported earlier by Prakash *et al.* (1969) at Delhi on wheat, Deol *et al.* (1985) on wheat at Ludhiana, Singh (1983) in the valley of Manipur on rice and Saini (1983) in Punjab on wheat crop. The reasons for these outbreaks as stated by these workers were the excessive rainfall coupled with introduction of new hybrid of wheat.

The life cycle of the armyworm was studied by Khanna (1972). He reported total larval period lasts for 3 to 4 weeks comprising six instars and the fecundity was of 500 to 700 eggs per female with incubation period 4 to 5 days and totally five generations were observed in a year. He also suggested the removal of grasses from bunds and hand collection of larvae and destroying them helps to control the pest. Neelgund and Mathad (1972b) studied the laboratory rearing and life history of the armyworm at Dharwad (Karnatak). They found that larval period involves 5 moults and 6 instars. They also mentioned pupal sex differentiation. Neelgund and Mathad (1974) carried out comparative studies of development of the armyworm reared on napier grass and artificial diet. Singh and Rai (1977) studied bionomics of the rice cutworm, *M. separata* at Varanasi (U.P.). They noted great variation in fecundity, larval growth, pupal period and adult longevity under laboratory and field conditions. Giraddi and Kulkarni (1985) studied the biology of armyworm under laboratory condition at Dharwad. They found that total life cycle occupied an average of 44 days ranging from 38 to 51 days.Patil *et al.* (1988) studied the influence of various diets on growth, development and reproduction of the armyworm

Generally cereals are more preferred by *M. separata.* However the evidence of breakdown of armyworm on other crops was reported by many of the scientists. However, Nair (1975) reported that armyworm also observed to infest gram (Pulse crop) in different localities in the country. Govindan *et al.* (1981) studied incidence of jawar armyworm, *M. separata* on maize cobs. They noted that the larvae known to be defoliator of different crop were observed causing serious damage to maize cobs in Karnataka. The larvae feed on silk and developing grains at tip. Tripathi *et al.* (1982a) had studied feeding behaviour of armyworm larvae on sunflower, soybean, pea and tomato. They reported that due to

antibiasis larvae did not feed and failed to develop on sunflower and soybean. Similar type of results were also observed by Tripathi *et al*, (1982b) in monocotyledonous plants like maize, pasture grass, sugarcane, sorghum, rice, fingermillet, wheat, Italian millet, oat and brown top millet Mote (1984) reported occurrence of armyworm on rabi sorghum. It is reported for the first time on the rabi (winter) crop of sorghum in 1982 where its incidence was heavy (3 to 13 larvae plant and 55 to 92 per cent plants affected), and complete defoliation occurred in some fields in the Satara district. Singh (1987) studied the effect of temperature sustenance and mating on the rice armyworm reproduction, wherein he found greater adult life span, fecundity and hatching at 15°C than at 30° C.

Reports are also available on incidence of armyworm on highbreed varieties. Kulkarni *et al.* (1977) carried out screening of high yielding sorghum varieties to armyworm. They noted that there was least incidence in SB-412 variety of sorghum and was significantly superior over the rest of entries tested. Giraddi and Kulkarni (1986) studied the reaction of 20 sorghum varieties to armyworm at Dharwad during kharif in 1981. They observed the varieties viz. SB-905 and SB-412 as most resistant to armyworm recording 1.09 and 1.33 larvae per plant.

The chemistry and function of a pheromone produced by the male of the southern armyworm was studied by Clearwater in 1972. He stated that the pheromone released by armyworm is benzaldehyde which functions as an arrestant, facilitating mating by preventing the escape of the female. The secretory cycle of a gland involved in a pheromone production in armyworm was studied by Clearwater and Sarafis in 1973 and suggested that the secretory cycle of this gland consists of three phases. Ogura (1975) studied the hormonal control of larval colouration in the armyworm, *Leucania separata.* Liu *et al.* (1985) tested the

effectiveness of sex pheromone traps (male) in China and stated that the attractiveness of Z-11-hexadecenal to males of armyworm was enhanced by the addition of 1 to 10% of (Z)-11-hexadecenyl acetate. In Japan, Miyahara (1987) used pheromone traps to monitor the spring immigration of the armyworm at Morioka where it do not overwinter. Similarly work on pheromones was done by Delisle and McNeil (1987) in *P.unipuncata* and Cusson *et al.*(1990)in *Pseudaletia unipuncata*

Phototatic behaviour of nocturnal moths and analysis of the cause of flight towards lamp was studied by Kao (1980) and active feeding behaviour by Kanda (1987). Many reports on behaviour of armyworm are also avalaible. Hirai (1984) studying migration, factors affecting time of taking off and flight period Lizhi *et al.* (1999) studied the influence of flight on reproduction and longevity of armyworm.

In addition to this following scientists also have contribution in the study of armyworm.Natural enemies of paddy cutworm, *M. separata* by Rizwi and Singh (1980), a preliminary study on the sterilization of the armyworm, *M. separata* with gamma-rays by Chao (1980), severe incidence of armyworm on rye in Kashmir Valley by Singh and Manchanda (1981), rice ear-cutting caterpillar; an injurious pest at panicle stage by Patel *et al.* (1981), field evaluation of some modern insecticides against armyworm, *M. separata* attacking paddy crop by Mathur and Upadhyay (1982), economic threshold and economic injury level of *M. separata* on rice by Khamparia *et al.* (1982), distribution; seasonal occurrence and natural enemies of armyworm attacking rice in China by Wu (1982), effect of leaf and panicle feeding by armyworm larvae on rice grain yield by Rice *et al.* (1982), observation on the life cycle of *M. separata* and its natural enemies in Wester Guangxi Zhuang Automonous Region by Wei (1982), morphogenetic effect of some

partially purified fractions and methanolic extracts of neem seeds on *M. separata* by Schmutterer *et al.*(1983), the host age selection by *Echtromorpha agrestoria notulatoria* (Fab), a parasite of *M. separata* by Nikam and Gosawi (1983), outbreak of armyworm, *M. separata* after flooding of paddy fields by Noda *et al.* (1984), role of temperature and photoperiod in the development of the meadow, *M. separata* by Berger (1984), a record of new parasite on armyworm by Mudiwale *et al.* (1984) and overwintering pottential of true armyworm by Fields and Mc.Neil (1984).

4. Work on Insect Protein and Proteases:

A) Insect Proteins:

In insects, as in other animals, one major biochemical event resulting from cellular activation is protein synthesis. Modern molecular biology has provided a clear overall picture of the mechanism by which genetic information encoded in DNA can be expressed as structural and catalytic proteins which play an essential role in growth, differentiation and function of all living cells. The sequences of genetic replication performed by the method of negative - positive image formation through hydrogen bonding between two pairs of purine and pyrimidine bases. There are two nucleic acid transcriptions RNA to DNA and RNA (m-RNA). As a result, polynucleotide sequences are produced which are then translated into amino acid sequences through hydrogen bonding between triplet nucleotide codons of messenger RNA and anticodon triplets of aminoacyl t-RNA. This translation is the expression of the genetic code and yields the proteins which characterize an organism.

During embryogenesis an intensive metabolism takes place which involves mainly breakdown of pre-existing yok reserves and conversion

of these into tissues and organ (Premkumar *et al.*, 1991). In the egg of the Japanese beetle Ludwig and Rothstein (1952) demonstrated a continuous conversion of soluble into non-soluble protein. Also, in the developing egg of the silkworm, structural proteins and soluble proteins vary inversely (Leonardi,1956).Changes in concentration of proteins and carbohydrates in the developing healthy and pebrine infected embryos of tropical Tasar silkworm, *Antheraea mylitta* was studied by Sinha *et al.*(1991).

During embryogenesis it seems reasonable that most of the free amino acids in the egg are formed through degradation of yolk proteins and that they are incorporated into the proteins of the growing embryo. Synthesis of amino acids however been detected in insect eggs (Bunde and Pepper, 1968; Wegener *et al.*, 1971). Thus, the level of free amino acids reflects the balance between yolk proteolysis, protein synthesis, amino acid synthesis, and possibly amino acid degradation. A higher concentration of amino acids during diapause has been found in orthopteran eggs (McFarland and Hogan, 1966; Bunde and Pepper, 1968; Roberts and Smith, 1971). Glutamine and glutamic acid vary inversely in some eggs, which indicates interconversion of these compounds (Vonder Grone-Gloor, 1959; Colombo *et al.*, 1962). The changes in tyrosin are correlated to cuticle formation (Colombo *et al.*, 1962). Changes in other individual amino acids may be correlated to special development events such as the initiation or termination of diapause and blastokinesis (McFarland and Hogan, 1966; Roberts and smith, 1971). Kunkel and Pan (1976) studied the selectivity of yolk protein uptake : a comparison of vitellogenins of two insects. Elliot and Gillott (1979) studied an electrophoretic study of proteins of the ovary, fat body and haemolymph in the migratory grasshopper, *Melanoplus sanguinipes*. Changes in vitellin and other yolk proteins during embryonic development in the

Bombyx mori was studied by Irie and Yamashita (1980). Oliveira *et al.*(1986) studied the uptake of yolk proteins in *Rhodnius prolixux.*

Tysell and Buterwarth (1978) studied different rate of protein granule formation in the larval fat body of *Drosophila melanogaster.* Changes in haemolymph proteins of the fall armyworm, *Spodoptera frugiperda* associated with parasitism by the braconid parasitoid ,*Cotesia marginiventris* (Cresson) was studied by Ferkovich *et al.*(1983). Tojo *et al.*(1985) studied the hormonal regulation of phase polymorphism and storage protein fluctuation in the common cutworm, *Spodoptera litura.* Jindra and Sehnal (1989) worked on larval growth, food consumption and utilization of dietary protein and energy in *Galleria mellonella.* Sinha *et al.*(1991) studied the changes in protein content in the haemolymph of healthy and pebrine infected larvae and pupae of *Antheraea mylitta D.*

The increase in structural proteins are related to the respiratory increase. An intense formation of muscle mitochondria was observed to occur in parallel (Agrell, 1952) as was also an incorporation of protein into the muscle mitochondria at the expense of soluble proteins (Lennie and Birt, 1967). Therefore, this later part of the metamorphosis should represent differentiation during which period the efficiency of the respiratory system increases. The electrophoretic pattern of pupal proteins in *Calliphora* as well as the behavior of different protein fractions indicates a possible splitting and subsequent synthesis in time with histolysis and histogenesis (Agrell, 1952). A peptide fraction shows a marked parallelism to the morphological changes (Agrell, 1961). New fractions of soluble proteins may arrive during metamorphosis (Stamm, 1962; Laufer, 1963; Patel, 1971). A specific migration of haemolymph proteins into the tissues of the butterfly prepupa has been observed (Loughton and West 1965; Chipendale, 1970; 1971). There may be a rapid turnover from larval to imaginal protein (Agrell, 1952). Tracer

studies on butterfly pupae indicate a high incorporation of amino acids in the imaginal tissues (Skinner, 1960). An analysis of the qualitative and quantitative variations of the basic proteins during metemorphosis of *Calliphora* indicated most of the basic proteins should be associated with ribosomal RNA (Agrell and Lindh, 1966) .Conversely polyamines seem to be preferentially associated to the nuclear RNA in *Calliphora* pupae (Heby, 1972). De. Bianchi and Terra (1976) investigated the haemolymph protein patterns during the spinning stage and metamorphosis of *Rhynchosciara americana.* Effect of protein synthesis inhibitors on muscle growth in the puparium of *Calliphora vomitoria* studied by Haulihan and Neutan (1978). Sinha, *et al.*(1991) worked on changes in protein content in the haemolymph of healthy and pebrine infected larvae and pupae of *Antheraea mylitta D.* Verma and Nath (1991) studied the changes in haemolymph protein picture during late larval pharate pupal stages of *Spodoptera litura.*

There is a decrease in the volume of haemolymph during adult development suggesting that the haemolymph proteins serve as an important source for protein synthesis of the adult organ (Chen, 1966). Proteins in relation to prothoracic hormonal activity have been studied by Williams (1951). According to them, the hormonal activity increases with simultaneous decrease in protein concentration. As the adult develops concentration of haemolymph protein decreases during adult life (Kim and Seo, 1981). The sex specific differences in composition of free amino acids was also observed in *Culex* species (Geigar 1961) and in *Aedes* species (Thayer and Terzian, 1970). The rates of turnover and incorporation of the amino acids into proteins has been reported for many insects like *T. molitor* (Ludwing and Jones, 1964), *D. subobscura* (Clarke and Maynard smith, 1966;), *D. Melanogaster* (Harrison and Holliday, 1967; Bauman 1969), *A. Aegypti* (Thayer and Terzian, 1970) and *P.*

regaina (Levenbook and Krishna, 1971). Hayes *et al.*(1970) worked on electrophoretic patterns of proteins in haemolymph from the adult cockroach, *Leucophaea maderae* (F) during a 24-hr. period. Parker (1971) compared the haemolymph proteins in two species of *Leptinotarsa* bettles. Proteins were separted from the haemolymph by using the PAGE. The electrophoretic patterns of haemolymph proteins from *L. decemlineata* and *L. juncta* adults demonstrated qualitative characteristics similar to those of their respective larvae. Effect of aging on amino acid turnover and rate of protein synthesis in the blowfly, *Phormia regina* was studied by Levenbook and Krishna (1971). According to them the rate of protein synthesis in young blowflies is higher than in aged individuals. Ruh and Willis (1974) studied synthesis of blood and cuticular proteins in late pharate adults of the cercopia silkmoth. Elliot and Gillot (1979) worked out organ specific study in the migratory grasshopper, *Melanoplus sanguinipes*. Phillips and Loughton (1982) studied cuticle proteins in adult *Locusta migratoria*.

B) Insect Proteases :

The diet must be reduced in size in order to enable their incorporation via semipermiable membranes in the alimentary epithelium. The enzymes produced are, broadly speaking adapted to the diet of the species. The most abundant enzyme catalyzing the breakdown of the predominant dietary constituent. There is also some experimental evidence that the secretion of digestive enzymes is controlled though neuroendocrine factors from the brain and that food of different composition, fed to a given species, tend to evoke preferentially the secretion of enzymes able to digest them most effectively. Enzymes responsible for the complete hydrolysis of proteins down to amino acids are the proteases. Proteases are secreted only in response to dietary

protein entering the midgut. A number of studies have shown a correlation between the total amount of protein entering the midgut and the amount of protease secreted. Engelmann's study on the roach, *Leucophaea maderae* clearly showed that the controlling factor in protease secretion is the amount of proteins in the gut. Digestion includes the processes whereby the food materials are broken down into smaller molecular forms such as monosaccharide sugars and amino acids which are then absorbed through the wall of the gut (Dadd, 1970). Such changes are catalyzed by the digestive enzymes, which are secreted by the salivary glands and columnar cell of the midgut.

All the digestive enzymes catalyze the hydrolytic cleavage of C-0 bonds. Some are known to catalyze not only hydrolytic removal of a particular group from their substrate, but in addition are also able to transfer such groups to suitable acceptors. The enzymes produced are, broadly speaking adapted to the diet of the species, the most abundant enzyme catalyzing the breakdown of the predominant dietary constituent.

There are two types of proteases Endopeptidases and Exopeptidases. Endopeptidases which catalyse the breakdown of proteins or peptones to polypeptides and which in insects, are almost invariably of the tryptic type acting best in alkaline media. Endopeptidases are defined as proteolytic enzymes clearing internal peptide bonds not adjacent to amino or carboxyl termini and exhibiting various degrees of amino acid specificity. In most cases endopeptidases also catalyse aminolysis and esterolysis.Endopeptidases (Proteinases) are divided into four subclasses on the basis of catalytic mechanism, as shown with specific reagent or effect of pH.

a) Serine proteinases :

Serine proteinases have a serine and histidine in the active site. The presence of serine is recognized by enzyme inactivation in the presence of DFP, PMSF and that of histidine through the use of ketones such as TPCK or TLCK for example Trypsin (EC 3.4.21.4) and Chymotrypsin (EC 3.4.21.1)

b) Cysteine proteinases::

Cysteine proteinases possess a cysteine in the active site and are inhibited by mercurial compounds for example Cathepsin B (EC. 3.4.22.1)

c) Aspartic proteinases: (EC 3.4.23)

Aspartic proteinases have a pH optimum below 5, owing to the involvement of a carboxyl residue in catalysis, which accounts for their inhibition by diazo compounds for example Cathepsin D (EC. 3.4.23.5).

d) Metalloproteinases : (EC. 3.4.24)

Metalloproteinases need a metal ion in the catalytic process and are inhibited by chelating agents such as EDTA. Exopeptidases include enzymes which hydrolyze single amino acids from the N-terminus (aminopeptidases, EC. 3.4.11) or from the C-terminus (Carboxy-peptidases, EC. 3.4.16.8) of the peptide chain and those enzymes specific for dipeptides (dipeptide hydrolases, EC. 3.4.13).

Three types of exopeptidases have been reported from insects the carboxypeptidases and aminopeptidases catalyse attack on the peptide chain at different points while the dipeptidases are responsiable for the breakdown of dipeptides. Endopeptidases appear to occur mainly in the lumen of the gut and exopeptidases in the epithelium, suggesting that

absorption may begin before hydrolysis of the protein. Exopeptidases remove terminal amino acid from either the carboxyl end (Carboxypeptidases) or from the amino end (minopeptidases) and usually, but not always exhibit a broad specificity for terminal amino acids.

During the last decade there have been substantial advances in our understanding of yolk protein biosynthesis and its hormonal control; however, despite the importance of the process the regulatory mechanism of its degradation still remains to be solved (Yamashita and Indrasith, 1988). Since, in these process proteins are of primary importance, several proteins have been found in arthropod eggs and some of them have been purified and characterized. It is now evident that proteolytic reactions play a key role not only in the regulation of intracellular protein turnover, but also in the control of many other physiological functions such as translocation and maturation of protein, fertilization, germination, oncogenic transformation and other morphogenetic process (Bond and Butler, 1987). Ribolla and De Bianchi (1995) studied the process of procathepsin from *Musca domestica* eggs. They proposed that in *Musca domestica* cathepsin zymogen activation occur in two steps. First an intramolecular cleavage of the procathepsin polypeptide which then undergoes autolysis to produce the mature enzyme. Cathepsin B like proteinase in *Drosophila* and its role in yolk degradation was invesgated by Medina *et al.*(1988). The results altogether suggest that the cathepsin B like proteinase is implicated in yolk degradation in *Drosophila*. Medina and Vallejo (1989) studied the aspartic proteinase in *Drosophila*. The proteinase is maximally active at PH 3.5 and has been characterized by its sensitivity to specific inhibitors and by the specificity of cleavage. The proteinase is detected in mature oocytes and remains essentially constant during embryogenesis. This suggests that the *Drosophila* aspartic

proteinase function mainly before embryogenesis. The sub cellular localization changes from the yolk granules, in oocytes, to the soluble fraction, in late embryos. The proteinase cleaves by basic residues, but arginine is prefored over lysine. Medina and Vallejo (1989) studied on the maternal origin of acid hydrolases in *Drospohila* and their relation with yolk degradation. The acid hydrolases are of maternal origin suggests that they have a role during embryogenesis and are involved in yolk degradation. The identification of yolk platelet associated hydrolases in the oocytes of *Rhodnius prolixus* was studied by Nussenzeig *et al.*(1992). They suggest that the yolk platelet from *Rhodnius prolixus*, a blood-sucking bug, are composed mostly of vitellin and it contain at least two hydrolytic enzymes, a phosphtase and Cathepsin D like proteinase. Both the proteinase and the phosphtase have an acid pH optimum. The proteinase appears to be bound to the yolk platelet membranes. Purification and characterization of a cysteine proteinase from the eggs of the cotton boll worm, *Helicoverpa armigera* was studied by Fan *et al.*(1998). High proteolytic activities were detected from oocytes of the cotton bollworm, *Helicoverpa armigera* at pH 3 to 4. The authors suggested that a cysteine proteinase might exist in the oocytes and serine residue is also necessary for its activation.

The ingested protein must be first broken down into amino acids before being absorbed. The site for both digestion and absorption is the midgut. The total activity of proteases increases rapidly during the first three days of intense larval growth and then falls off during later development. With the onset of pupation it declines to a very low level, which persists throughout the pupal stage (Chen, 1978). Food digestion may occur outside the midgut, for example, in the crop or foregut. Digestive enzymes in this case either come from the salivary gland or are passed forward from the midgut, since there is neither secretion of

enzymes nor absorption in the crop and foregut. There is very little evidence for the production in the salivary gland of proteases used for digestion of food. Price (1974) investigated an acid protease in blowfly (*Calliphora erythrocephala*) salivary glands and found maximal activity when larvea normally cease to feed. In this case the protease seems to leave the glands internally rather than being ejected in the saliva. Proteases are sometimes detected in feces (Engelmann, 1969) and probably represent stable enzymes that survive digestion and are excreted. Purification and characterization of trypsin like proteinase from midget of larva hornet, *Vespa orientalis* studied by Hagenmaier (1971). He reported that, hornet protease is homologous with the other serine proteases. According to experimental results the pH profile, temperature activity, the mode of action as shown by inhibitors and the cleavage specificity on B-chain of insulin are all quiet similar to mammalian trypsin. Ahmad *et al.*(1976) studied the alkaline protease in the larvea of the armyworm, *Spodoptera litura*. He reported that the alkaline protease activity in the gut of *Spedoptera litura* was found to increase with the onset of pupation. Eguchi and Iwamoto (1976) worked on alkaline proteases in the midget tissue and digestive fluid of silkworm, *Bombyx mori*. According to them, the proteolytic enzymes in the alimentary canal in *Bombyx mori* are predominantly localized in the gut contents. These proteases, however, are somewhat different in thermostability and in other properties of enzymes. The bound form of tissue proteases may be a source of digestive fluid protease. Proteolytic activity in the digestive fluid of larvae of *Trichoplusia ni* was investigated by Pritchett *et al.*(1981). They observed both tryptic and chymotryptic activities. Baker (1976) studied the properties of midgut proteases in larvae of *Attagenus megatoma*. The midgut protease activity exhibited high temperature and alkaline pH optima. The total protease levels declined in starved larvae

but increased after 48 hr of feeding. Characterization of an acidic proteinase from the posterior midget of *Rhodnius prolixus stal* was carried out by Houseman and Downe (1982). They reported that the *Rhodnius prolixus stal* contained Cathepsin D in the posterior midgut to breakdown ingested blood proteins. The presence of Cathepsin B and lysosomal carboxypeptidase B that have also been detecd in the postorior midgut of *R. prolixus* and other blood sucking Hemiptera. Christeller *et al.*(1989) worked on partial purification and characterization of the major midgut proteases of grass grub larvea, *Costelytra zealandica.* They reported that trypsin can be considered the major target in attempts to interfere with protein digestion in grass grub larva. Meenakshisundaram and Gujar (1998) worked on alkaline proteases from some Lepidopteran larvea. It is evident from the present work that the test insects possess alkaline proteases in the midgut region which are almost having similar properties with respect to the optimum conditions of pH, temperature, time and differing in their substrate and inhibitor specificity which probably make the insects to thrive on certain selected host plant utilizing different plant proteins qualitatively and survival of herbivour insects.

During transformation into the pupa and throughout the pupal and pharate adult instars insects are particularly vulnerable. Since at these periods they are provided with very limited powers of movement and defense, special methods of protection are necessary. The transformation of the larva to imago represents varied degree of histolysis of larval tissues and a corresponding degree of histogenesis of the imaginal organs.

Yano *et al.*(1995) studied the regulation of the expression of Cathepsin B in *Sarcophaga peregrina* (Fleshly) at the translation level during metamorphosis. In this study authors demonstrated that the amount of Cathepsin B in haemocytes was controlled at the translational level i.e. larval haemocytes stored a significant amount of untranslated

Cathepsin B mRNA. Yang and Davies (1971) studied Trypsin and Chymotrypsin during metamorphosis in *Aedes aegypti*. Protease activity and cell death during metamorphosis in the salivary gland of the *Chironomous tentans* was studied by Henrikson and Clever (1972). Homma (1997) studied proteases participating in the metamorphosis of flesh fly.

The development of holometabolous insects via metamorphosis permits a unique examination of the ontogeny of digestive enzymes from larval stages to adult. Differences in the array of digestive enzymes are anticipated and have been described in insects that inhabit totally different ecological niches as larvae and as adults. Gooding and Huang (1969) studied trypsin and Chymotrypsin from the beetle, *Pterostichus melanrius*. These proteinases from the digestive tract have an optimal activity near pH 8 and temperature 47^0C when denatured haemoglobin is used as a substrate. The two proteinases occur in about equal concentrations in both males and females. Muraleedharan and Prabhu (1978) worked on food intake and midgut protease activity in the red cotton bug, *Dysdercus cingulatus fabr*. Martin and Kukor (1981) studied the digestive enzymes of detritus feeding stonefly nymphs (*Plecoptera-ptronarcyidae*). Partial purification and characterization of cysteine proteinase from metamorphosing tadpole tails of *Rana catesbeiana* was studied by Fujita *et al.*(1989). Partial purification and characterization o the major midgut proteases of grassgrub larvae (*Costelytra zealandica*, Coleoptera-Scarabaidae) was studied by Christeller *et al.*(1989). Pan *et al.*(1991) worked on the changes in composition and proteolytic enzyme activities of *Artemia* during early development. Ferreira *et al.*(1994) worked on the propertis of the digestive enzymes and the permiability of the peritrophic membrane of *Spodoptera frugiperda* (Lepidoptera) larvae. Protease mediated prophenoloxide activation in the haemolymph of

American cockroach, *Periplaneta amaricana* was studied by Thangaraj *et al.*(1995). Regulation of digestive enzyme levels in insects was studied by Lehane *et al.*(1995). Moffatt *et al.* (1995) studied the synthesis and secretion of trypsin in the midgut of *Stomoxys calcitrans*. Purification and characterization of gut alkaline proteases from some Lepidopteran larvae was studied by Meenakshisundaram and Gujar (1998). Blackmore *et al* (1995) studied the protein stimulation of trypsin secretion from the opaque zone midgut cells of *Stomoxys calcitrans*. Rosenfield, (1998) identified proteases from midgut and haemolymph of adult *Anopheles stephensi* mosquitoes. The peak midgut protease activity is shown by the fifth day old insect, which also consumes the maximum amount of food. It appears the proteins in the food stimulate midgut protease activity in this insect.

5. <u>Work on Protein and Proteases of Armyworm :</u>

Proteins provide the chief structural elements o the muscles, glands and other tissues (Wigglesworth, 1972). Clearwater (1972) studied the chemistry and function of a pheromone produced by the male of the southern armyworm. He stated that pheromone released by armyworm is benzaldehyde which functions as on arrestant, facilitating mating by preventing the escape of the female. Clearwater and Sarafis (1973) studied the secretory cycle of a gland involved in pheromone production in armyworm and suggested that the secretory cycle of this gland consists of three phases Cusson *et al.* (1990) studied *invitro* biosynthesis of juvenile hormone by corpora allata of armyworm *Pseudaletia unipuncta* virgin females as a function of age, environmental conditions, calling behaviour and ovarian development. They also studied the ovarian

development in female armyworm, *P. unipuncta* and its relationship with pheromone release activities.

Enzymes responsible for the complete hydrolysis of the proteins down to the amino acids are the proteases. Zhi-qing *et al.* (2008) studied the effect of terpinen 4- 01 on the phosphatase, glutathione s- transferase (GSTs), Cytochrome P450 and polyphenol oxidase activity of 5^{th} instar larvae of *M. separata.*

6. Aim of the Present Work :

A number of workers have investigated various aspects of *M. separata,* mainly efficacy of different insecticides for the control of armyworm, nuclear polyhedrosis virus in armyworm, biology of armyworm under laboratory and field conditions etc., but the biochemical and physiogical aspects of this animal are yet to be studied. There exists a lacuna in the field of biochemistry and physiology of this animal. The proteins and proteases enzymes are very important in insect which are mainly concerned with supply of structural components during development. However, the information on the proteins and proteases activity during the development of armyworm, *M. separata* is rather scanty. Therefore in the present study attempts have been made to provide information on proteins and proteases activity during embryogenesis, larval growth, metamorphosis and adult development of *M. separata.* The results are discussed with regard to the changes undergone during embryogenesis, larval growth, metamorphosis and adult development of *M.separata.*

7. Plan of Proposed Work :

1. To develop sensitive assay for demonstrating presence of proteins and proteases in *M. separata* that hydrolyse the proteins.
2. To study the effect of various factors such as pH, temperature, incubation time, enzyme concentration and substrate concentration, which that modulate the enzyme activity.
3. To study the alteration in protein during embryogenesis, larval growth, metamorphosis and adult development of *M. separata*.
4. To study the alteration in the proteases activity during embryo genesis, larval growth, metamorphosis and adult development of *M. separata.*
5. To provide information on physiological role of proteins and proteases during embryogenesis, larval growth metamorphosis, adult development of *M. separata.*