STUDIES ON THE PROTEASE ACTIVITY IN THE DIGESTIVE SYSTEM OF *IPHITA LIMBATA*
(HETEROPTERA : PYRRHOCORIDAE)

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CERTIFICATE

This is to certify that this thesis is an authentic record of work carried out by Mr. K. S. Sajan, under my supervision and guidance in partial fulfilment of the requirements of the Degree of Doctor of Philosophy under the Faculty of Science of the University of Calicut. No part of this thesis has been presented before for any other degree.

Dr. K. V. Lazar

DECLARATION

I do here by declare that this thesis has not previously form the basis for the award of any degree/diploma

K S Sajan

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CONTENTS

Foreword

FOREWORD

With the exception of a few highly specialized species insects feed on a diet of macromolecules, many of which do not penetrate the gut wall. In order that these molecules may be used by the tissues, they must be reduced to an absorbable form by the process of digestion and the digestive products then cross the gut wall and are distributed to the tissues. The associated processes of digestion and absorption in insects have been reviewed by Day and Waterhouse (1953), Treherne (1967), Dadd (1970), House (1973) and Terra (1988). As the digestive enzyme of an insect is adapted to its food, studies on the digestive enzyme become very important and form the basis for the physiological study of insect nutrition. (Dadd, 1970; Gooding, 1972; Wigglesworth, 1972; Prema and Mohamed, 1980; Chapman, 1985; and Abdul Jabbar and Mohamed 1989, Woods and Kingsolver, (1999).

Mostly digestion occurs in the midgut where varieties of enzymes are available in abundance (Engelmann, 1969; Persuad and Davey, 1971; Hori *et al.*, 1981; Noriega *et al.*, 1996). By virtue of salivary gland secretions or the regurgitations of the enzyme from the midgut, digestion commences in the foregut in certain insects. Rare instances of extra intestinal digestion are also reported in some insects (Chapman, 1982). The nature of the enzyme secreted depends mostly on the nature of the food. Herbivorous insects secrete more carbohydrases (Day and Powning, 1949; Wharton *et*

al., 1965; Hori, 1973; Agarwal and Behadur, 1978, 1981). Whereas carnivorous insects secrete predominantly proteases (Gooding, 1975; Billingsley and Hecker, 1991). Day and Waterhouse (1953) stated that the nature of the proteolytic enzymes present in the gut homogenate of insects is in generally similar to that of invertebrate animals.

It is generally suggested that the greater the diversity of the digestive enzymes exhibited by a species, the greater the diversity of food that can be utilised by it (House, 1974). Since practically very kind of natural organic material is eaten by some insect or other it is not unexpected that the list of digestive enzymes found within Insecta is a long one (Gilmour, 1961). Though it is believed in general that the gut of herbivorous insects exhibits the activity of carbohydrate hydrolases and the carnivorous insects produce protein digestive enzymes. Fraenkel (1940) pointed out that the mere presence of an enzyme may not necessarily signify that the organism actually utilises the substrates of that enzymes. However, Dadd (1979) is of opinion that enzyme detected and their relative strengths reflect reasonably well the type of food normally consumed. Therefore it is planned in the present study to investigate the activity of protease enzyme and the influence of various diets on it in the midgut and hindgut of *Iphita limbata* (Heteroptera: Pyrrhocoridae) a heteropteran plant bug.

Plan of study

The alimentary canal of *I. limbata* is a straight tube with distinct, foregut, midgut and hindgut. The plan of study was to employ the homogenate technique to identify the kinetic properties of the enzyme protease in the gut of *I. limbata*, to quantify its activity and to study the effect of various diets on its activity. The assays were carried on both sexes separately and on the two distinct regions of the alimentary canal, viz., hindgut and midgut. A distinctive feature of the study was the separate assays on the tissue and lumen content of both parts of the gut. The availability of these data would permit a conclusion to be drawn about the secretion and action of the enzyme, the region of maximum activity, to compare the activity of the enzyme with respect to different diet and thereby arrive at the nature of proteolytic enzyme in the digestive physiology of the insect.

Presentation of material

The thesis commences with an introductory chapter. After a brief general account of the process of digestion in animals, especially in insect, the author has reviewed relevant literature on proteases in insects. It introduces the classification of enzyme, their kinetic properties, difference in activity related to different regions of alimentary canal, gut tissue, lumen content, sex, diet etc. The introductory chapter closes with a review

of the role of diet on the control of enzyme secretion and/or the competitive inhibition of digestive enzymes with other food molecules.

The introductory chapter is followed by a chapter on the material and methods employed during the experiments. The chapter which then follows is devoted to the presentation of data of the results obtained, pertaining to the analysis of the protease activity, its kinetic properties, differences related to sex, region of gut, gut tissue and lumen content, diet and control mechanism of enzyme secretion.

This charter is followed by discussion and interpretation of the results obtained.

The thesis ends with bibliography.

INTRODUCTION

K.S. Sajan “Studies on the protease activity in the digestive system of iphita limbata (Heteroptera : Pyrrhocoridae)” Thesis. Department of Zoology , University of Calicut, 2002

INTRODUCTION

Digestive enzymes in insects

As early as 1874 digestive enzymes in insects have been reported (Plateau, 1874). His studies revealed that the cockroach, the saliva is able to digest starch, foregut does not secrete any enzyme and midgut digests proteins and emulsified fat. Since this pioneer study a number of studies on the digestion in insects have been appeared (see reviews, Waterhouse, 1957; Gilbert, 1967; Wyatt, 1967; Dadd, 1970; Wigglesworth, 1972; Hori, 1973; House, 1974; Chippendale, 1978; Turenen, 1979; Terra, 1988). The present review is focussed in the various aspects of digestion in insects that have immediate bearing on the lines along which the author's study was planned and executed.

It is evident from the above reviews, together with many recent reports, that largely, most of the studies have been limited to the mere detection of the various digestive enzymes, using appropriate substrates. Quantitative data on the enzymes using specific substrates at optimal assay conditions are available only with respect to a few. Besides defining the gross lytic capacity of various digestive organs, gross measurements have been made of the relative secretory activity in relation to feeding, growth and metamorphosis (Dadd, 1970). Studies with purified enzyme preparations are also reported (Dedet *et al.*, 1982; Peaucellier, 1983; Branca *et al.*, 1999; Lam *et al.*, 2000).

The physiological conditions in the alimentary canal and the pattern of digestive enzymes show great variation among insects and both these depend mainly upon the food and feeding habits of the individuals. In plant-sucking bugs, the salivary enzymes are prominent and they have been studied in a large number of species (Hori, 1975; Colebatch *et al.*, 2002). Among coccinellids tested, Sakurai (1968) found that the activity of protease, lipase and trehalase is higher in entomophagous individuals than in phytophagous ones and this tendency may be correlated to their food habits. The digestive enzyme complement will vary with their taxonomic position also. It is generally believed that the greater the diversity of the digestive enzymes exhibited by a species, the greater the diversity of food that can be utilized by it (House 1974). Since practically every kind of natural organic material is eaten by some insect or the other, it is not unexpected that the list of digestive enzymes found within insects is a long one (Gilmour, 1961). House (1974) concluded that an insect is adequately equipped with the digestive enzymes needed to digest the components of its natural food material. Insects do indeed possess a wide complement of enzymes capable of lysing the main classes of nutrients in their diverse foods. Since these major nutrients are complex carbohydrates, proteins and lipids, the major digestive enzymes in insects comprise of the respective hydrolases – carbohydrases, proteases and lipases. Although Fraenkel (1940) has pointed out that the mere presence of an enzyme may

not necessarily signify that the organism actually utilizes the substrate of that enzyme detected and their relative strengths reflect reasonably well the type of food normally consumed (Dadd, 1970). Thus, carnivorous insects generally have predominantly proteolytic enzymes and deficient in amylases and saccharases, where as omnivorous and herbivorous ones usually possess powerful and varied carbohydrases. Insects possessing limited and specific diets usually have a narrow complement of digestive enzymes. For instance, nectar feeding lepidopteran adults possess a predominant sucrase and the cloth moth larvae possess a keratinase, which helps in the digestion of keratin present in their diet. However, a few anomalies for the complementarity between food and digestive enzymes exist among insects. The digestion of cellulose in termites, starch in aphids and wax in wax moth by intestinal bacteria are considered as anomalies because the individuals themselves are incapable of elaborating the respective enzymes. However, because of the fact that the animals provide the essential conditions by harbouring the bacteria within their alimentary canal, this may be considered as a complementarities rather than an anomaly.

In general the digestive enzymes such as amylase, maltase, invertase, tryptase, peptidase and lipases are commonly found in the salivary secretions and regions of the digestive tract of insect (Abbot, 1926; Hobson, 1931; Feltcher and Haub, 1933; Ballentane, 1940; Day and

Powning, 1949; Saxena, 1954 a, b, 1955, 1958; Eisner, 1955; Ahmad *et al.*, 1976, 1980; Prema and Mohamed, 1980; Houseman and Downe, 1982, a, b; Shukle *et al.*, 1985; Wieman and Nielsen, 1988; Abdul Jabbar and Mohamed, 1989; Christeller *et al.*, 1990; Bauman, 1990; Lim *et al.*, 1991; Gillikin *et al.*, 1992; Milne and Kaplan, 1993; Gotz *et al.*, 1993; Xu *et al.*, 1994; Mc Chie *et al.*, 1995; Nagaraju and Abraham, 1995; Usian *et al.*, 1995; Cheng *et al.*, 1996; Noriega *et al.*, 1996; Oppert *et al.*, 2002).

Digestive enzyme in phytophagous insects

Several studies on digestive enzymes have been carried out in phytophagous insects based on the hydrolysis of various substrates by whole homogenates of the gut tissues, including their lumen content. These qualitative tests are indicative only of the presence of the various enzymes in the particular system. The mere detection of the various enzymes does not provide a complete picture of the functional pattern of enzyme activity in an individual. A comparison of the potential activities of the different enzymes in a given insect is possible only from the comparative data on total and specific activities as determined *in vitro* in optimal assay systems. Studies of this kind among various phytophagous insects have been reviewed by Waterhouse (1957), Dadd (1970), Wigglesworth (1972), House (1974), Chippendale (1978), Foissac *et al.*, (2002).

Distribution of digestive enzymes along the alimentary system

The various components in the food are broken down by the hydrolysis catalysed by the respective enzymes in the regions where they are sufficiently active. It is often found that some of the complex components of the food have to be subjected to the successive action of a series of enzymes before they are transformed into products suitable for absorption by the epithelium. Among continuous feeders, the midgut is found to be the major site of digestion. The discontinuous feeders are characterized by a storage organ, which may be either a modified crop, or the proximal part of the ventriculus (Wigglesworth, 1972). Though the midgut is the main site of digestion, reports are available among insects where the preliminary or perhaps more complete digestion occurs in the crop (Wigglesworth, 1972; House, 1974). In some biting flies, blood directly passes into the midgut, which then functions both as a storage organ and digestion site (Dadd, 1970). Matsumoto *et al.*, (1997) studied distribution in various tissues including alimentary tract and germ cells in *Sitophilus zeamais* (maize weevil). In alimentary organs, cathepsin L-like cysteine proteinases are distributed in the gastric caeca, but not in the midgut. It is also present in genital organs, especially in oocytes and nurse cells, where it exists at high levels. These results indicate that it plays a variety of physiological roles including a role in food digestion. Compartmentalization of proteinases, amylases and pH in the midgut of *Nauphoeta cinerea*

(Blattoptera : Blaberidae) was studied by Elpidina *et al.*, (2001) in order to understand the organization of protein and starch digestion. Total proteolytic activity measured with azocasein was maximal at pH 11.5 both in anterior (AM) and posterior (PM) halves of the midgut, but the bulk of activity (67%) was found in PM. Total AM and PM preparations were fractionated on a Sephadex G-50 column and further analysed by means of activity electrophoresis and specific inhibitors and activators. The major activity in PM was classified as an unusual SH-dependent proteinase with M (r) 24 kDa; pH optimum with synthetic substrate BApNA at 10.0. The enzyme was 43-fold activated in the presence of 1 mM dithiothreitol, insensitive to synthetic inhibitors of serine and cysteine proteinases, strongly inhibited by STI and displayed four active bands on zymograms. In PM, activities of trypsin-like, chymotrypsin-like, subtilisin-like and cysteine proteinases were observed. Aspartic and metalloproteinases were not detected. In AM, activity of unusual SH-dependent proteinase also dominated and activity of chymotrypsin-like proteinase was observed, but their levels were much lower than in PM. Distribution of amylase activity, exhibiting an optimum at pH 6.0, was quite the opposite. The major part of it (67%) was located in AM. Treatment of amylase preparation with proteinases from AM and PM reduced amylase activity two fold. Enzyme activity is observed in the midgut of different insects. (Agarwal, 1981 a, b; Brey *et al.*, 1995; Algimantas *et al.*, 1999; Winnie Lam *et al.*, 1999; Silva *et*

al., 2001). Digestive enzymatic activities were also observed in the hindgut region of the insects (Nachman *et al.*, 1996; Wijffels *et al.*, 1997; Gontijo *et al.*, 1998).

The simple detection of a particular enzyme in various regions of the insect gut does not provide complete knowledge about its digestive capacity on a particular substrate in these regions. Since the degree of digestion of a substrate is correlated with the strength of the particular enzyme activity, quantitative study of the enzyme is essential to arrive at the site of digestion of a component in the food. These types of studies on the distribution of digestive enzymes in the alimentary canal were carried out in a number of insects (Agarwal, 1981; Brey *et al.*, 1995).

Enzyme activity manifested by the gut tissue and lumen content

Enzyme activity as determined in whole homogenate of a given region of the gut is a net value made up of two components: enzyme secreted into the lumen or otherwise present in the particular region of lumen and enzyme confined to the epithelial cells of the gut wall. Enzymes secreted into the lumen are those actually synthesized in the cells bordering the lumen, or those elaborated in glands and secreted through ducts opening into the lumen. An enzyme elaborated in the epithelial cells and passed into the lumen may have residual activity in the cells. As such, the enzyme activity determined in the homogenates separately of the tissue represents the activity of those enzymes, which are strictly intracellular

and those enzymes, which are predominantly secreted outside, but whose residual activity, or even a zymogen form activated under the *in vitro* conditions of the assay, is present intracellularly. Similarly, an enzyme detected in a particular region of the lumen may not always have originated in that region; it might have been secreted into a preceding part of the gut and passed along with food. In *Glossina morsitans morsitans* the levels of trypsin and chymotrypsin activities in the gut lumen increase, following blood feeding and change significantly in the gut cells throughout the digestion cycle. (Yan *et al.*, 2001). In *Phlebotomus papatasi* aminopeptidase activity was associated mainly with the midgut wall, whereas trypsin activity was confined to the midgut lumen (Dillon and Lane., 1993). The salivary enzymes are active in the foregut of a number of species (Swingle, 1925; Champlain and Fisk, 1956; Dadd, 1970; Wigglesworth, 1972), but in others, these are more active in the midgut (Saxena, 1954 a; 1963; Khatoon, 1967; Chapman, 1973; Takanona and Hori, 1974). It has also been reported that enzymes originating in the midgut are regurgitated into the crop for digestion in *Blatella germanica* and *Periplaneta americana* (Day and Powning, 1949; Eisner, 1955) and in *Locusta migratoria* (Khan, 1963).

Carbohydrates are absorbed as oligosaccharides in addition to monosaccharides (Dadd, 1970); accordingly, several oligosaccharidases are recorded to be present in the gut wall. At the same time, many

polysaccharidases essentially requiring high pH are more active in the lumen than in the epithelium of the gut (Khan, 1963; Applebaum *et al.*, 1964 a; Ishaaya *et al.*, 1971; Nishide and Kusano, 1976; Terra *et al.*, 1979). However, vary limited information is available on the enzyme activity of the gut epithelium and content separately except in the studies conducted in *B. orientalis* (Swingle, 1925), *Bombyx mori* (Horie, 1959), *Chilo zonellus* (Pant *et al.*, 1959), *Peries rapae* (Nishide and Kusano, 1976) *Rhynchosciara americana* (Terra *et al.*, 1979) *Helicoverpa zea* (Johnson and Felton 2000) *G. morsitans morsitans* (Yan *et al.*, 2001) and *Opisina arenosella* (Harshini *et al.*, 2002).

Enzyme activity in lumen content

A likely source of enzyme activity in the lumen content, which does not seem to have received adequate attention of workers in the field, is of feed material origin. All raw food naturally contains the proper types and proportion of enzymes necessary to digest it (Beazel, 1941; Murray *et al.*, 1990; O'Keef *et al.*, 1991). In phytophagous larva/insect, particularly the continuous feeders, which feed voraciously and in which the feed material stays in the gut for a few hours only, there is every possibility that the enzymes present in the plant tissue will be released on cell damage, during the duration in the buccal cavity and /or in the proventriculus and continuously act on the endogenous substrates. Applebaum *et al.*, (1964 a) were seized of this possibility and tested for amylase activity in the feed

material (cotton leaves) used by *Prodenia litura.* The midgut lumen of this insect is highly alkaline. Tested under the condition of assay of the insect amylase, that is, at the pH of 9.5, the authors could not detect amylase activity in the cotton leaves and concluded that amylase of the leaf origin was not operative in the digestion of starch by *Prodenia* and that amylolysis in the larva was effected solely by enzyme elaborated in its tissues. In *Tenebrio molitor* when the insect was fed on wheat, the ∝-amylase was inhibited and digestion was mainly dependant on the β-amylase present in the wheat (Applebaum, 1961, 1964 b). Yang and Davies (1968 a) found that in blood-fed *Aedes aegypti* the amylase resulted mainly from the ingested blood rather than from midgut secretion. It is possible that this exogenous activity, originating from the feed material may have physiological significance in the nutrition of the phytophagous insect and may operate synergistically with the tissue enzyme of the animal. During assays *in vitro* with added substrate, the transformation products of a particular enzyme will be those due to the activity of the enzyme in the plant tissue and the enzyme of insect origin. Ground plant tissues have generally a slightly acidic pH, but some enzyme activity could be expected even at the alkaline pH characteristic of the lumen content of lepidopterous larvae.

Diurnal alteration in the activity of enzymes

Another aspect which has not received due attention is a possible diurnal alteration in the activity of enzymes. It was already pointed out that the activity of insect enzymes may depend upon, or be influenced by, the composition of the diet (Wigglesworth, 1972); also, the enzyme activity in the alimentary system of the starved animal shows a marked change in few hours following feeding (Hori, 1973). The components of the leaves, particularly the type of carbohydrates, can vary markedly diurnally. Leaf starch, elaborated and accumulated during photosynthesis, is broken down in the dark and sucrose content increases. In case the digestive enzyme is subject to some degree of induction by the substrate, one should expect to find a diurnal change in the enzyme activity of gut tissue and salivary gland and therefore of lumen content also. In the case of starch digestion, the larvae may be comparatively starved in relation to starch during certain hours of the dark period. The changes in the enzymatic activity related to time of blood feeding is studied by Klowden (1990). Simpson and Bernays (1983) reviewed the dietary rhythm of locusts.

Influence of hydrogen ion concentration in the environment

The hydrogen ion concentration in the gut constitutes one of the most important physiological factors for an enzyme to manifest its optimal activity. According to Waterhouse (1949), the pH in the alimentary canal varies with the systematic position in which a particular insect belongs;

thus, high midgut alkalinity is characteristic of lepidopterous larvae. The pH in the gut lumen is much higher than that of the gut wall in these insects. This wide variation in pH influences the major sites of digestive reactions. Therefore, certain enzymes, which require high pH optima, will be more active in the lumen and others with their low optimal pH tend to operate better at the lower pH prevailing intracellularly in the epithelium (Horie 1959; Wyatt, 1967). It is found that the pH optima using casein as substrate were about pH 6.8 for the rice weevil and pH 5.2 for the red flour beetle (Liang *et al.*, 1991).

Blood meal digestion in the midgut of *P. papatasi* and *Phlebotomus langeroni* was studied by Dillon and Lane (1993) and found that the optimal activity occurred at pH 8-9 for all enzymes examined in both species. In *N. cinerea* the pH of the midgut contents was 6.0-7.2 in anterior midgut, 6.4-7.6 in the first and 8.8-9.3 in the second halves of posterior midgut. Thus, pH in anterior midgut is in good agreement with the optimal pH of amylase, located in this compartment, but the activity of proteinases, including the ability to degrade amylase, in such an environment is low. Active proteolysis takes place in the second half of posterior midgut, where pH of the gut is close to the optimal pH of proteinases (Elpidinia *et al.*, 2001).

A trypsin-like enzyme purified by Milne and Kaplan (1993) from the spruce budworm *Choristoneura fumiferana* gut juice has a molecular mass

of 25 kDa and its pH activity profile indicates a pKa of 8.0. The effect of pH on the digestive enzymes were done by many workers with different type of insects (Peterson *et al.*, 1995; Blanco-Labra *et al.*, 1996; Parker and Roberts, 1996; Gontijo *et al.*, 1998; Regel *et al.*, 1998; Smartt *et al.*, 1998; Zhu and Baker, 1999; Zhu and Baker, 2000; Schernthaner *et al.*, 2002; Nogueira de Melo *et al.*, 2001). Some of these studies on hemipteran insects show that they have an optimum pH in acidic range (Colebatch *et al.*, 2001).

***In vivo* applicability of *in vitro* findings**

The biochemical observations on the individual enzymes by using synthetic substrates and provision of artificial conditions, may not furnish a true picture of the digestive physiology in the intact animal, since *in vitro* hydrolysis may not have total bearing on the situation *in vivo*. This is well illustrated in the case of an enzyme with acidic pH optimum, which occurs in both gut epithelium and lumen content. Its activity as determined *in vitro* in the lumen content will not have a real bearing on the physiology of the animal, since this enzyme would be much less active under the alkaline conditions prevailing in the lumen. Similarly, an enzyme with pronounced alkaline pH optimum will not be exerting this optimal action under the slightly acidic conditions prevailing in the tissue; its activity as determined *in vitro* will not be truly representative of the *in vivo* activity. This limitation will have to be borne in mind when separate assays are

carried out on the tissue and the lumen contents of gut. It has also to be remembered that *in vitro* the products of the enzymatic reaction accumulate and tend to inhibit the forward reaction, whereas *in vivo* the products of digestion are constantly being absorbed and do not generally get a chance of accumulate. For this reason, the assay conditions should be so adjusted, especially with respect to the period of incubation, that what is attempted to be measured is the initial rate of the particular reaction.

Relevance of the enzyme kinetics

Characterization in respect of specificity of action and optimal assay conditions has been attempted only for a few digestive enzymes in insects. Comparisons of activity among enzymes and in different regions of the alimentary system are meaningful only if the different kinetic properties are charted. An enzyme is characterized based on a number of biochemical parameters. Lin and Richards (1956) pointed out that comparisons of various enzymes merely based on the requirements for the expression of the activities and substrate specificities do not prove the identity of an enzyme in different insects, but may perhaps do so, if a number of additional properties are also similar. A β-glycosidase isolated from *L. migratoria* was found to break down both β-glucoside and β-galactoside; the similarity of various kinetic characteristics in the action on β-glucoside and β-galactoside suggested that one and the same enzyme was acting on

both the substrates (Morgan, 1975 a). Kinetic properties are used to compare enzymes by Moon *et al.*, (1997) in *Plasmodium falciparum*. The hydrolysis of a substrate by different tissue material need not always be by the same enzyme if there are variations in the different profiles and kinetic data. There are reports among insects where the hydrolysis of the same substrate shows different optimal conditions and kinetic parameters when preparations are made from different regions of the digestive system. The amylase from *B. mori* possesses an optimum pH of 9.2 – 9.5 in the digestive fluid (Hori, 1959; Ito *et al.*, 1962), 8.0 – 11.3 in the gut tissue (Mori, 1930; Shinoda, 1930 b; Kuroda, 1953) and 6.6 – 7.1 in the salivary gland (Matsumura, 1930). Invertase showed optimum pH of 7.0 and 6.0 respectively in the midgut brei and hindgut brei of *Sericesthis geminata* (Soo Hoo and Dudzinski, 1967). The optimum pH is 4.7 for the midgut invertase and 6.0 – 7.0 for the salivary invertase of *Oncopeltus fasciatus* (Bongers, 1970). Trehalase from the salivary glands and midgut of *Sesamia inferens* showed optimum activity at 50°C and 60°C respectively. The trehalase concentration at which maximum hydrolysis took place was different for the salivary and midgut enzyme preparations of the same insect (Agarwal, 1976 a, b).

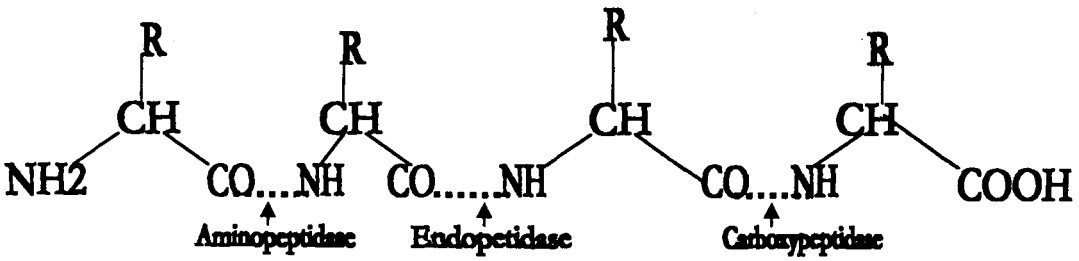
Nature of digestive enzymes

Since digestion is a biochemical process whereby complex organic molecules such as polysaccharides, proteins and lipids are broken down to

their simpler components such as monosaccharides, amino acids and fatty acids and glycerol, the enzymes taking part in this process are of hydrolytic type. Here the literature pertaining to peptide-hydrolysing enzymes was reviewed.

Peptide hydrolases

The digestive peptide hydrolases fall into two groups, the first of which is mainly concerned with the degradation of large molecules of food protein to yield smaller fragments and the second group completing the process initiated by the first group and leading eventually to the liberation of amino acids. The former group is called endopeptidase (proteinases) since they attack the interior peptide bonds of a protein molecule. Thus, endopeptidase will be loosely defined as proteolytic enzymes cleaving internal peptide bonds not adjacent to amino or carboxy termini and exhibiting various degrees of amino acid specificity, in most cases endopeptidases also catalyze amidolysis and esterolysis. The smaller peptides formed, are acted upon by the second group of peptide hydrolases, the exopeptidases, di-and /or tripeptidases to give rise to individual amino acids. Exopeptidases remove terminal amino acids either from the carboxy end (Carboxypeptidases) or from the amino end (aminopeptidases) and usually but not always exhibit a broad specificity for terminal amino acids.



The proteinases are divided into sub-subgroups based on the catalytic mechanism, as shown by active centre studies, or the effect of pH. The enzyme, serine-proteinases have an active centre containing serine and histidine. The enzyme, SH-proteinases have a cysteine in the active centre. The enzyme, acid proteinases have a pH optimum below 5.0, due to the involvement of an acidic residue in the catalytic process.

Trypsin

The common proteinases in insects are activating at natural to alkaline pH and these resemble mammalian trypsin. Trypsin acts at peptide linkages involving the carboxyl group of either an arginine or a lysine unit.

The trypsin in insects does not hydrolyse native proteins in the food beyond the protease or polypeptide level (House, 1974). Insect trypsin is not known to exist in a zymogen or precursor form, trypsinogen, nor is enterokinase activity detected in insect tissues. Trypsin was identified in a number of insects (Lara *et al.*, 2000; Konarev *et al.*, 2002).

Chymotrypsin

These enzymes attack the peptide link on the carboxyl side of an aromatic amino acid or leucine present in the protein molecule.

Six types of chymotrypsin c-DNAs were identified in *H. zea* and *Agrotis ipsilon* (Mazumdar-Leighton and Broadway, 2001). Analogous to trypsin, the chymotrypsin in insects is also usually alkaline and a chymotrypsinogen-like protein is reported in the midgut of *Rhyzopertha dominica* (Zhu and Baker 2000). Chymotrypsin is reported in many insects (Gatehouse *et al.*, 1997; Valaitis *et al.*, 1999).

Pepsin

Pepsin resembles chymotrypsin in attacking the peptide link adjacent to aromatic amino acid, but on the amino side of the aromatic amino acid present in the protein molecule.

The presence of pepsin has been reported in a few species of Diptera (Greenberg and Paretsky, 1955; Fraser *et al.*, 1961; Sinha, 1975; Ozkizilcik and Chu 1996). A pepsin precursor, pepsinogen, is not known to occur in insect tissues.

Cathepsins

Cathepsins are intracellular proteinases seen in animal tissues. A number of cathepsins are known, the more important being cathepsin B and cathepsin D. These proteinases are of rare occurrence in insect gut,

except for some isolated reports (Khan, 1964; Gooding, 1969; Houseman and Downe, 1980). The enzyme is reported in *G. morsitans morsitans* (Yan *et al.*, 2002) A cathepsin D was characterized in colorado potato beetle, *Leptinotarsa decemlineata*, (Brunelle *et al.*, 1999). A cathepsin L-like enzyme is expressed in *Drosophila melanogaster* (Tryselius and Hultmark, 1997). Matsumoto *et al.*, (1997) identified and characterized a gene family comprising at least four genes encoding cathepsin L-like cysteine proteinases (SCPs) in *S. zeamais*.

Exopeptidases

The peptidases are divided according to their specificity into those hydrolysing single amino acids from the N-terminus of the peptide chains, those hydrolysing single residues from the C-terminus, those specific for dipeptide substrates and those splitting off dipeptide units from either the N-terminus or the C-terminus.

Carboxypeptidases

Carboxypeptidases catalyses the splitting of the terminal peptide bond at the carboxyl end of the protein or peptide molecules to set free the amino acid at the carboxyl end. Carboxypeptidases have been detected in some Diptera (Sinha, 1976; Yan *et al.*, 2002). The enzyme has been purified and characterized by Gooding and his colleagues from the extracts of blood sucking insects (Gooding *et al.*, 1973). Xiong and Jacobs-Lorena (1995) characterized the putative promoter region of a black fly midgut

carboxypeptidase gene. Carboxypeptidases are also studied in a number of insects (Ramos *et al.*, 1993; Bernasconi, 1994; Stone *et al.*, 1994).

The occurrence of a specific zymogen-procarboxypeptidase is not yet reported in insects unlike in the vertebrates.

Aminopeptidases

Aminopeptidases act in the same pattern as that of carboxypeptidases, but with the difference, that it splits off the amino acid from the amino-terminal of the peptide molecules. The DNAs of two distinct gypsy moth (*Lymantria dispar*) larval gut aminopeptidases, APN1; APN2, were cloned and sequenced by Garner *et al.*, (1999). Aminopeptidase activity was partially characterized by Billingsley (1990) from midguts of *Anopheles stephensi*. The activity of aminopeptidase has also been reported from other insects (Lenz *et al.*, 1991; Francis and Bulla, 1997; Algimantas *et al.*, 1999; Plinio *et al.*, 1999; Johnson and Felton, 2000).

Dipeptidases

The dipeptides, formed during the breakdown of proteins by the action of the proteinases followed by carboxypeptidases and aminopeptidases and those exopeptidases splitting off dipeptidyl units, are hydrolysed to individual amino acids in the gut in the presence of dipeptidases.

The studies on all these different types of protein digesting enzymes are reviewed under the heading proteases.

Proteases

The literatures of proteolytic enzymes in insects have been reviewed by House, 1974; Law *et al.*, 1977; Applebaum, 1985; Chapman, 1985; Terra, 1988; Terra and Ferreira, 1994; Lehane, 1994).

Complex molecules of proteins ingested though the food are broken down in the digestive tract by different proteases. The hydrolysis of proteins commences by splitting of the internal bonds of the long peptides or proteins, irrespective of the molecular weight. These enzymes are the endopeptidases, which include trypsin, chymotrypsin, pepsin and cathepsin. According to Desnulle (1960), all these enzymes are not 'proteinases' specifically attacking large protein molecules, since they are able to split short peptides, provided that one of the residues linked by the bond has a typical side chain and that the terminal groups (amino or carboxyl) are not too close to this bond, or are blocked by some radicals. The products of hydrolysis by the proteinases are acted on by the peptidases.

The frequently occurring insect proteinase, active at neutral to alkaline pH, resembles vertebrate trypsin rather than other proteinases (Wigglesworth, 1972). Pepsin, with its low optimum pH, is generally absent in insects. The pH of insect gut, which is comparatively high, is not suitable for the peptic activity unlike that of vertebrate stomach, which is highly acidic. Trypsin and chymotrypsin are the predominant

endopeptidases found in the insect gut. The author uses the terms 'proteases' to signify the collective group of enzymes acting on proteins and leading to the liberation of the free amino acids. These enzymes comprise of the proteinases and the peptidases and they have mostly their optimum pH in fairly alkaline range (Day and Water house, 1953; Champlain and Fisk, 1956; Wigglesworth, 1972). However, some insects show optimum pH in acidic range. In general the properties of either enzyme determined in different insects were so similar that any differences were of minor character (Powining *et al.*, 1951). Mammalian trypsin is secreted as a precursor and is activated by an enterokinase. Such activation is required by chymotrypsin and pepsin also. Whether such an activating mechanism occurs in insects is unknown (Gilmour, 1961), expect for the isolated report on the positive effect of enterokinase on some insect proteinases, (Schlottke, 1937 a).

As a matter of fact although proteinase activity in insect is often characterised as trypsin – like proteinases they are most likely multiple proteolytic enzymes (House, 1974). This has been demonstrated by electrophoretic and chromatographic techniques by several investigators, the main contribution in this field being by Gooding (1969, 1974 a and 1977 b, c) and Jany *et al.*, (1977,1978 a, b). These authors identified trypsin and chymotrypsin as the major endopeptidases though some other bands of unknown specificity were also found which are designated as

proteinase IV and proteinase VI those resembled trypsin and chymotrypsin in some characters but not all. Electrophoretic studies on the midgut extract of *Musca domestica* showed three trypsin like enzymes (Patel and Richards, 1960) and the authors suggested the possibility of one of these being related to micro flora of midgut. A perusal of the literature revealed that most of the investigations are carried out in insects that are carnivorous, either parasites or predators at one or other stage of life cycle. Since of the diet of these insects possesses high protein content and they are expected to have high concentration of proteolytic activity, of different types.

Proteinase has been investigated in many insects of different orders (Dadd, 1970; Wigglesworth, 1972; Barnard, 1973; House, 1974; Chippendale, 1978; Pernas *et al.*, 1998; Harsulkar *et al.*, 1998, Oppert *et al.*, 2002). Despite numerous studies on insect digestion many aspects of digestive process including the synthesis and secretion of proteases are still poorly understood (House 1974).

Gut extracts of several insects contain carboxypeptidases, aminopeptidases and dipeptidase (Duspiva, 1936; Schlottke, 1937 a, b, c; Lichtenstein, 1947; Tatchell, 1958; Khan, 1962; Khatoon, 1965; Hori, 1973; Sinha, 1976; Hall, 1986; Hiraizumi *et al.*, 1992). The most common proteolytic enzymes found in the digestive tract of insects are active in neutral or alkaline pH and thus resemble mammalian trypsin

(Wigglesworth, 1928; Champlain and Fisk, 1956; Yang and Davies, 1968 b, c; Gooding and Huang 1969; Baker and Fabrick, 2000). The use of synthetic substrates to differentiate between the endo-and exopeptidases that make up the total complement of peptide hydrolases has resulted in a greater understanding of protein digestion in many insect species (Gooding, 1972; Ward, 1975 a, b, c; Baba *et al.*, 2001; Elpidina *et al.*, 2001). Purification and characterization of gut peptide hydrolases have received much attention during the past few years (Dahlmann *et al.*, 1978; Kunz, 1978 a, b; Stone *et al.*, 1994; Algimantas *et al.*, 1999; Spinella *et al.*, 1999) and these enzymes have been compared with mammalian proteolytic enzymes (Giebel *et al.*, 1971; Kramer *et al.*, 1973; Miller *et al.*, 1974; Grogan and Hunt 1977).

During the past decade, many important investigations on the insect gut peptide hydrolases were undertaken (Miller *et al.*, 1974; Knecht *et al.*, 1974; Ward, 1975 a, b, c; Eguchi and Iwamoto, 1976; Grogan and Hunt, 1977; Dahlmann *et al.*, 1978; Kunz, 1978 a, b; Hoseman and Downe 1980, 1981, 1982a; Billingsley and Downe, 1986,1988; Adedire, 1990; Koiwa *et al.*, 2000; Yan *et al.*, 2001). Of the proteolytic enzymes facilitating the break down of the complex blood proteins in the blood-sucking insects, trypsin is of primary importance (Yang and Davies, 1968 c; Owhashi *et al.*, 2001).

Generally, in insects, irrespective of the nature of their food and feeding habits, a trypsin like enzyme occurs in the digestive tract. According to Shinoda (1930 a) and Schlottke (1937 a), the proteinases such as trypsin are found mainly in the gut content and peptidases may occur within the epithelium, suggesting that complete hydrolysis of protein in the gut lumen is not essential for absorption. However, from the results on dipeptidase in *Utethesia pulchella* larvae, Khatoon (1965) concluded that dipeptidases are not only intracellular in the digestive tract, but are present also in the lumen content, so that the total hydrolysis of protein may occur in the midgut lumen prior to absorption.

Presence of different types of proteases is studied in insects. A thiol activated digestive proteinase, a cathepsin – B like endoproteinase, an exoproteinase and a cathepsin –D like acidic proteinase, a cDNA encoding a cathepsin L-like protein are studied from the posterior midgut of *Rhodnius* (Houseman 1978; Houseman and Downe, 1980, 1981, 1982a; Billingsley and Downe, 1988; Lopez-Ordonez, 2001). The thiol activated digestive protein shows highest activation with dithiothreitol followed by cystein, glutathione and mercaptoethanol in descending order of activation ability. The proteinase occurs in the midgut lumen and a maximal activity occurs 5-10 days after ingestion of the blood meal (Houseman, 1978). Cathepsin –B plays a major role in primary extra cellular digestion of blood proteins (Billingsley and Downe, 1988). Cathepsin –D can be separated

from cathepsin – B using DEAE ion exchange chromatography (Houseman and Downe, 1982a). This enzyme has been identified in posterior midgut of insects from six families of Hemiptera (Houseman and Downe, 1983). A number of peptidases have been characterised in insects. Though no proteinase activity was observed in the midgut of *Dysdercus fasciatus*, activity of peptidases was recorded (Ford, 1962). Extract of the first two ventriculi of *D. fasciatus* has amino and carboxypeptidases and third ventriculus had aminopeptidases, but no dipeptidases in any part of the gut (Khan and Ford, 1967). The digestive midgut of *Cimex hemipterous* and *Cimex lectularius* contains cathepsin – B aminopeptidase and an acidic proteinase that hydrolyses haemoglobin at an optimum pH of 3.4 (Houseman and Downe, 1982 b). The posterior midgut of the seed feeding pentatomid *E. euschistoides* contains the proteinase cathepsin –B and an aminopeptidase (Houseman *et al.*, 1984). Saxena (1954c) found neither polypeptidases nor digestion of proteins to amino acids in the alimentary canal of *Leptocorisa vericornis*. In the bug, *Lygus disponsi*, some properties of proteases were studied by (Hori., 1970). In four species of heteropteran bugs studied, all contain proteinase in the salivary glands, which indicates that protein is an essential component in the diet of both phytophagous and predaceous Heteroptera (Rastogi, 1962). The activities of proteinase at pH 7.6 and 3.1 were investigated in the salivary glands of adults *Lygus rugulipennis*. The proteinase with pH optimum at 7.6 is assumed to be

present in the saliva, while the one with pH optimum at 3.1 is presumably lysosomal enzymes of the gland tissue (Varis *et al.*, 1983). In haematophagous insect the major hydrolases were studied by Gooding (1972, 1975), Gooding *et al.*, (1973), Terra *et al.*, (1988) and Ramos *et al.*, (1993). The salivary glands of *D. fasciatus* had lipase, amino and carboxypeptidases, α- glucosidases, β- lucosidases and a weak amylase and aminopeptidase but no proteinases, dipeptoidase or cellulase (Ford, 1962; Khan and Ford, 1967). Extracts of the first two ventriculi of *D. fasciatus* had amino and carboxypeptidases, the third ventriculus has aminopeptidases but no dipeptidase in any part of the gut (Khan and Ford, 1967).

The presence of proteases have been reported in Lepidopteran insects (Shinoda, 1930a; Horie, *et al.*, 1963; Khatoon, 1967; Ishaya *et al.*, 1971; Eguchi *et al.*, 1972; Ward, 1972; Eguchi and Iwamoto, 1975,1976., Young, 1978; Carlini *et al.*, 1997; Lara *et al.*, 2000; Estebanez *et al.*, 2001; Mazumdar-Leighton and Broadway, 2001). Some of the digestive proteases lepidopterous insects are purified and characterised (Yoe and Kim, 1987; Broady; 1989; Johnson *et al.*, 1991; Milne and Kaplan; 1993; Xu *et al.*, 1994; Novillo *et al.*, 1999). The biochemical properties of crude and partially purified proteases were studied in the larvae of *Mithimna seperata* (Bai and Sha, 1989). Two digestive trypsin-like proteinases were isolated and characterized by Novillo *et al.*, (1999) from the larvae of the stalk corn

borer, *Sesamia nonagrioides*. Among coleopteran insects, the purification and characterization or identification of the proteolytic enzymes have been done only in a few species (Gatehouse *et al.*, 1985; Weiman and Neilsen, 1988; Kitch and Murdock, 1986; Campos *et al.*, 1989, 2002; Christellor *et al.*, 1989; Thie and Houseman, 1990 a, b; Mc Chie *et al.*, 1995; Zhu *et al.*, 2000). In Kola weevil *Sophrorhinus inspratus* both trypsin like and chymotrypsin like enzymes were detected (Adedire, 1990). In the grass grub larvae, *Costelytra zeafandica*, the dominant endopeptidase in the midgut is trypsin, which is present in four forms. In addition to this chymotrypsin esterase, leucine aminopeptidase and carboxypeptidase A and B are present (Christeller *et al.*, 1989). Trypsin like enzymes were detected in a number of other insects (Jany *et al.*, 1978a, b; Graf and Briegel, 1985; Houseman *et al.*, 1987, 1989; Birk *et al.*, 1989; Broadway 1989; Bai and Sha, 1990; Sandeman *et al.*, 1990; Christeller *et al.*, 1990, 1992; Jonston., 1991; Stiles. *et al.*, 1991; Billingsley and Hiecker, 1991; Zinckler and Potzer, 1992; Milne and Kaplan, 1993; Hoerler and Briegel, 1995; Khalaf *et al.*, 1995; Valaitis *et al.*, 1999). Dipterans have received more attention on this aspect than other orders. In dipterans, most of the studies are on *A. aegypti* (Shambaugh, 1954., Gooding, 1966; Briegel and Lea, 1979; Graf and Briegel, 1985, 1989; Graf *et al.*, 1986, 1991; Felix *et al.*, 1991; Beng *et al.*, 1992., Noriega *et al.*, 1997, 1999; Kaplan *et al.*, 2001). In *Stomoxys calcitrans* two types of trypsin like enzymes were

isolated by Hatano and Hori (1989). The precursor of one of these enzymes appears to be autocatalytic (Moffat and Lehane, 1990). A chymotrypsin like proteinase was also detected in *S. calcitrans* (Schneider *et al.*, 1987). Posterior midgut of this insect contains both proteases and peptidases necessary for proteolytic break down of ingested blood meal (Houseman *et al.*, 1987). Using highly degenerate, serine-protease-specific PCR primers and a midgut-specific cDNA library it was estimated that a minimum of 24 independent serine proteases were expressed in the midgut of *S. calcitrans.* (Lehane *et al.*, 1998). Hamilton *et al.*, (2002) reported about a novel seriene protease from the midgut of *S. calcitrans.* The general protease trypsin aminopeptidases in *P. papatasi* and *P. langeronni* were studied and their role in blood digestion in optimized assay was investigated by Dilon and Lane (1993). Trypsin like activities was detected in the midgut of *Glossina palpalis* (Steiles *et al.*, 1991). In the blow fly larvae, *Lucilia cuprina*, a chymotryptic protease is released (Sandeman *et al.*, 1990). Biosynthesis of chymotrypsin like enzymes was observed in 1-8 day old pupae of *Lutzomyia anthophora* (Mehmood and Borovsky, 1992). Chymotrypsin like serine proteinase was identified as a major gut proteinase from larvae of the Hessian fly *Mayetiola destructer* (Shukle *et al.*, 1985). Chymotrypsin like proteinase was also detected in *Callosobruchus maculates* (Gate house *et al.*, 1985). *Trichoplusia ni, Pieris raphae* (Broadway, 1989), *Mythimna seperata, Heliothis armigera* and *Galleria mellonella* (Bai and Sha, 1990),

Choristoneura occidentalis (Valaitis *et al.*, 1999), *R. dominica* (Zhu and Baker, 2000).

A chymotrypsin like endoproteinase from the gut of the cockroach is isolated and partially characterised by Baumann (1990). Besides an alkaline protease in the midgut of *P. americana*, an acid active cathepsin like protease elaborated in low concentration from salivary glands (Agarval and Behadur, 1981). In the order Thysanura, three types of protease enzymes were observed in *Thermobia domestica* (Zindler and Potzer, 1992). One is trypsin like and the other two are the charge isomer of cystein proteinases. From the gut of the thick *Rhiphicephalis appendiculatus*, two aspartic proteases are characterised (Vundla *et al.*, 1992). These enzymes hydrolysed denatured haemoglobin at acid pH. The proteolytic action was observed in the midgut of many other dipterans (Briegel and Lea, 1975; Lehane.1976, 1977; Langley *et al.*, 1978; Muse, 1984; Sharma *et al.*, 1984; Bowles *et al.*, 1988; Collet, 1989; Endege *et al.*, 1988; Billingsley, 1990; Sandeman *et al.*, 1990; Billingsley and Hiecker, 1991; Yonemura *et al.*, 1991; Mahmod and Borowsky 1993; Cazares-Raga *et al.*, 1998; Yan *et al.*, 2002). In *Acheta domestica* most of the proteases activity occurred in the crop and ventriculum (Teo and Woodring, 1994). Proteolytic enzymes were also identified and characterized in a number of insects (Houseman 1978; Houseman and Downe, 1982 a, b, 1983; Houseman *et al.*, 1984; Grogan and Hunt, 1986; Wolfson, 1987; Kawamura *et al.*, 1987; Jimnez and

Giliam, 1989; Sumenkova *et al.*, 1989; Thie and Houseman 1990b; Overney *et al.*, 1998; Oppert *et al.*, 2002).

Effect of food on digestive enzymes.

Though the influence of feed material on the enzymes was studied in a number of insects, there are only a few on phytophagous or seed and fruit feeding insects. The activity levels of a range of midgut enzymes in *Dysdercus cingulatus* vary in a manner, which roughly parallels change in food intake with one obvious exception in the case of B-glycosidase activity (Chapman, 1985). Muraleedharan and Prabhu, 1978, 1979a, b, 1981) have studied the proteolytic activities in *D. cingulatus* female adult insects fed on soaked cottonseeds and showed definite patterns of quality of food ingested and midgut protease activity during the first gonotrophic cycle. When the insect was allowed to feed on cottonseed, protease activity was proportional to the amount o food ingested. Zaidi (1985) observed that the proteolytic activity of this insect and its predator *Antillochus coqueberti* was highest at pH 2.0 for both species (especially the males) and was absent in the predator at pH 7.0. As a pest of Malvaceae *D. cingulatus* required enzymes that acted in acidic as well as neutral pH in order to digest plant proteins; while its predator required enzymes that acted only at acidic optimum. It was suggested that *A. coqueberti* may ingest saliva containing proteolytic enzymes into eggs, nymphs or adults of *D. cingulatus*, before beginning to suck the blood contents. Geering – Sacher (1972) studied the

proteolytic activity in the midgut of *D. fasciatus* using a very sensitive method, the hide powder azure serving as a substrate. He has demonstrated the cycles of proteinase production in different gut sections of males and females. Differences in proteinases activity are also found to exist in the second and third ventriculus of the midgut of females.

A direct positive correlation between food intake and enzyme activity was observed in *Catopsilla crocale* (Christopher and Mathavan, 1985). A possible relationship between feeding and regulation of enzyme activity was also found in this insect. Feeding on a different food plant or a nutrient solution did not cause significant change in salivary enzymes of *L. rugulipennis* (Varies *et al.*, 1983) except some depletion during prolonged feeding. In haematophagous hemipterans, such as *Rhodnius prolixus* Persuad and Davey, (1971) observed that protease activity in the posterior midgut reached a peak after 4-6 days of a blood meal. A good correlation was also seen between protease activity and the protein content of the posterior midgut. In *Bombyx mori* (Jadav and Kallapur, 1988) midgut-tissue protease activity was significantly increased during active feeding periods up to the 7^{th} day of larval development. However, in the subsequent periods of 8^{th} and 9^{th} day of development the enzyme activity in the midgut significantly reduced because of starvation before they began to spin the cocoons. Starvation lead to a reduction in protease activity in *Nauphopta* and *Leucophaea maderae* (Engelman and Geraertsm, 1980). All

enzyme activities were reduced by starvation in *L. rugulipennis* (Varies *et al.*, 1983).

It has been demonstrated that the proteins in the food stimulate midgut protease production in insects (Shambaugh, 1954; Engeman, 1969; Ishaya *et al.*, 1971; Akov, 1972; Briegel, 1975). Different types of protein diets including chicken blood, bovine serum albumen (BSA), Gluteraldehyde cross linked BSA, BSA fragments prepared by both pepsins cyanogens bromide cleavage, non soluble proteins in the form of gluteraldehyde fixed erythrocyte ghosts and small peptides from neutralised liver digests and their relation to the production of the enzyme trypsin in the midgut of the mosquito *A. aegypti* was studied by Felix *et al.*,. (1991). They observed a stimulated trypsin activity with chicken blood and BSA diets. The synthesis of trypsin was initiated by gluteraldehyde cross-linked BSA and by fragments of BSA, but a delayed enzyme activity was produced by non-soluble protein diets. Small peptidase did not induce trypsin activity. Noriega *et al.*, (1999) found that in *A. aegypti*, the levels of midgut trypsin activity after feeding are directly proportional to the protein concentration in the meal. The mechanisms of this up-regulatory event were investigated by analyzing the expression of the late trypsin gene under different dietary conditions. Transcription of the gene was dependent on both the quality and quantity of protein in the meal. Their results support the suggestion that the primary mechanism that regulates

the synthesis of trypsin in the mosquito midgut is transcriptional regulation of the gene. This regulatory mechanism enables the midgut to maintain the appropriate balance between protease synthesis and the protein content of the meal.

Alarcon *et al.*, (2002) studied the digestive proteases during development of larvae of red palm weevil, *Rhynchophorus ferrugineus*. The results obtained from larvae reared on different substrates have made possible a comparative assessment of the influence of diet on the development of the digestive enzymatic system.

Hormonal control of digestive enzymes

Thomsen and Moller (1963) demonstrated that protease production in the gut of adult female *Calliphora* is controlled by the medial neurosecretory cells in the brain. A hormonal control of digestion in the tsetse fly, *Glossina morsitans* was studied by Langley (1966, 1967). In rabbits, the serotonin hormone controls the total digestive physiology of the small intestine (Salvador *et al.*, 2000).

Secretagogue regulation of digestive enzymes.

Since food plays a stimulatory role, it may act either directly as a secretagogue stimulus, or indirectly by activating the nervous and /or endocrine system, through distension of the gut. Hosbach *et al.*, (1972) have observed that *Drosophila* the protease activity in the larval gut is directly related with protein concentration in the food. Similarly in

Leucophaea and *Sarcophaga*, Engelmann (1969) and Engelmann and Wilkens (1969) noted that the level of proteolytic enzyme activity is proportional to the quantity of ingested proteinacious food. However Engelmann (1969) ruled out the above possibility since removal of endocrine organ had no influence the production of gut enzymes a hormonal control of protease synthesis.

Further evidence for a secretagogue stimulation of protease secretion in mosquitoes has been provided by Breigel and Lea (1975). Their results clearly demonstrated that proteolytic activity is not affected by stretching of the midgut or albumen, but related to the presence of protein. Thus, a neural stimulus with respect to protease secretion following food ingestion is excluded.

Another aspect of protease regulation in *Aedes* is the occurrence of active trypsin in excreta (Briegel, 1975). When the protease secretion reached a maximum and about 80% of the protein is digested, proteolytic activity in the midgut of the insect declined rapidly. The rapid decline in proteolytic activity was suggested to be due to the cessation of enzyme synthesis and excretion. Following depletion of the protease a new cycle of production is triggered by the next blood meal.

Prandial/ paracrine control of digestive enzymes.

Lehane *et al.*, (1995) proposed that the direct interaction of an element of meal with digestive enzyme producing cells resulting in

increased rates of enzyme synthesis or secretion should be referred to as a prandial mechanism to avoid the confusion over the use of the term secretagogue. Most of the studies suggest that paracrine or prandial mechanisms are the main factors, which control digestive enzyme synthesis and secretion in insects. Distinguishing between the two mechanisms is a significant challenge. In many insects soluble proteins are potent stimulants of proteinase synthesis and secretions probably though the prandial/ paracrine pathways. The details of the mechanisms involved are unknown. Feeding can affect the control of digestive enzyme synthesis at either transcriptional or translational level. Similar results are obtained by the studies in *S. calcitrans* (Blackmore *et al.*, 1995; Moffatt *et al.*, 1995).

According to Hoerler and Briegel (1995) two components of trypsins, viz., a constitutive and an inductive component, are present in *Anopheles albimanus*. The constitutive trypsin is synthesised shortly after eclosion and is retained the midgut epithelial cells. The inductive trypsin is synthesised and released continuously after the injection of a blood meal among coccinellids tested. Sakurai (1968) found that the activity of protease, lipase and trehalase was higher in entomophagous individuals than in phytophagous ones which was correlated to their food habits. Carnivorous insects generally have predominantly proteolytic and lipolytic enzymes and will be deficient in amylases and saccharases. According to

Shambough (1954) there exists a striking interaction between the amount of blood injected by the female mosquitoes and subsequent protease activity. Similarly, there was a significant correlation between trypsin activity and protein content of the midgut in *Hybomitra affinis* (Thomas and Gooding, 1976). In *Ceratitis capitata*, the midgut protease increases with the increase of protein content of the diet (Limos *et al.*, 1992). In the phytophagous larva of *S. mauritia*, Prema and Mohamed, (1980) have demonstrated a lower digestive protease activity indicating lesser utilization of protein when compared to the higher activities of carbohydrases like amylase or invertase.

Sexual dimorphism in protease activity.

Sexual dimorphism in respect of the midgut protease was evident in the female larvae of *B. mori*, showed significantly higher enzyme level than that of male. The protein requirement of the female is always higher than that of the male on account of egg production (Jadhav and Kallapur, 1988). Contrary to this Sarangi (1986) observed in *B. mori* that there was no significant difference in proteinase activity between males and females of both pure races and the hybrids but in the cockroach nearly twice the proteolytic activity of the male was found in females (Baumann, 1990). In *Heliothis zea* high level of proteinase activity was observed in the whole midgut homogenate (Lenze *et al.*, 1991). In *S. calcitrans* trypsin like

proteinase activity was increased after a blood meal (Byrovsky, 1985; Schneider *et al.*, 1987).

Genetic studies on proteolytic enzymes.

Yan *et al.*, (2002) described the molecular characterization of three insect gut proteases: cathepsin B (GmCatB), zinc-metalloprotease (GmZmp) and zinc-carboxypeptidase (GmZcp). The cDNA for GmCatB encodes a protein for 340 amino acids with a predicted molecular mass of 38.2 kDa, while the 854 bp GmZmp cDNA encodes a protein of 254 amino acids with a molecular mass of 29 kDa. The GmZcp cDNA is 1319 bp in length and has a 354 amino acids open reading frame encoding a 40 kDa protein. All three cDNAs have signal peptide sequences associated with their N-terminal domains and structure analysis indicates that GmCatB and GmZmp are expressed as zymogens with pro-domains proteolytically removed for activity. The activation domain associated with the carboxypeptidase sequences is lacking in GmZcp. While GmCatB transcription is constitutive, teneral flies express very low levels of transcripts for GmZmp and GmZcp prior to the first bloodmeal. Transcription of all genes is induced and remains high throughout the digestion cycle within a few hours following the first bloodmeal ingestion. Both GmCatB and GmZcp are parasite responsive, with the expression of both genes being higher in trypanosome-infected flies.

Six chymotrypsin cDNAs were identified from larval midguts of *H. zea* (Mazumdar-Leighton and Broadway., 2001). Colebatch *et al.*, (2002) conducted cDNA cloning of a salivary chymotrypsin-like protease and identified additional cDNAs encoding putative digestive proteases from the green mirid, *Creontiades dilutus*. Many studies were conducted on the purification of enzymes by cloning techniques (Noriega *et al.*, 1996; Gatehouse *et al.*, 1997; Vizoli *et al.*, 2001; Girard and Jouanin 1999; Kotani *et al.*, 1999; Zhu and Baker., 1999, 2000; Gorman *et al.*, 2000; Zhu *et al.*, 2000; Yan *et al.*, 2001; Zeng *et al.*, 2002a; 2002b).

Using ELISA Hamilton *et al.*, (2002) provided direct evidence that the midgut defensins of the blood-sucking fly, *S. calcitrans*, are secreted into the gut lumen. They showed that midgut defensin peptide levels increase up to forty fold in response to a blood meal but not to a sugar meal. Their data suggested that the midgut defensin genes are post-transcriptionally regulated and that their function is protection of the stored blood meal from bacterial attack while it awaits digestion.

MATERIALS AND METHODS

K.S. Sajan “Studies on the protease activity in the digestive system of iphita limbata (Heteroptera : Pyrrhocoridae)” Thesis. Department of Zoology , University of Calicut, 2002

Materials and methods

MATERIALS AND METHODS

Experimental animal

I. limbata (Heteroptera: Pyrrhocoridae) is a phytophagous bug, which infests mainly on cottonseeds. The bug also feeds on a variety of plant sap, fruits and seeds. The bugs are oval with bright red and black markings. Females are generally larger than males and could be distinguished by their external genitalia. The bugs are odd in their prolonged mating deportment. In extreme case, the mating period lasts for 20-30 days. Another peculiar feature of these insects is their dormant nature during the months of August to December. The bugs show cannibalistic tendencies in early instars.

The adults of *I. limbata* were collected from the Calicut University campus and were reared in the insectary of the department (Plate I). They were transferred into a glass through containing moist sand at the base. The sand was heated before use in order to avoid infection. Petroleum jelly was smeared on the inner edge of the glass troughs in order to prevent the escape of insect from glass troughs. These insects were starved for 2-3 days. The bugs were then separated into different groups and each group was maintained on different food such as soaked cottonseeds (*Gossypium herbaceum*), ripe banana fruit

PLATE – I A colony of *Iphita limbata* reared in the laboratory fed on green gram

PLATE – I

(*Musa paradisiaca*), soaked green gram (*Phaseolus aureus*), 10 % sugar solution in distilled water, 5 % casein suspension in distilled water and distilled water. The glass troughs were covered with muslin and kept in big insect cages insulated from ants by water barrier.

The insects were maintained for one week and then transferred to another trough containing these different diets. Insects required for experiments were drawn from the trough just before the experiment. Adults of males and females were separated and used in this study.

Preparation of enzyme extract

Insects were anaesthetised one by one by keeping them for 3-4 minutes in small specimen bottles with cotton soaked in solvent ether. When motionless, they were taken out and washed in insect ringer. They were dissected in ice-cold ringer to expose the alimentary canal. A diagrammatic sketch of the alimentary canal of *I. limbata* is given in figure A. Ice blocks were kept around the dissection tray in order to maintain the temperature. The complete alimentary canal was removed from the insect and the midgut was carefully separated from the hindgut by cutting with a pair of scissors. The lumen content of both regions was carefully ejected out in to a watch glass. The gut tissue after ejecting the lumen content, were carefully washed in ice-cold

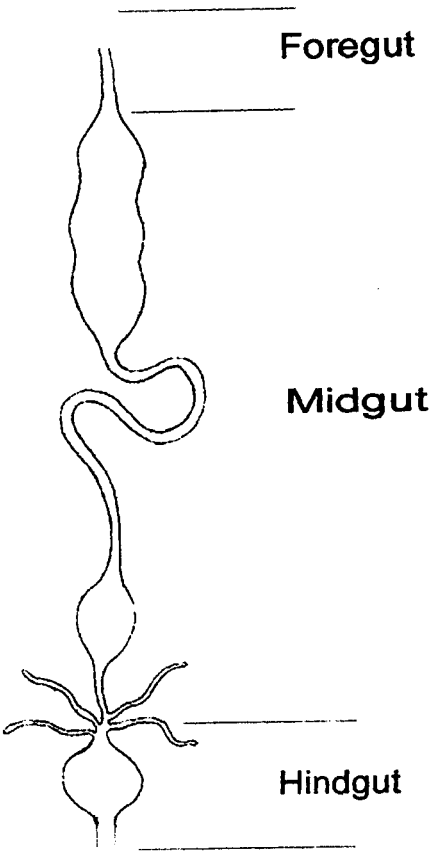


Figure A: Alimentary canal of the plant bug *Iphita limbata*

ringer and used for the experiments. The homogenates of tissue and lumen content were then prepared as explained below.

Preparation of 'homogenates'

A homogenate of a tissue is, by definition, a suspension of the formed elements of the cells in a diluted cytosol mileau, which is isotonic with the cell contents and which is inert. What was attempted in the present study was a cell-free preparation, with maximum exposure of intracellular enzymes and for this purpose, water was used as the dispersion medium. Nevertheless, the word 'homogenate' will be retained for purpose of convenience.

Distilled water obtained from all-glass still was used as dispersion medium for the cell-free preparations. This water was chilled before use. The dispersion was generally 5% (fresh weight of tissue/volume) in strength. Since the dissection was carried out in an aqueous medium, the moister adherent to the outside of the tissue would lead to inaccuracy in weighing of tissue. As a routine, the author has preferred to adjust the final volume of the enzyme preparation in reference to the definite number of gut regions. This was justified by the fact that the tissues used for an experiment were from animals of almost uniform size. In actual practice, such a homogenate corresponded closely to a 5% (w/v) preparation. Gut tissues, the gut

(tissue plus lumen content) and expelled content were ground in a homogenizer type of apparatus. During grinding, the homogenizer was kept surrounded by ice.

Using a glass homogenizer

A hand-operated all glasses device of the Potter-Elvehjem type was employed, to homogenize a maximum of 100-mg tissue at a time. The medium was added intermittently during grinding, which lasted for about 10 minutes. The homogenized tissue was transferred to graduated centrifugal tubes, the homogenizer was washed twice with minimum ice-cold distilled water, and the washing added to the main preparation and adjusted the volume appropriately. The homogenate is centrifuged for ten minutes at 4000 rpm in a refrigerated centrifuge and the supernatant was used as the enzyme extract. The ground preparation thus obtained was stored in a refrigerator (0 - 4°C) and used within 30 – 60 minutes

Any effect of the anaesthetic on tissue enzymes was disregarded.

Tissues and samples employed for studies

The kinetic properties of the digestive enzymes were studied using the different regions of the alimentary system from adult insects. The optimum assay conditions, which emerged from this study, were

employed to quantify the enzymes in the epithelium and lumen content of the different parts of the gut regions of the green gram fed adult insects.

Determination of protease activity

The assay system consisted of 1 ml of 75% vitamin free casein of 2%, 1 ml of buffer (Acetate buffer pH-5), 1 ml of enzyme extract and 3 drops of toluene as antiseptic. Incubations were performed for 1 hour at 40°C in a thermostatically controlled water bath. After the incubation, reaction was stopped by adding 3 ml of 0.3 M trichloro acetic acid solution. Samples were mixed well and heated for 5 minutes in a water bath at 100°C to ensure complete coagulation of the remaining protein after the enzymatic hydrolysis. The mixture was filtered through Whatman number-3 filter paper to obtain a clear filtrate. The degree of proteolysis was assayed by measuring the increase in tyrosine by the Folin and Ciocalteu's reagent using the method of Lowry *et al.*, (1951). The intensity of the colour was measured in a Shimadsu UV mni UV spectrophotometer at 540 nm wavelength of the visible spectrum. In the control assay system all the ingredients were the same as those in the experimental system, except for the substitution of the active enzyme preparation by a previously denatured enzyme preparation, the

inactivation having been effected by heating in a bath of boiling water for 15 minutes.

Unit of enzyme activity.

One unit of enzyme activity corresponded to the formation of one micromole of the tyrosine under the conditions of the assay. Activity units were represented by the amount of tyrosine produced by the enzymatic action per milligram of gut/tissue of gut/lumen content.

The relative activity of the enzyme for the following parameters a) midgut over hindgut, b) female insects over male insects and c) one type of food over the other were expressed as a percentage difference in activity.

Determination of the kinetic properties

Optimum pH

The pH at which the maximum enzymatic hydrolysis of casein occurred was found out by performing the assay at different hydrogen-ion concentrations, keeping the other conditions constant.

Optimum substrate concentration, K_m value and V_{max}

A series of assay mixtures were set up having increasing concentration of the substrate. The K_m value and V_{max} for the

particular enzyme were calculated by the Lineweaver-Burk double reciprocal plot.

Optimum reaction temperature

The reaction mixtures were incubated at various temperatures ranging from 17°C to 50°C, the other conditions remaining the same.

Analysis of data

Students't test is used for estimating the significance of difference between the variables. P values higher than 0.05 were considered as not significant (NS).

RESULTS

K.S. Sajan “Studies on the protease activity in the digestive system of iphita limbata (Heteroptera : Pyrrhocoridae)” Thesis. Department of Zoology , University of Calicut, 2002

Results

RESULTS

Kinetics / optimum conditions for activity

Hydrogen-ion concentration

Midgut

Hydrolysis of casein was followed at pH ranging from 3.0 - 9.8 (pH 3.0 - 5.0 acetate buffer, 5.5 and 6.0 citrate phosphate buffer and 7.0 and 8.0 phosphate buffer and 9.8 sodium glycinate buffer). The pH activity relationships in midguts of male and female insects are shown in table 1 and figure 1.

Table 1
Figure 1

The enzyme activity was low at pH 3.0, increased gradually, recording a peak at pH 5.0. The activity was relatively high between pH 4.5 and 7.0 but declined sharply at alkaline conditions.

Hindgut

The pH activity relationships in the hindgut of male and female insects are shown in table 2 and figure 2.

Table 2
Figure 2

The pH enzyme activity relationship of hindgut protease showed a similar pattern of changes to that of midgut protease, but with a lower magnitude. The optimum pH for protease activity in the midgut and hindgut was the same.

Table 1. Effect of pH on the protease activity of the midgut

pH grades	Activity units *	
	Females	Males
3.0	10.832 ± 0.57	6.923 ± 0.54
4.0	12.139 ± 0.23	7.820 ± 0.56
4.5	35.833 ± 0.87	11.748 ± 0.54
4.8	41.337 ± 0.98	13.591 ± 0.35
5.0	45.461 ± 1.13	14.972 ± 0.95
5.5	38.438 ± 0.90	12.620 ± 0.12
6.0	30.766 ± 0.76	10.051 ± 0.05
7.0	13.826 ± 0.56	5.821 ± 0.13
8.0	9.165 ± 0.03	3.746 ± 0.01
9.8	6.999 ± 0.11	2.782 ± 0.45

*The values are the means of six determinations with ± SE

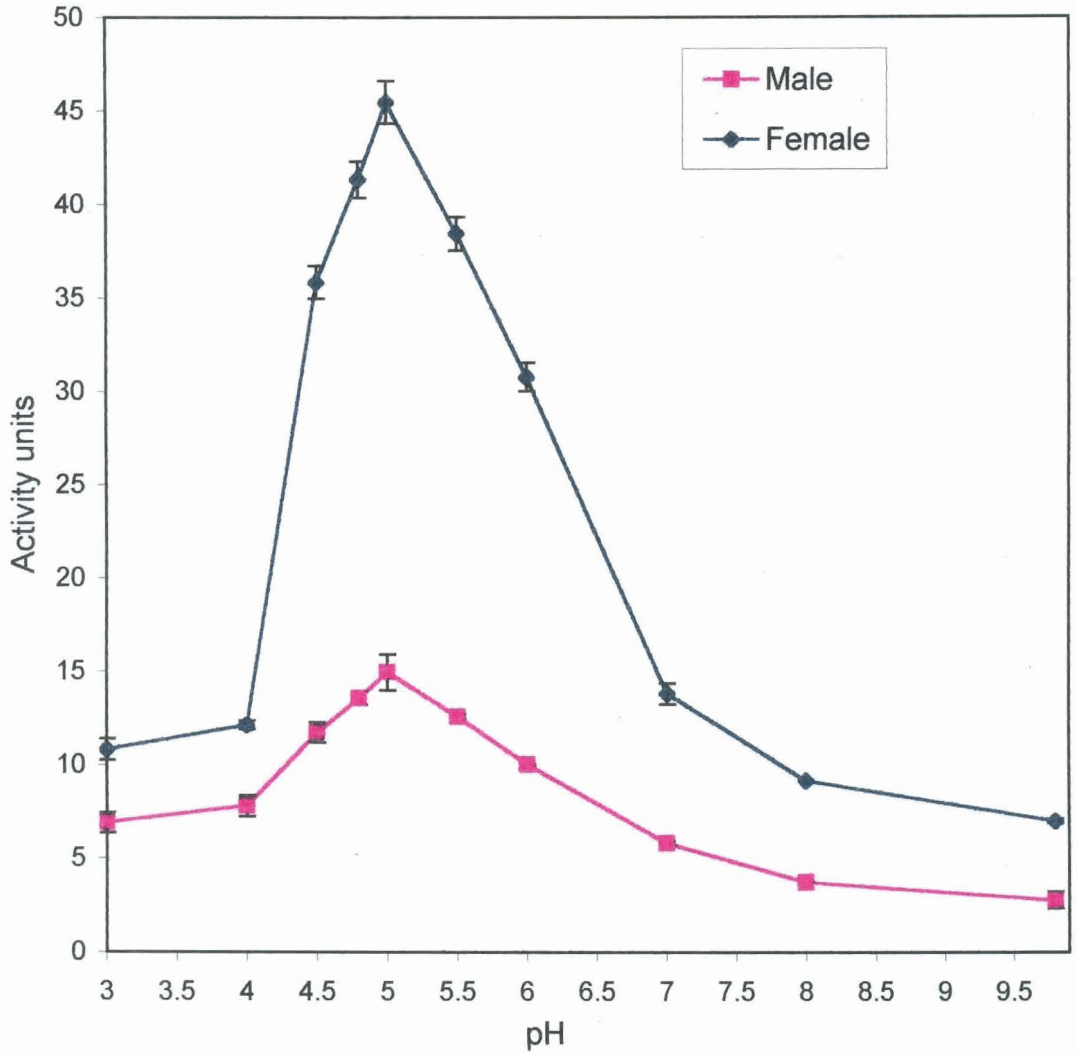


Figure 1.Effect of pH on the protease activity of midgut

Table 2. Effect of pH on protease activity of the hindgut

pH grades	Activity units *	
	Females	Males
3.0	6.704 ± 0.24	3.92 ± 0.01
4.0	9.368 ± 0.54	5.621 ± 0.02
4.5	20.097 ± 0.58	8.934 ± 0.54
4.8	23.457 ± 0.98	10.471 ± 0.58
5.0	30.786 ± 1.23	13.823 ± 0.78
5.5	21.614 ± 0.65	9.628 ± 0.54
6.0	14.500 ± 0.54	6.374 ± 0.45
7.0	5.294 ± 0.24	2.982 ± 0.23
8.0	4.327 ± 0.02	2.372 ± 0.06
9.8	2.041 ± 0.09	0.931 ± 0.01

*The values are the means of six determinations with ± SE

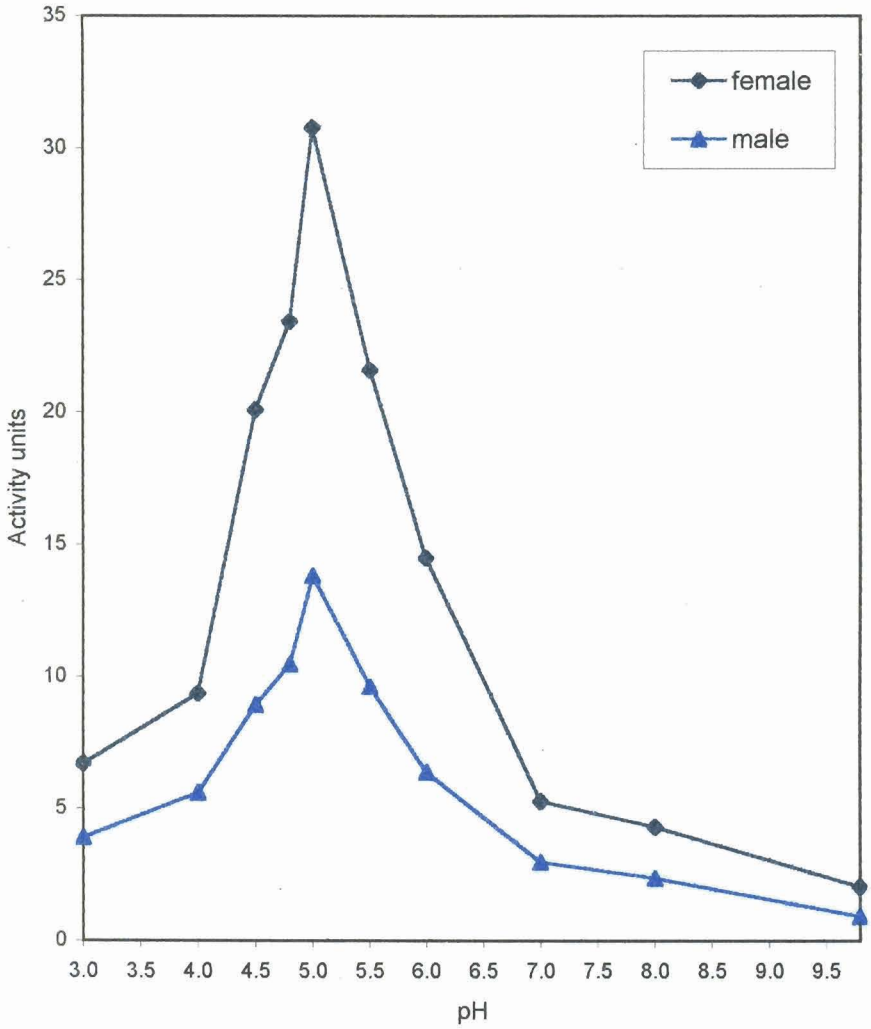


Figure 2. Effect of pH on the protease activity of hind gut

Substrate concentration

Midgut

The activity–substrate concentration relationships ranging from 0.25% to 2.5% casein of both male and female midgut protease, under the assay conditions given in the chapter 'materials and methods' are presented in table 3 and figure 3.

Table 3
Figure 3

A rectangular hyperbolic relationship was observed for reaction velocity, against an increase in substrate concentration from 0.25% to 2.5%.

A Lineweaver–Burk double reciprocal plot of the velocity against substrate concentration is illustrated in figure 3A.

Figure 3A

The K_m value estimated from the Linewever-Burk plot was 0.67 % for the midgut protease in female insects and was 1.07 % for male insects using casein as substrate. The $1/V_{max}$ was 0.0125 for female insects and was 0.0325 for male insects.

The K_m values varied widely with different sexes. It was higher for females and lower for males. The value for V_{max} with casein hydrolysis were 30.77 and 80.00 activity units in males and females respectively.

Table 3. Effect of substrate concentration on protease activity of the midgut

Concentration grades	Activity units *	
	Female	Male
0.25%	0.269 ± 0.01	0.083 ± 0.02
0.50%	17.602 ± 0.21	5.812 ± 0.05
0.75%	24.975 ± 1.25	8.281 ± 1.23
1.00%	46.103 ± 1.45	15.356 ± 1.45
1.50%	52.255 ± 1.67	17.416 ± 1.65
2.00%	60.318 ± 1.84	20.116 ± 1.84
2.50%	61.008 ± 2.13	20.347 ± 2.54

*The values are the means of six determinations with ± SE

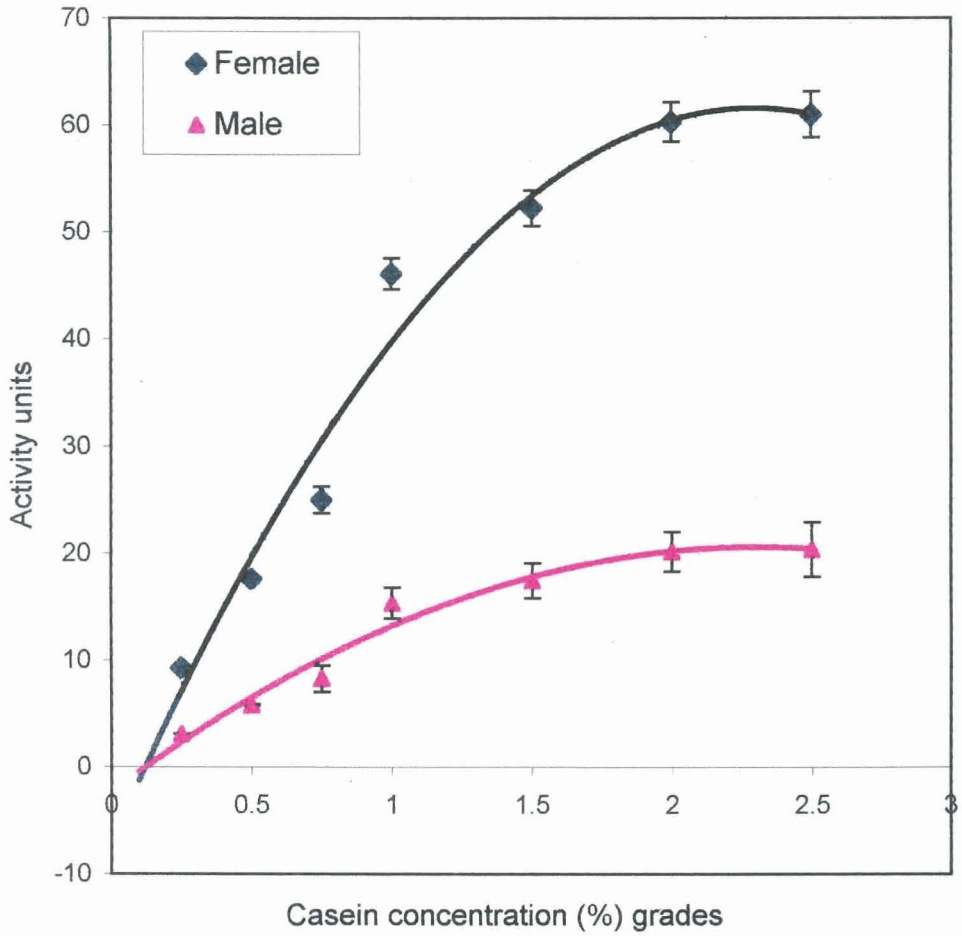


Figure 3. Effect of substrate concentration on the protease activity of midgut

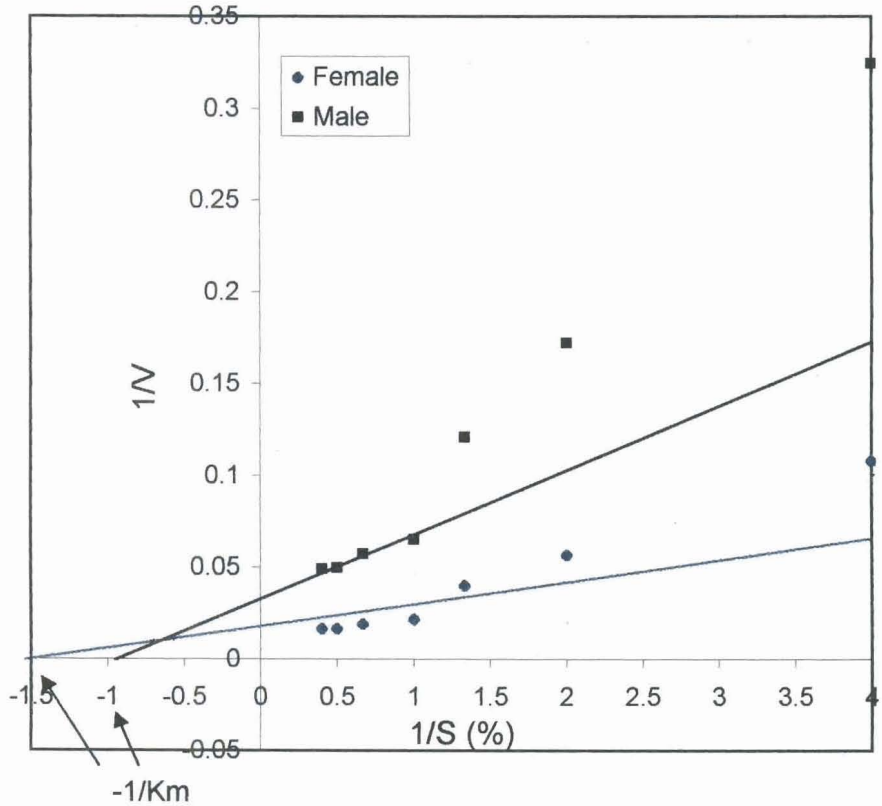


Figure 3A . Lineweaver -Burk plots for general protease in midgut

Hindgut

The enzyme activity- substrate concentration relationships in the hindgut of male and female insects are shown in table 4 and figure 4.

Table 4
Figure 4

With increase in substrate concentration from 0.25% to 0.5%, a sudden increase in activity was observed in both male and female midgut enzyme properties (41.24 times in females and 60.72 times in males). The activity increased gradually and plateau was observed both in females and males when substrate concentration increased from 2% - 2.5%. The Lineweaver-Burk plot of the substrate concentration against reaction velocity is shown in figure 4A.

Figure 4A

The K_m value estimated from the Lineweaver-Burk plot was 2 % for hindgut protease in female insects and 2.67 % using casein as substrate. The $1/V_{max}$ was 0.0421 % for female insects and 0.737 % for male insects.

The K_m values varied widely with different sexes. It was higher for females and lower for males. The values for V_{max} with casein hydrolysis were 13.57 and 23.75 activity units in male insects and female respectively.

Table 4. Effect of substrate concentration on protease activity of the hindgut

Concentration grades	Activity units *	
	Female	Male
0.25%	0.1406 ± 0.051	0.029 ± 0.025
0.50%	5.7989 ± 0.046	1.761 ± 0.098
0.75%	9.5348 ± 0.546	3.012 ± 0.845
1.00%	17.9384 ± 0.845	5.826 ± 0.845
1.50%	27.9366 ± 1.259	9.174 ± 1.254
2.00%	33.1627 ± 1.874	10.924 ± 1.980
2.50%	33.3329 ± 1.654	10.981 ± 2.150

*The values are the means of six determinations with ± SE

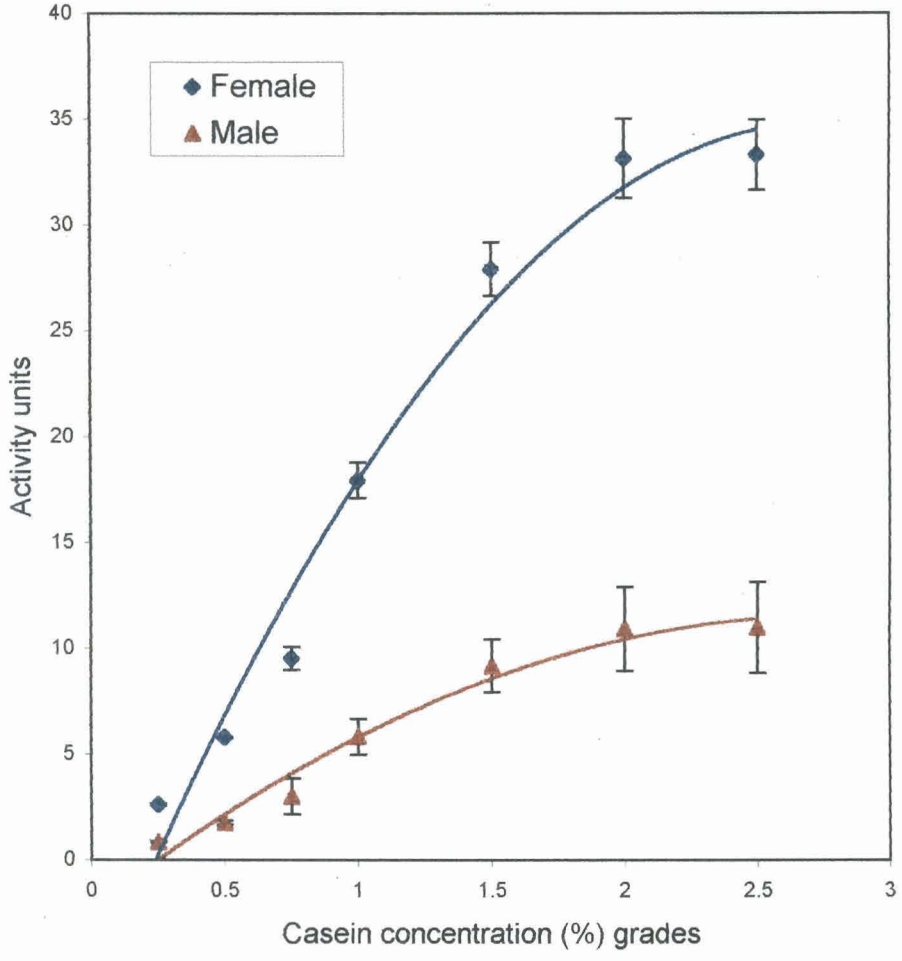


Figure 4. Effect of subsrtate concentration on the general protease activity of hindgut

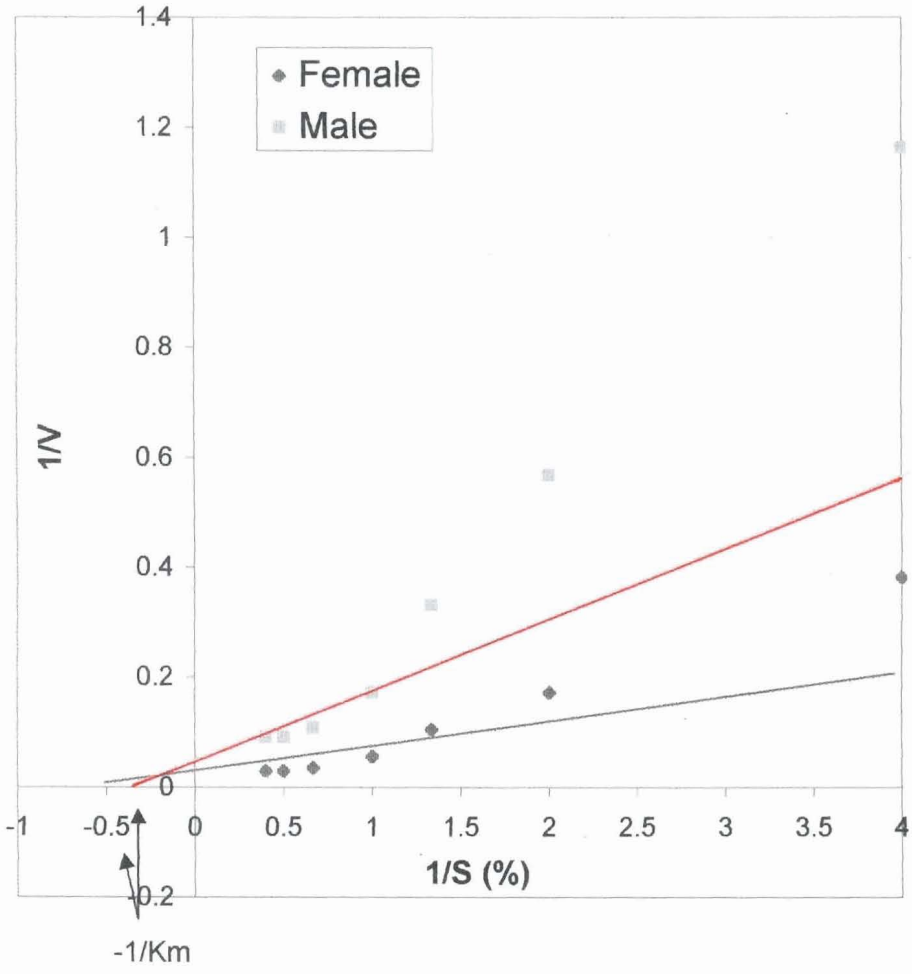


Figure 4A. Lineweaver-Burk plots for general protease in hind gut

Reaction temperature

Midgut

Assays for casein hydrolysis, using midgut protease of both male and female insects under optimal conditions of pH and substrate concentration were carried out at temperatures ranging from 17°C - 55°C. The results are illustrated in table and 5 figure 5.

Table 5
Figure 5

The enzyme preparations of midgut regions of both the sexes showed a gradual increase from 17°C to 47°C followed by a dip by 38% in females and 22% in males at 55°C.

Hindgut

The results of hindgut protease activity of males and females are given in table 6 and figure 6.

Table 6
Figure 6

Though the activity lowered in hindgut enzyme preparations, both male and female insects showed the same optimal temperature as that of midgut enzyme preparations, i. e., 47°C.

Difference in protease activity between males and females

Midgut

The differences in protease activity between male and female insects are presented in table 7and figures 7 and 7A.

Table 5. Effect of temperature on the protease activity of the midgut

Temperature	Activity units *	
	Females	Males
17 °C	2.586 ± 0.025	1.964 ± 0.021
29 °C	12.331 ± 0.098	3.831 ± 0.036
37 °C	35.322 ± 2.250	11.530 ± 1.254
47 °C	42.639 ± 1.260	13.980 ± 1.980
50 °C	9.885 ± 0.012	3.012 ± 0.150

*The values are the means of six determinations with ± SE

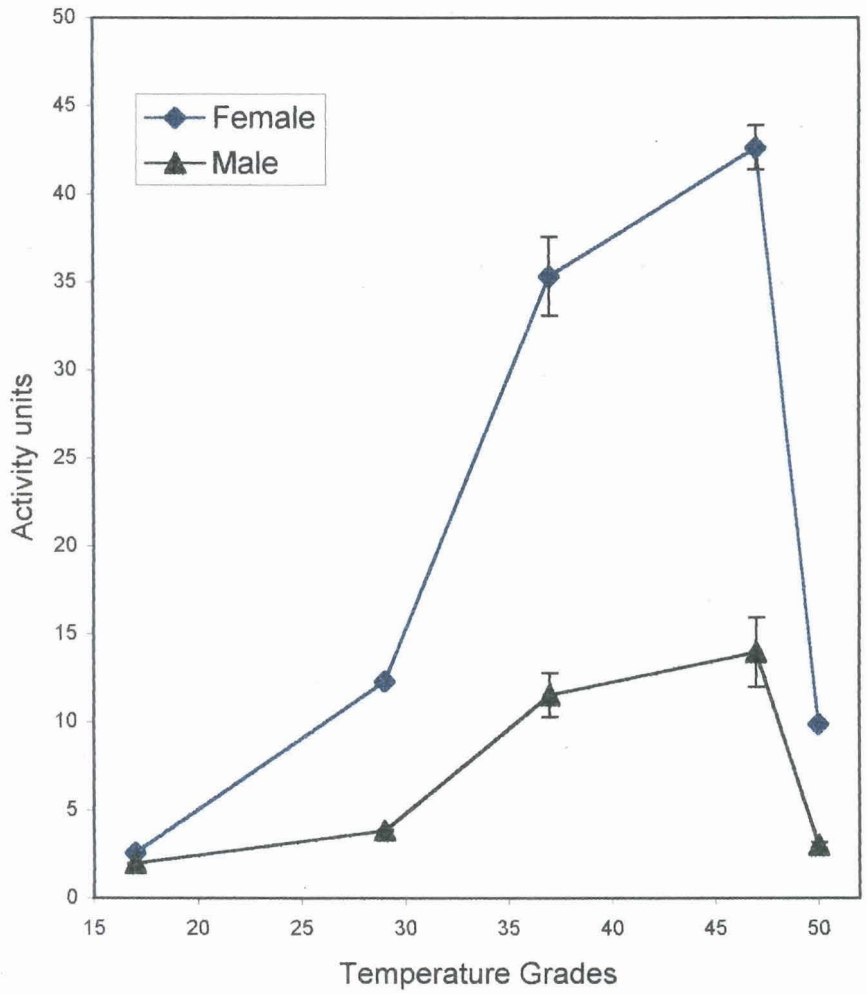


Figure 5. Effect of temperature on protease activity of midgut

Table 6. Effect of temperature on protease activity of the hindgut

Temperature grade	Activity units *	
	Females	Males
17°C	0.961 ± 0.021	0.821 ± 0.021
29°C	6.692 ± 1.240	2.05 ± 0.035
37°C	15.445 ± 1.350	4.981 ± 1.240
47°C	17.335 ± 1.240	5.614 ± 1.250
50°C	2.425 ± 0.025	0.621 ± 0.025

*The values are the means of six determinations with ± SE

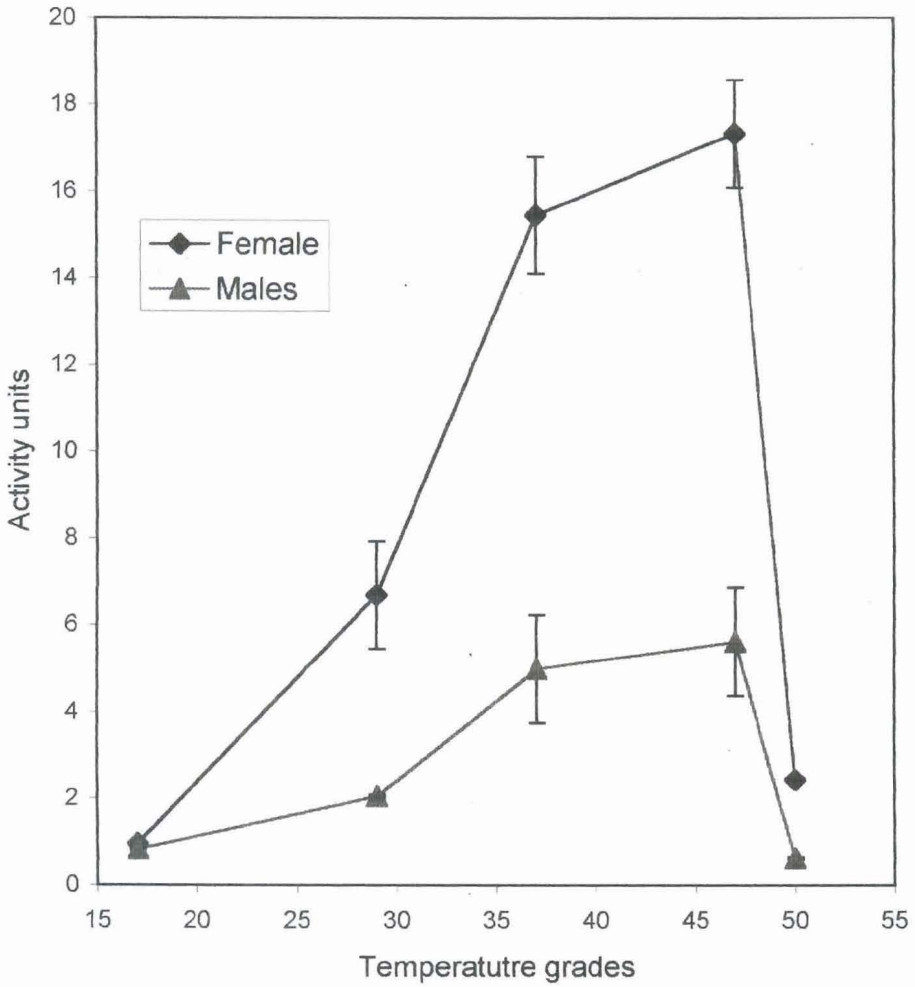


Figure 6. Effect of temperature on protease activity of hindgut

Table 7
Figures 7 and 7A

Midgut

Among the 3 types of enzyme preparations the highest activity was exhibited by female midgut lumen content enzyme preparation which was 4.77 fold and 1.67 fold more than the activity expressed by the gut tissues and gut with content respectively. The enzyme activity of the male insects was lower than the female insects in all the 3 types of enzyme preparations.

In general, proteolytic activity of the gut tissue was low compared to the other two types of enzyme preparations.

Hindgut

A difference in proteolytic activity between males and females was also exhibited in the case of hindgut enzyme preparations. The females showed significantly higher proteolytic activities than the males. Among the 3 types of enzyme preparations the female lumen content showed the maximum proteolytic activity which was 3.52 fold and 1.55 fold more than that of gut tissue and gut with content respectively.

Relative protease activity in different gut regions

The difference in enzyme activity exhibited by different gut regions and three types of enzyme preparations are given in table 8 and figure 8 and 8A.

Table 8
Figures 8 and 8A

Table 7. Relative protease activity between male and female insects

Gut region		Activity units *		#Relative activity	$Significance
		Female	Male		
Midgut	Gut with content	42.428 ± 5.21	14.207 ± 0.324	298.63	P<0.01
	Gut tissue	14.849 ± 1.23	5.682 ± 0.564	261.30	P<0.02
	Lumen content	70.854 ± 6.54	21.311 ± 1.687	332.47	P<0.01
Hindgut	Gut with content	20.223 ± 1.95	8.254 ± 1.423	245.00	P<0.01
	Gut tissue	8.898 ± 0.22	3.301 ± 0.254	269.50	P<0.02
	Lumen content	31.345 ± 2.36	9.905 ± 1.240	316.45	P<0.01

*The values are the means of six determinations with ± SE.

The relative activities are expressed as the percentage difference in proteolytic activity in females over males.

$ Significance of difference between the activities of males and females.

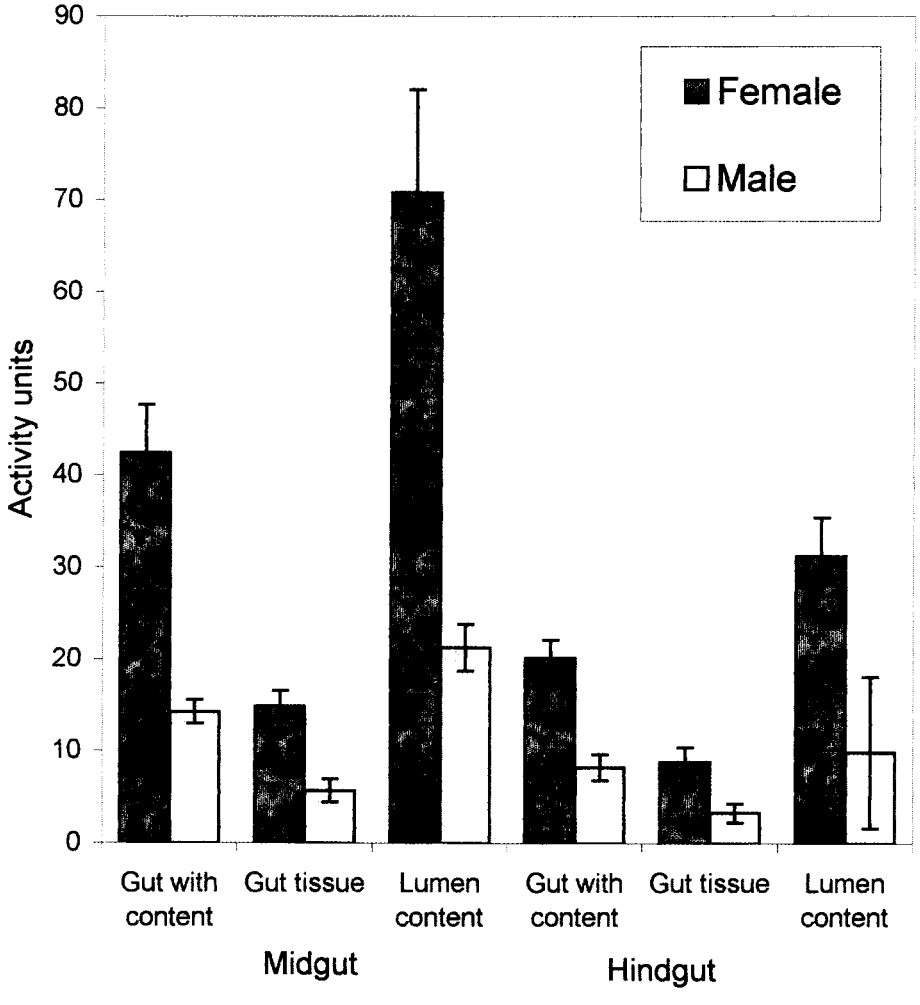


Figure 7. Difference in protease activity between male and female insects

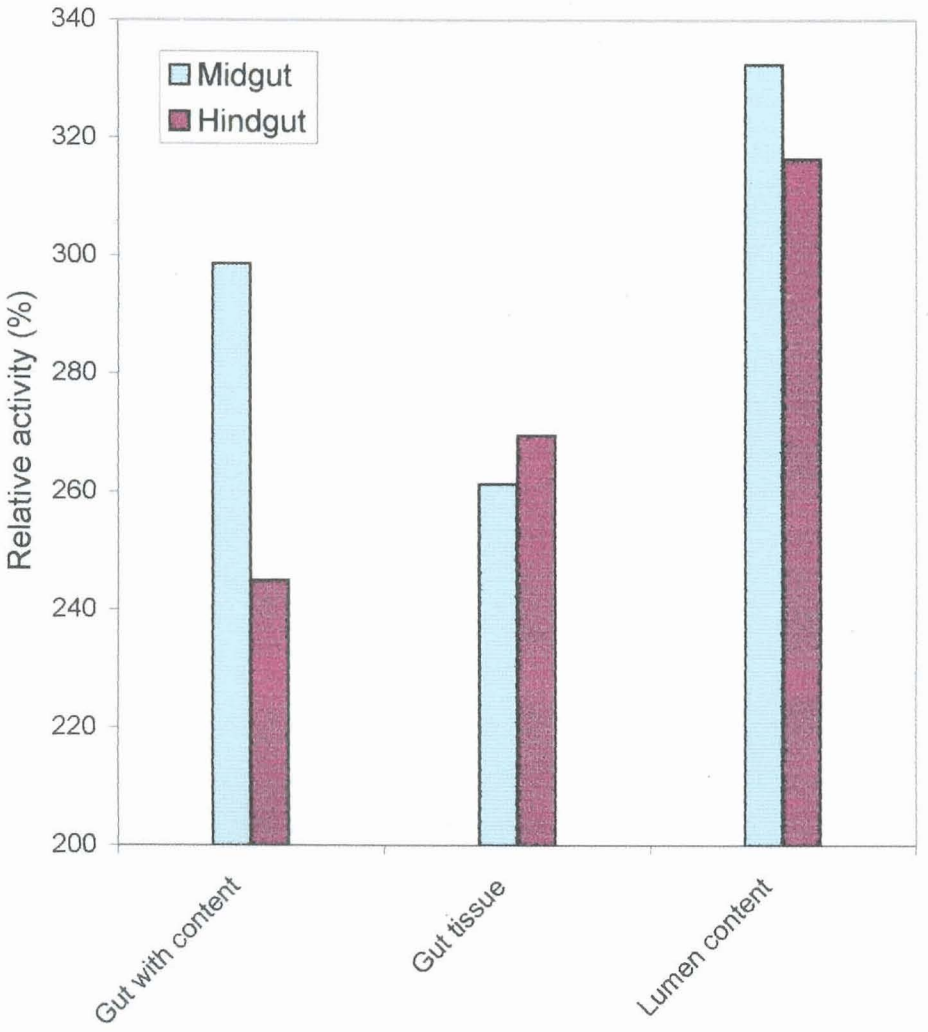


Figure 7A. Relative activity of females over males

Table 8. Relative protease activity between different gut regions

Sexes		Activity units *		#Relative activity	$Significance
		Midgut	Hindgut		
Female	Gut with content	42.428 ± 5.21	20.223 ± 1.95	209.80	P<0.01
	Gut tissue	14.849 ± 1.23	8.898 ± 0.22	166.88	P<0.05
	Lumen content	70.854 ± 6.54	31.345 ± 2.36	226.05	P<0.01
Male	Gut with content	14.207 ± 1.32	8.254 ± 1.42	172.12	P<0.05
	Gut tissue	5.682 ± 0.56	3.301 ± 0.25	172.10	P<0.05
	Lumen content	21.311 ± 1.69	9.905 ± 1.24	215.15	P<0.02

*The values are the means of six determinations with ± SE

The relative activities are expressed as the percentage difference in proteolytic activity in midgut over hindgut.

$ Significance of difference between the activities of midgut and hindgut

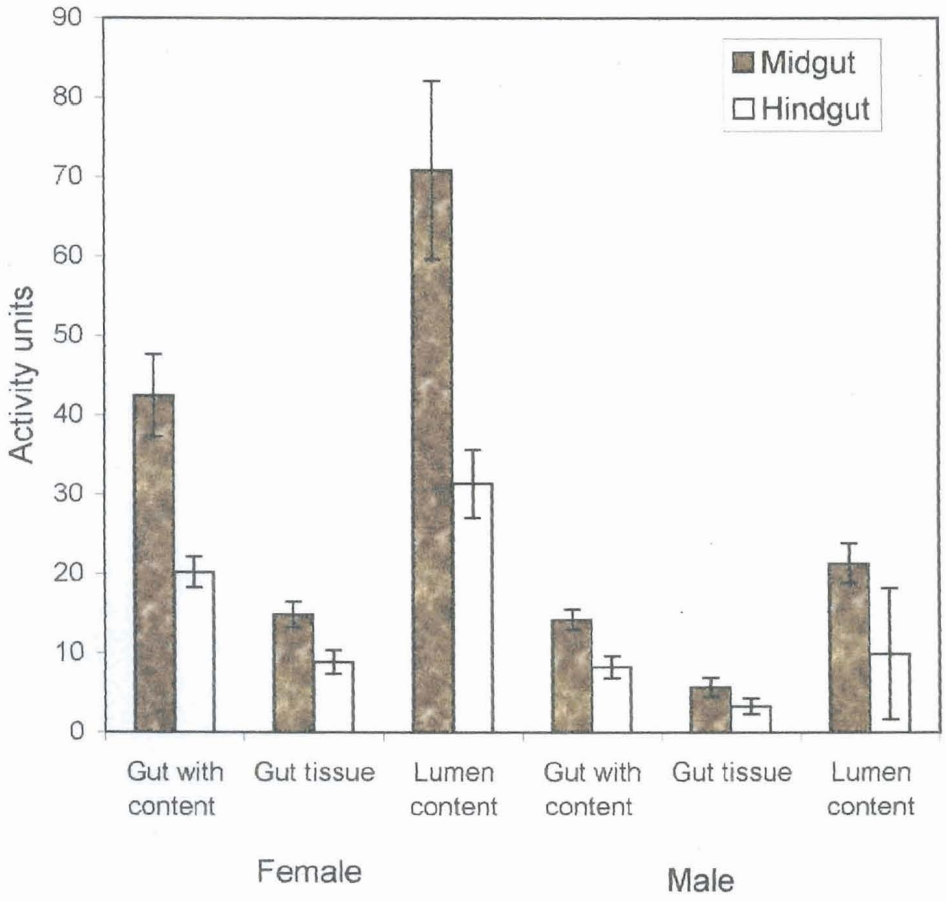


Figure 8. Difference in protease activity in different regions of gut

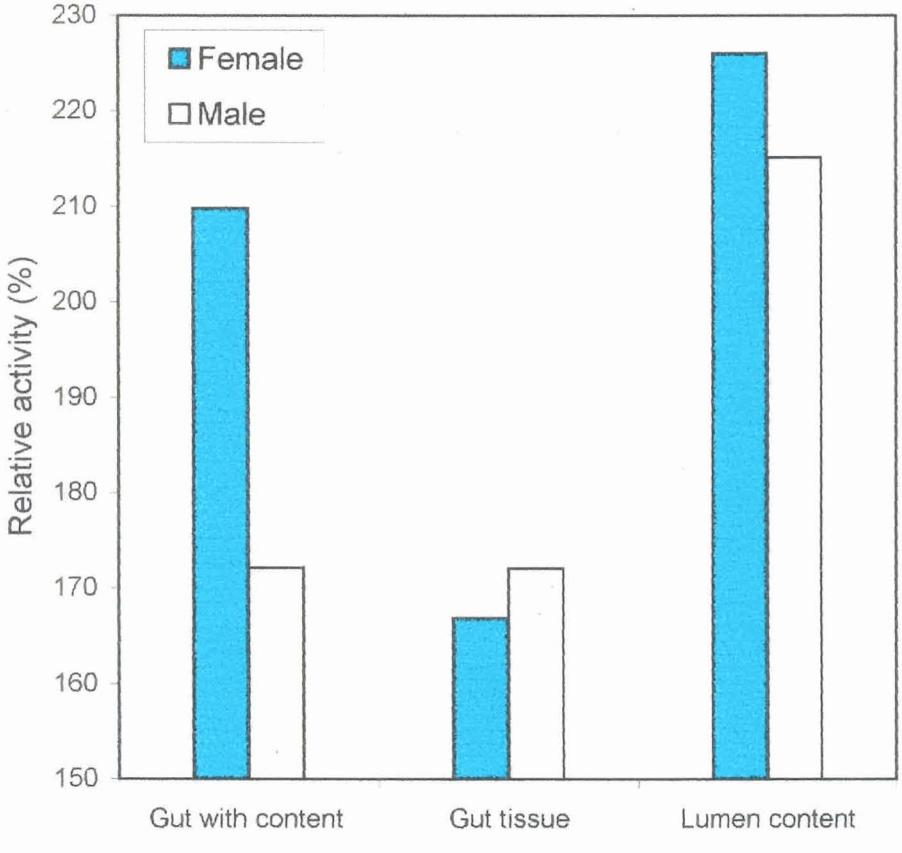


Figure 8A. Relative activty of midgut over hindgut

55F

All the three different types of enzymatic preparations of females showed significantly higher activities in the midgut regions than that of the hindgut regions. The difference in activity of the lumen content (226%) was more than the other two enzymes preparations. Male insects also exhibited more or less similar differences in proteolytic activity between mid – and hindgut regions.

Effect of food on protease activity with respect to sex

Midgut protease activity

Influence of different types of food on the activity of midgut protease enzyme in relation to sex is shown in table 9 and figures 9 and 9A.

Table 9
Figures 9 and 9A

The activities of midgut proteases in male and female insects fed with different diets were: cottonseed > green gram > casein suspension > distilled water> banana > sugar solution. The relative protease activities exhibited by insects fed on different diet are shown in tables 10 and 11 and figures 10 and 11.

Tables 10 and 11
Figures 10 and 11

The enzyme activity exhibited by female insects fed with soaked cottonseeds was the highest one recorded in the present study. The activity exhibited by female was about 2 fold to that of the male. Male and female insects fed with soaked green gram exhibited only a marginal reduction in

Table 9. Effect of food in protease activity of the midgut

Dietary regimen	Activity units *		#Relative activity	$Significance
	Female	Male		
Cotton seed	43.766 ± 7.23	20.457 ± 4.21	213.94	P<0.001
Green gram	42.428 ± 5.21	14.207 ± 1.32	298.63	P<0.001
Casein suspension	26.041 ± 4.35	11.955 ± 2.13	217.83	P<0.01
Banana	8.328 ± 1.30	3.205 ± 0.84	259.82	P<0.05
Sugar solution	6.878 ± 0.24	2.332 ± 0.14	294.94	P<0.01
Distilled water	8.797 ± 0.56	3.786 ± 0.15	232.34	P<0.001

*The values are the means of six determinations with ± SE

The relative activities are expressed as the percentage difference in proteolytic activity in females over males

$ Significance of difference between the activities of males and females

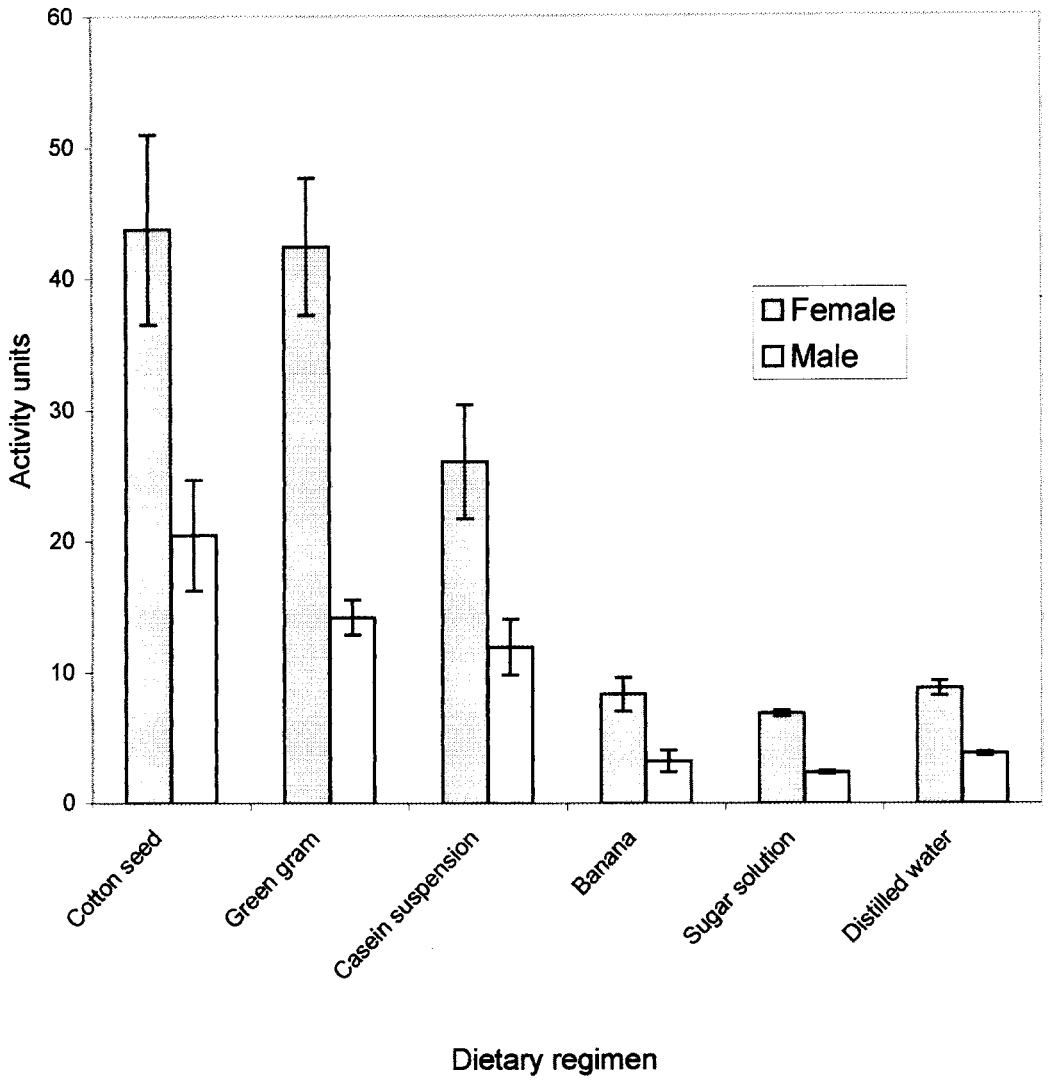


Figure 9. Effect of food on protease activity of midgut

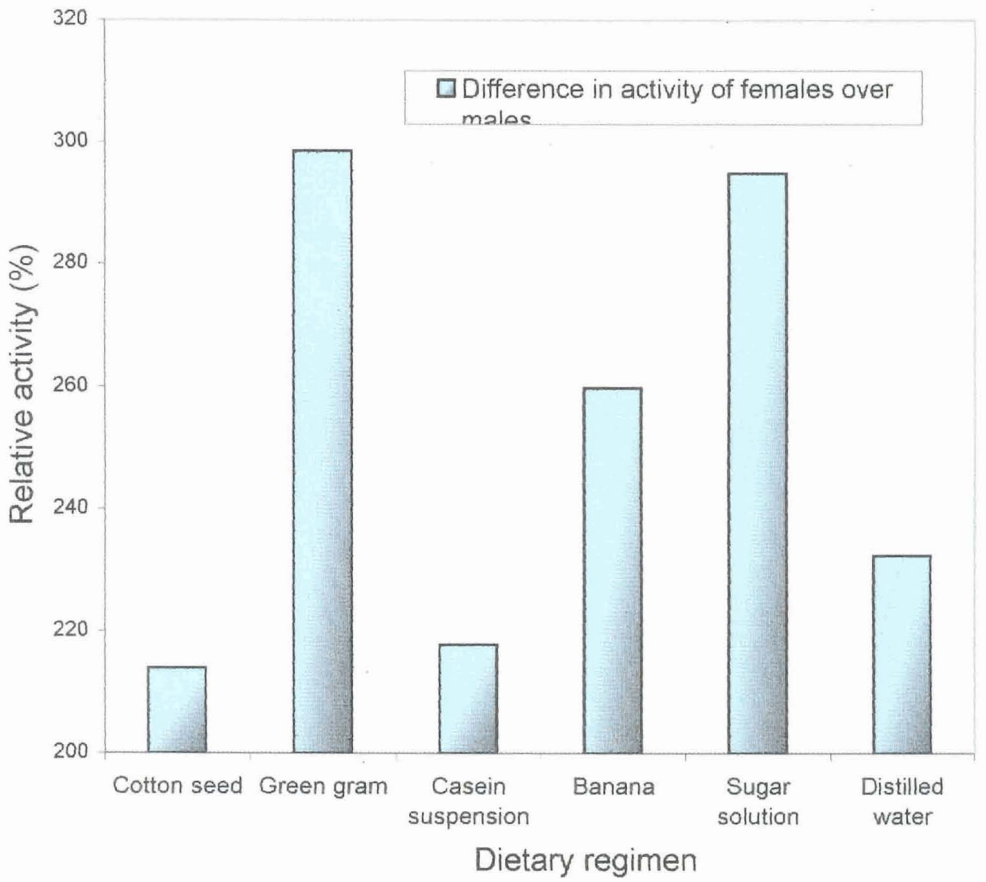


Figure 9 A. Relative activity of midgut protease in females over males

Table 10. Relative activity of protease in the midgut of female insects maintained on different diets.

Dietary regimen		* Relative activity	#Significance
A	B		
Cotton seed	Green gram	96.941	NS
	Casein suspension	59.501	P<0.05
	Banana	19.030	P<0.001
	Sugar solution	15.716	P<0.0001
	Distilled water	20.102	P<0.001
Green gram	Casein suspension	61.379	NS
	Banana	19.631	P<0.01
	Sugar solution	16.212	P<0.01
	Distilled water	20.736	P<0.02
Casein suspension	Banana	31.982	P<0.02
	Sugar solution	26.413	P<0.01
	Distilled water	33.783	P<0.05
Banana	Sugar solution	82.585	NS
	Distilled water	105.631	NS
Sugar solution	Distilled water	127.905	P<0.05

*The relative differences in protease activity in insects maintained in diets given in column A to that of column B are expressed as a percentage of activity in the latter over the former.

#Significance of difference between the activities exhibited by insects maintained on different diets given in the column.

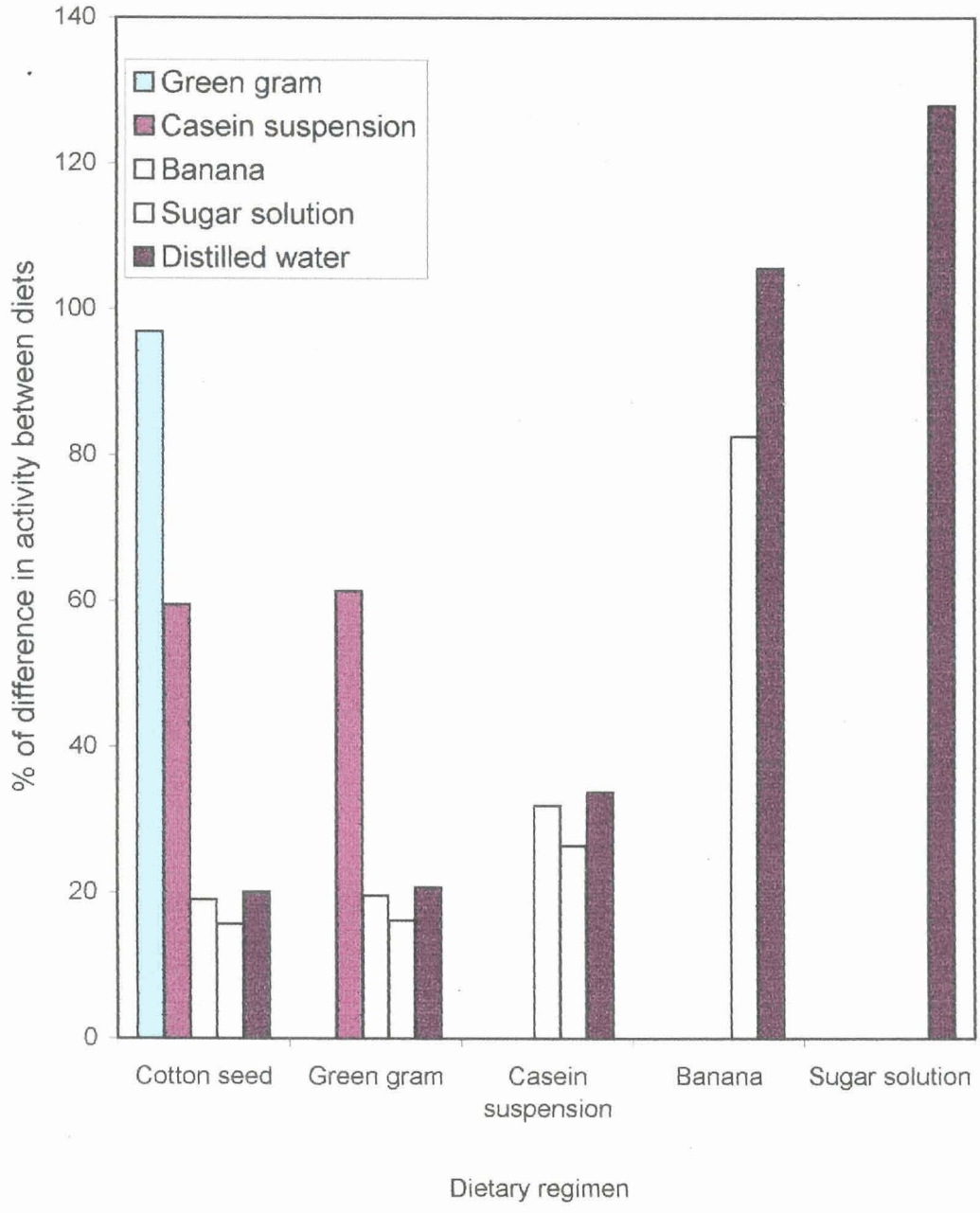


Figure 10. Percentage of midgut protease activity exhibited by female insects fed with different diets represented by bars over to that of diets represented in the X axis

Table 11. Relative activity of protease in the midgut of male insects maintained on different diets

Dietary regimen		*Relative activity	#Significance
A	B		
Cotton seed	Green gram	69.45	NS
	Casein suspension	58.44	P<0.05
	Banana	15.67	P<0.01
	Sugar solution	11.40	P<0.001
	Distilled water	18.51	P<0.01
Green gram	Casein suspension	84.15	NS
	Banana	22.56	P<0.02
	Sugar solution	16.41	P<0.01
	Distilled water	26.65	P<0.02
Casein suspension	Banana	26.80	P<0.02
	Sugar solution	19.50	P<0.01
	Distilled water	31.66	P<0.05
Banana	Sugar solution	72.74	NS
	Distilled water	118.11	NS
Sugar solution	Distilled water	162.37	P<0.05

* The relative differences in protease activity in insects maintained in diets given in column A to that of column B are expressed as a percentage of activity in the latter over the former.

Significance of difference between the activities exhibited by insects maintained on different diets given in the column.

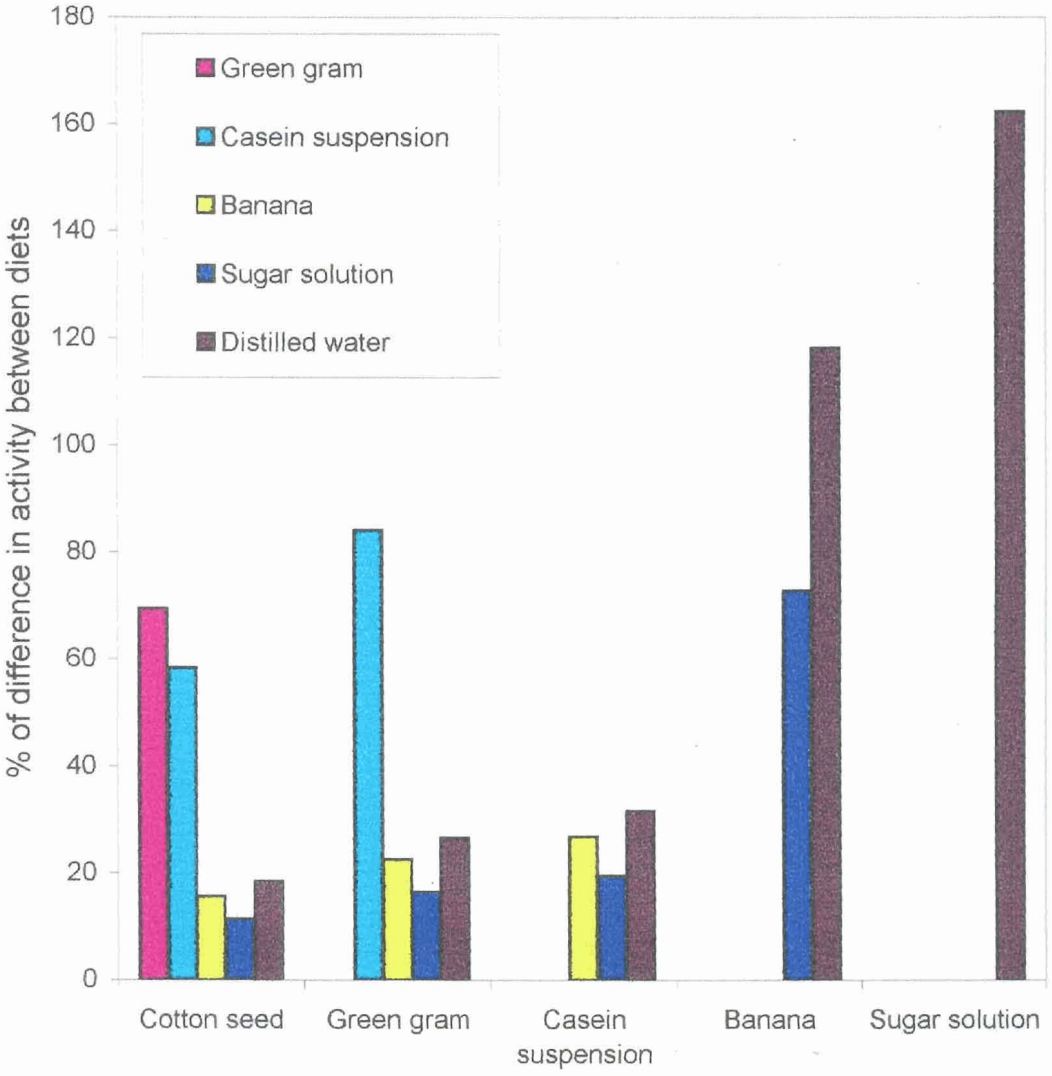


Figure 11. Percentage of midgut protease activity exhibited by male insects fed with different diets represented by bars over to that of diets represented in the X axis

proteolytic activity compared to cottonseed fed insects. The protease enzyme activities of the male and female insects fed with casein suspension were significantly ($p<0.05$) lower than those fed with cottonseeds. When the insects were fed with banana, sugar solution and distilled water the activity was very significantly reduced over cottonseed fed insects.

A relatively higher protease enzyme activity was exhibited by insects fed on soaked green gram. The activity in male insects was only 33.48% activity of that of female.

The protease enzyme activity of the male and female insects fed with casein suspension was lower to that of insects fed with soaked green gram, but these differences are not significant. When the insects are fed with banana, sugar solution and distilled water both male and female insects showed an activity lower to that of insects fed with green gram.

When the insects fed with casein suspension, the enzyme activity of male was reduced to 45.9% over that of female. The proteolytic activities of the male and female insects fed with banana, and distilled water were less than those of the insects fed with casein suspension. In the case of sugar solution fed insects a less significant ($p<0.05$) reduction in activity was observed.

The protease activity of insects fed on banana was very low. When both sexes were compared a less significant ($p<0.05$) reduction of 38.48%

in activity was exhibited by males. When the proteolytic activities of insects fed with sugar solution and distilled water were compared with that of banana fed insects both male and female insects showed no significant difference in activity.

The activity of protease enzyme was very low when the male and female insects were fed with sugar solution. The male insects showed only 33.9% of proteases activity of females. Compared to sugar solution fed insects, the distilled water fed insects showed an increased activity. The male insects fed with distilled water have only 43% of activity of that of females fed with distilled water.

Proteolytic activity of gut lumen content and gut tissue of midgut

Influence of different types of food on the activity of midgut lumen content and gut issue in relation to sex is shown in figure 12 and table 12.

Table 12
Figure 12

The pattern of enzyme activity in the midgut lumen content of female insects maintained on different diet were cottonseed >green gram > casein suspension > distilled water > banana > sugar solution. The enzyme activities in the female gut tissue and male lumen content were cottonseed > green gram > casein suspension > banana> distilled water > sugar solution.

Table 12. Effect of food in protease activity of the midgut of the lumen content and gut tissue*

	Female		Male	
Dietary regimen	Content	Tissue	Content	Tissue
Cotton seed	79.523 ± 9.23	15.234 ± 1.254	25.571 ± 3.254	6.114 ± 0.254
Green gram	70.855 ± 11.21	14.850 ± 1.654	21.311 ± 2.547	5.683 ± 1.240
Casein suspension	34.375 ± 5.26	13.247 ± 0.985	16.139 ± 1.654	2.511 ± 0.235
Banana	7.589 ± 1.30	9.251 ± 1.240	4.214 ± 1.113	2.540 ± 0.845
Sugar solution	7.561 ± 1.24	6.252 ± 1.114	3.124 ± 0.954	2.587 ± 0.652
Distilled water	9.301 ± 0.56	8.085 ± 1.214	3.240 ± 0.354	2.145 ± 0.265

*The values are the means of six determinations with ± SE

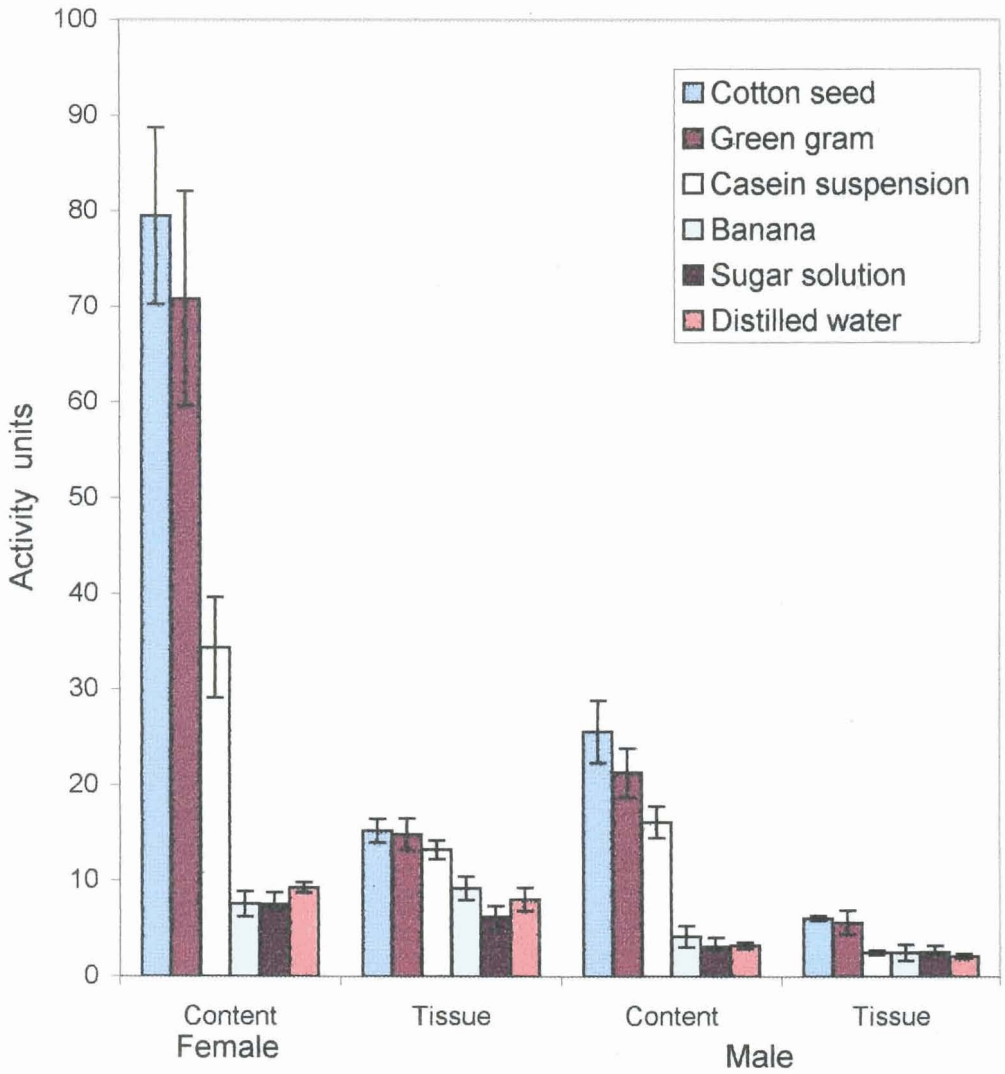


Figure 12. Proteolytic activity of lumen content and gut tissue of midgut

The relative activities of the proteolytic enzyme of gut lumen content and gut tissue exhibited by insects fed on different diets are shown in table 13 and 14 and figure 13 and 14.

Table 13 and 14
Figure 13 and 14

Though the activity pattern with reference to different diets was more or less same as that of midgut protease activity, it was noted that the activity in the gut tissue and lumen content of sugar solution fed males showed no significant difference from that of males fed on distilled water. In male insects contrary to the pattern observed in the midgut protease activity, the homogenate of gut tissue exhibited a significant difference between the insect fed with green gram/casein suspension. Similarly, the gut tissue of male insects fed with banana, sugar solution and distilled water showed no significant difference in the activity to that of casein fed males. The casein fed females showed a significant difference in protease activity of the gut lumen content with that of green gram fed ones but no significant difference from insects fed with cottonseed.

Hindgut protease activity

Influence of different types of food on the activity of midgut protease enzyme in relation to sex is shown in table 15 and figure 15 and 15A.

Table 15
Figure 15

Table 13. Relative activity of protease in the tissue and lumen content of the midgut region by female insects maintained on different diets.

Dietary regimen		*Relative activity in lumen content	Significance#	*Relative activity in tissue	Significance#
A	B				
Cotton seed	Green gram	89.100	NS	97.479	NS
	Casein suspension	43.227	P<0.05	86.957	NS
	Banana	9.543	P<0.001	60.726	P<0.05
	Sugar solution	9.508	P<0.001	41.039	P<0.05
	Distilled water	11.696	P<0.02	53.072	P<0.05
Green gram	Casein suspension	48.515	P<0.05	89.207	NS
	Banana	10.711	P<0.001	62.297	P<0.05
	Sugar solution	10.671	P<0.001	42.101	P<0.05
	Distilled water	13.127	P<0.01	54.445	P<0.05
Casein suspension	Banana	22.077	P<0.02	69.835	P<0.05
	Sugar solution	21.996	P<0.02	47.196	P<0.02
	Distilled water	27.057	P<0.05	61.033	P<0.05
Banana	Sugar solution	99.631	NS	67.582	NS
	Distilled water	122.559	NS	87.396	NS
Sugar solution	Distilled water	123.013	P<0.05	129.324	P<0.05

*The relative differences in protease activity in insects maintained in diets given in column A to that of column B are expressed as a percentage of activity in the latter over the former.

#Significance of difference between the activities exhibited by insects maintained on different diets given in the column.

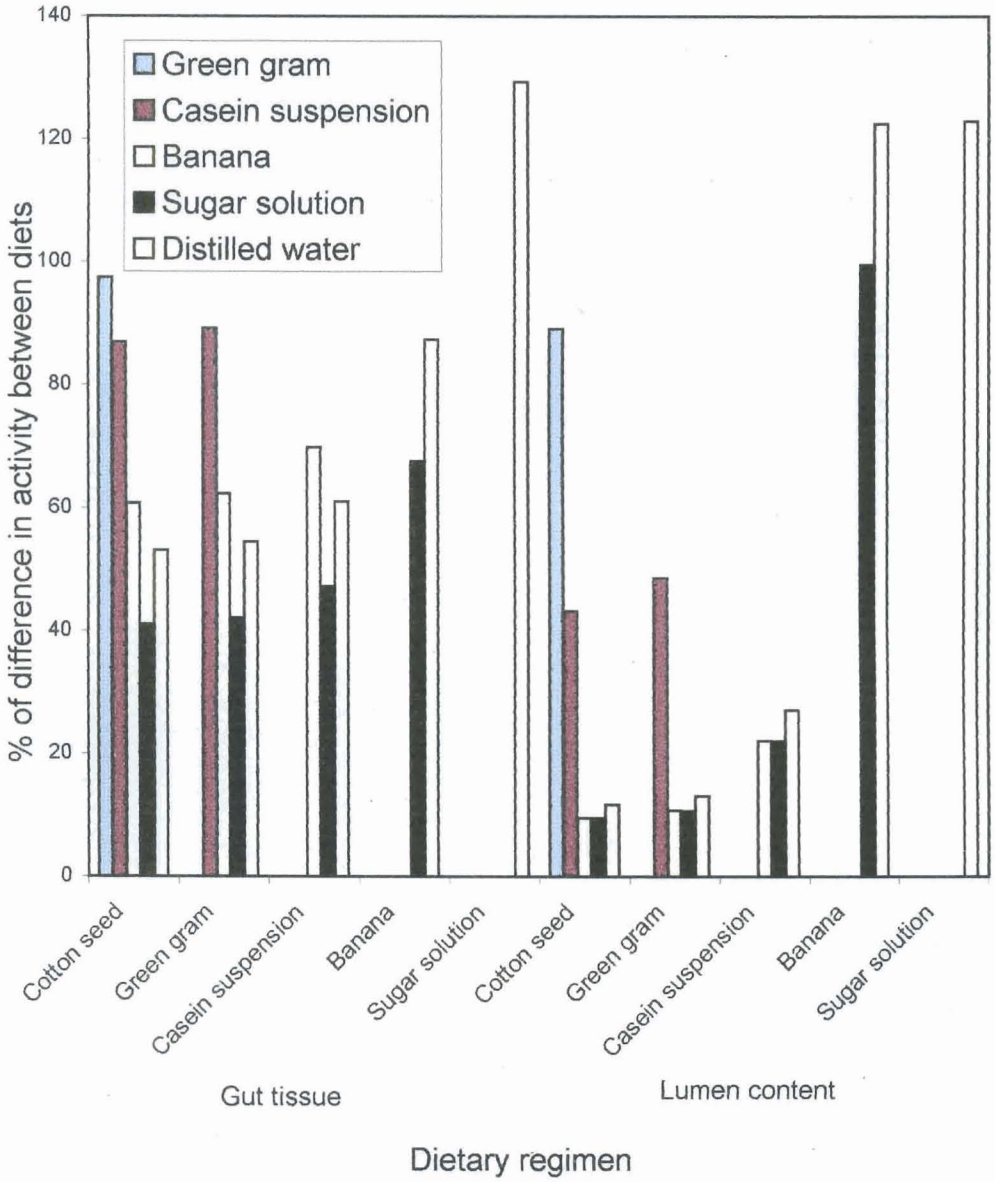


Figure 13. Difference in protease activity in the lumen content and gut tissue of the midgut by female insects fed on different diets.

Table 14. Relative activity of protease in the tissue and lumen content of the midgut of male insects maintained on different diets.

Dietary regimen		*Relative activity in lumen content	Significance#	*Relative activity in tissue	Significance#
A	B				
Cotton seed	Green gram	83.34	NS	92.95	NS
	Casein suspension	63.11	P<0.05	41.07	P<0.05
	Banana	16.48	P<0.001	41.54	P<0.05
	Sugar solution	12.22	P<0.001	42.31	P<0.05
	Distilled water	12.67	P<0.001	35.08	P<0.05
Green gram	Casein suspension	75.73	NS	44.19	P<0.05
	Banana	19.77	P<0.05	44.70	P<0.05
	Sugar solution	14.66	P<0.05	45.52	P<0.05
	Distilled water	15.20	P<0.05	37.74	P<0.02
Casein suspension	Banana	26.11	P<0.01	101.17	NS
	Sugar solution	19.36	P<0.001	103.04	NS
	Distilled water	20.07	P<0.01	85.43	NS
Banana	Sugar solution	74.13	NS	101.85	NS
	Distilled water	76.89	NS	84.45	NS
Sugar solution	Distilled water	103.71	NS	82.92	NS

* The relative differences in protease activity in insects maintained in diets given in column A to that of column B are expressed as a percentage of activity in the latter over the former.
#Significance of difference between the activities exhibited by insects maintained on different diets given in the column.

42 594

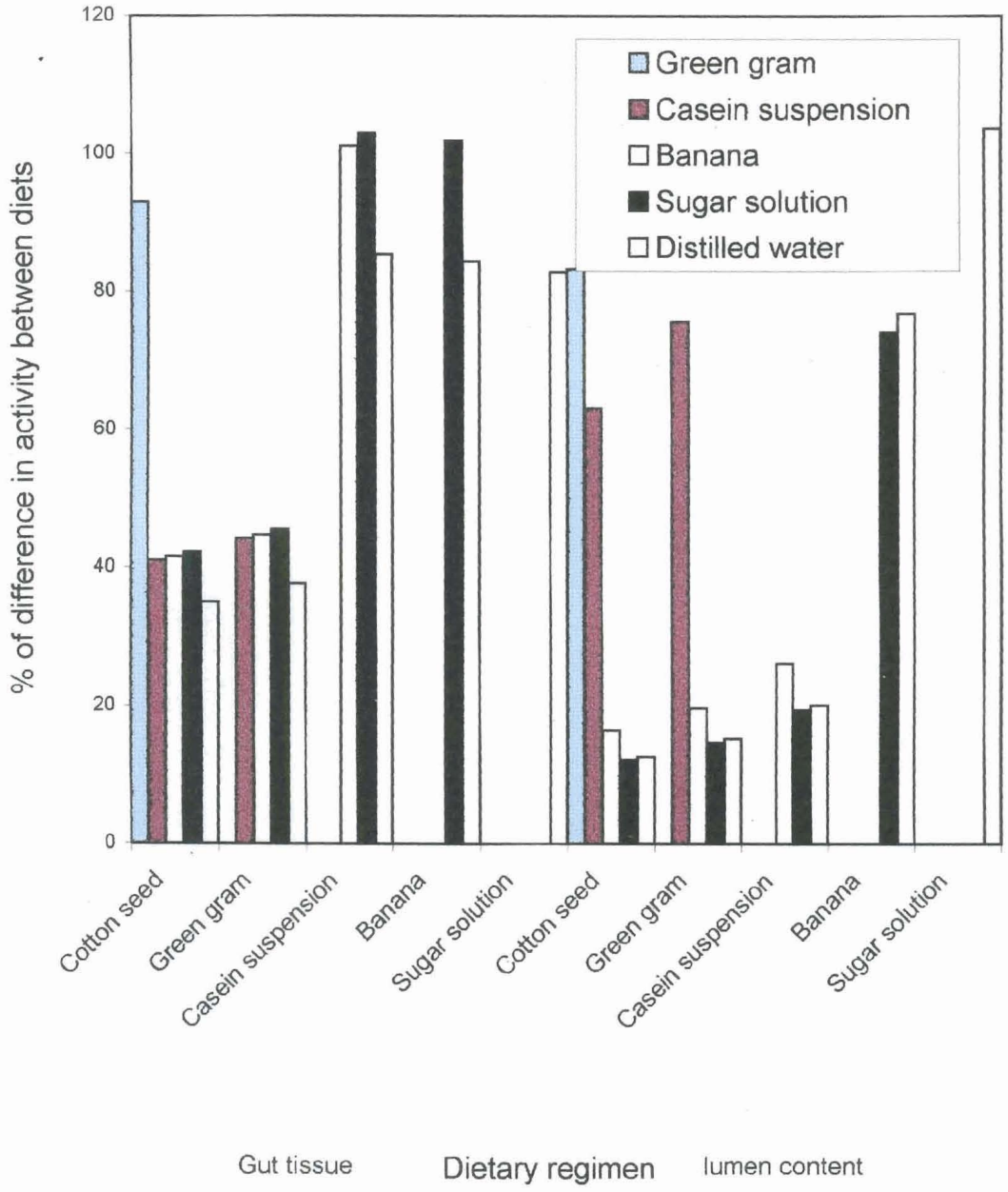


Figure 14. Difference in protease activity in the gut tissue and lumen content of the midgut by male insects fed on different diets.

Table 15. Effect of food in protease activity of the hindgut

Dietary regimen	Activity units *		#Relative activity	$significance
	Female	Male		
Cotton seed	23.652 ± 2.13	11.826 ± 1.57	200.00	P<0.001
Green gram	20.223 ± 1.95	8.254 ± 1.42	245.00	P<0.001
Casein suspension	12.252 ± 1.24	6.283 ± 0.54	195.00	P<0.05
Banana	4.685 ± 0.56	2.296 ± 0.84	204.00	P<0.05
Sugar solution	0.316 ± 0.12	0.131 ± 0.11	241.25	NS
Distilled water	3.500 ± 0.21	1.866 ± 0.15	187.56	P<0.05

*The values are the means of six determinations with ± SE

The relative activities are expressed as the percentage difference in proteolytic activity in females over males

$ Significance of difference between the activities of males and females

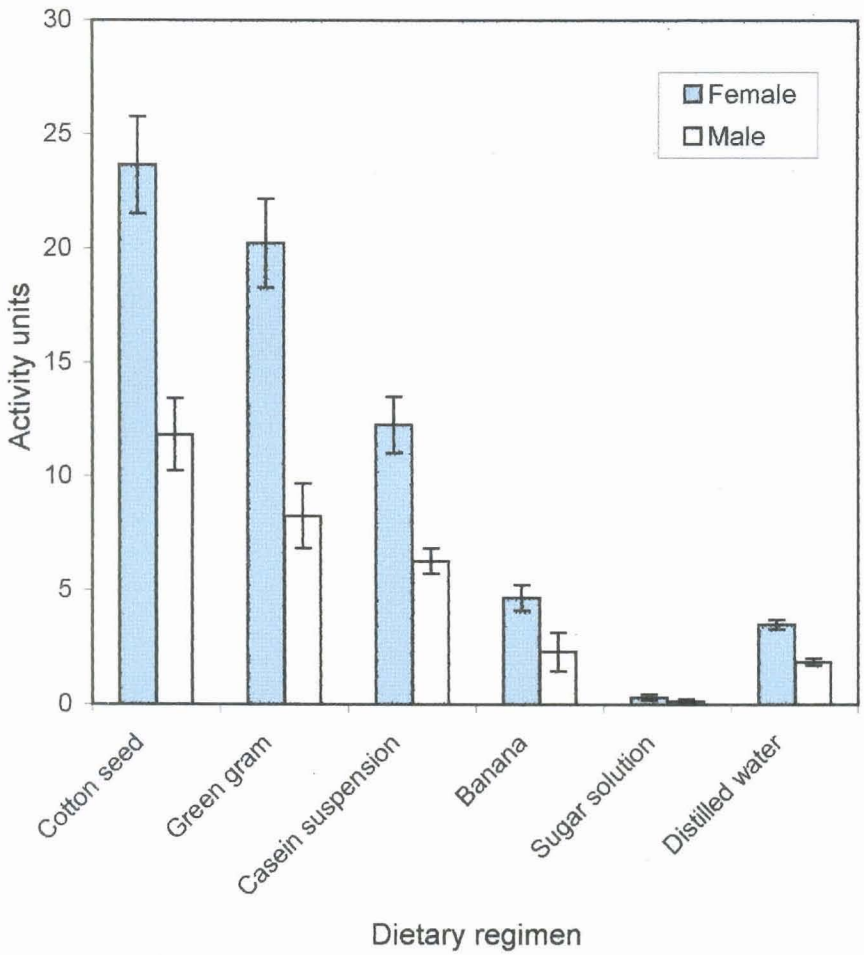


Figure15. Effect of food on protease activity of hindgut

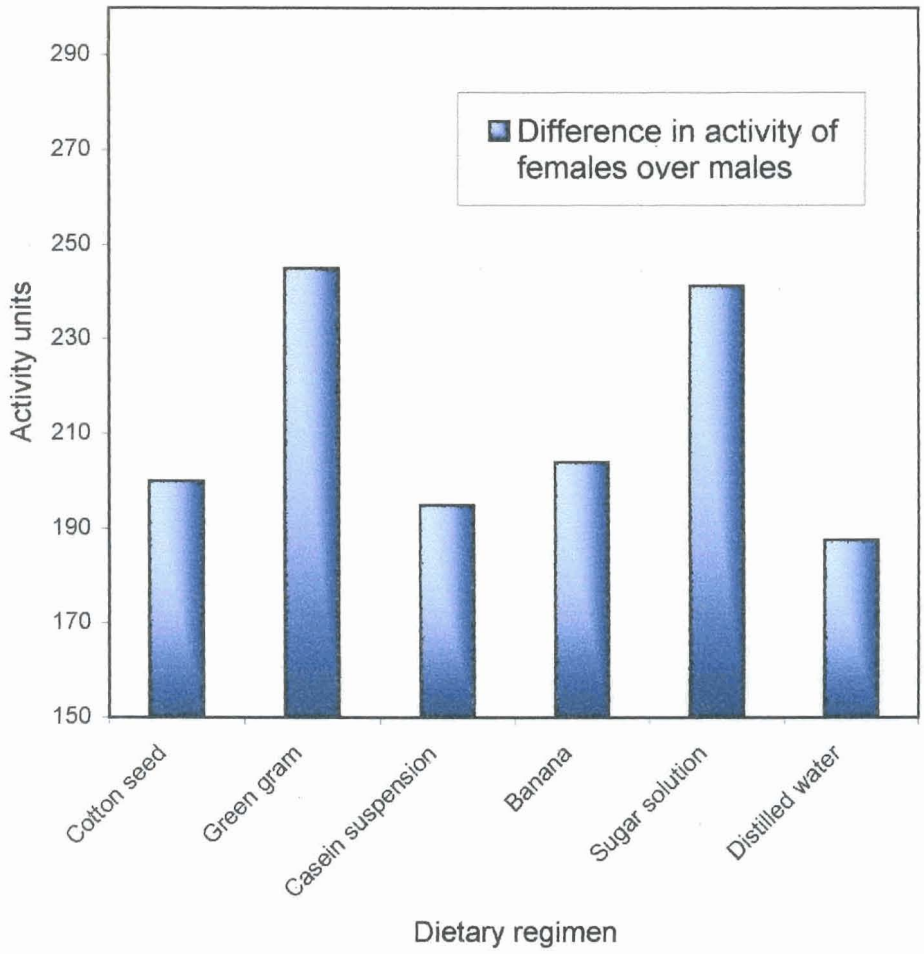


Figure 15A. Percentages of hindgut protease activity of females over males

The patterns of protease activities in the hindgut of male and female insects maintained on different diets were the same. The relative activities were cottonseed > green gram > casein suspension > banana > distilled water> sugar solution. The relative activity difference between male and female insects fed on sugar solution was not significant.

Figure 15 A

The relative activities of hindgut protease exhibited by insects fed on different diets are shown in table 16 and 17 and figure 16 and 17.

Table 16 and 17
Figure 16 and 17

The pattern of proteolytic activity of hindgut with reference to different diet was generally similar to that of midgut. Sugar solution fed insects showed a significant reduction in activity, i.e., 6.764% in females and 5.75% in males, than banana fed insects.

Casein suspension fed males showed a reduction (58.44%) in activity than cottonseed fed males, as in the case of total hindgut activity but this difference was not statistically significant at 5% level.

Proteolytic activity of gut lumen content and gut tissues of hindgut

Influence of different types of food in the activity of hindgut lumen content and gut tissue in relation to sex is shown in table 18 and figure 18.

Table 18
Figure 18

Table 16. Relative activity of protease in the hindgut of female insects maintained on different diets.

Dietary regimen		*Relative activity	#Significance
A	B		
Cotton seed	Green gram	85.499	NS
	Casein suspension	51.802	P<0.05
	Banana	19.808	P<0.01
	Sugar solution	1.340	P<0.0001
	Distilled water	14.798	P<0.001
Green gram	Casein suspension	60.588	NS
	Banana	23.168	P<0.05
	Sugar solution	1.567	P<0.001
	Distilled water	17.308	P<0.01
Casein suspension	Banana	38.239	P<0.02
	Sugar solution	2.587	P<0.001
	Distilled water	28.568	P<0.01
Banana	Sugar solution	6.764	P<0.01
	Distilled water	74.708	NS
Sugar solution	Distilled water	1104.421	P<0.01

* The relative differences in protease activity in insects maintained in diets given in column A to that of column B are expressed as a percentage of activity in the latter over the former.

\# Significance of difference between the activities exhibited by insects maintained on different diets given in the column.

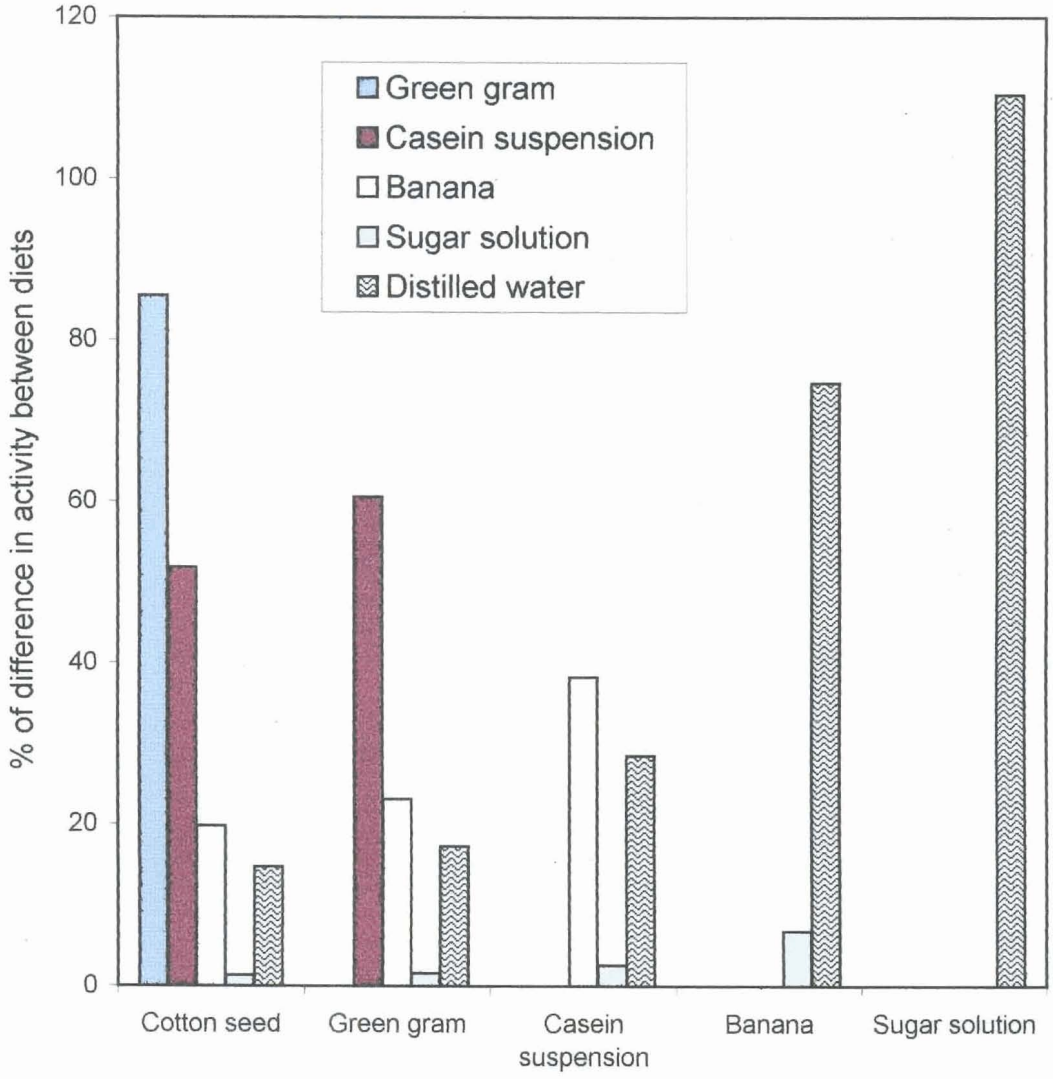


Figure 16. Percentage of hindgut protease activity exhibited by female insects fed with different diets represented by bars over to that of diets represented in the X axis

Table 17. Relative activity of protease in the hindgut of male insects maintained on different diets

Dietary regimen		*Relative activity	#Significance
A	B		
Cotton seed	Green gram	69.80	P<0.05
	Casein suspension	53.13	NS
	Banana	19.42	P<0.02
	Sugar solution	1.11	P<0.0001
	Distilled water	15.78	P<0.001
Green gram	Casein suspension	76.12	NS
	Banana	27.82	P<0.02
	Sugar solution	1.59	P<0.001
	Distilled water	22.61	P<0.02
Casein suspension	Banana	36.55	P<0.05
	Sugar solution	2.09	P<0.01
	Distilled water	29.70	P<0.02
Banana	Sugar solution	5.72	P<0.01
	Distilled water	81.26	NS
Sugar solution	Distilled water	1420.57	P<0.001

* The relative differences in protease activity in insects maintained in diets given in column A to that of column B are expressed as a percentage of activity in the latter over the former.

Significance of difference between the activities exhibited by insects maintained on different diets given in the column.

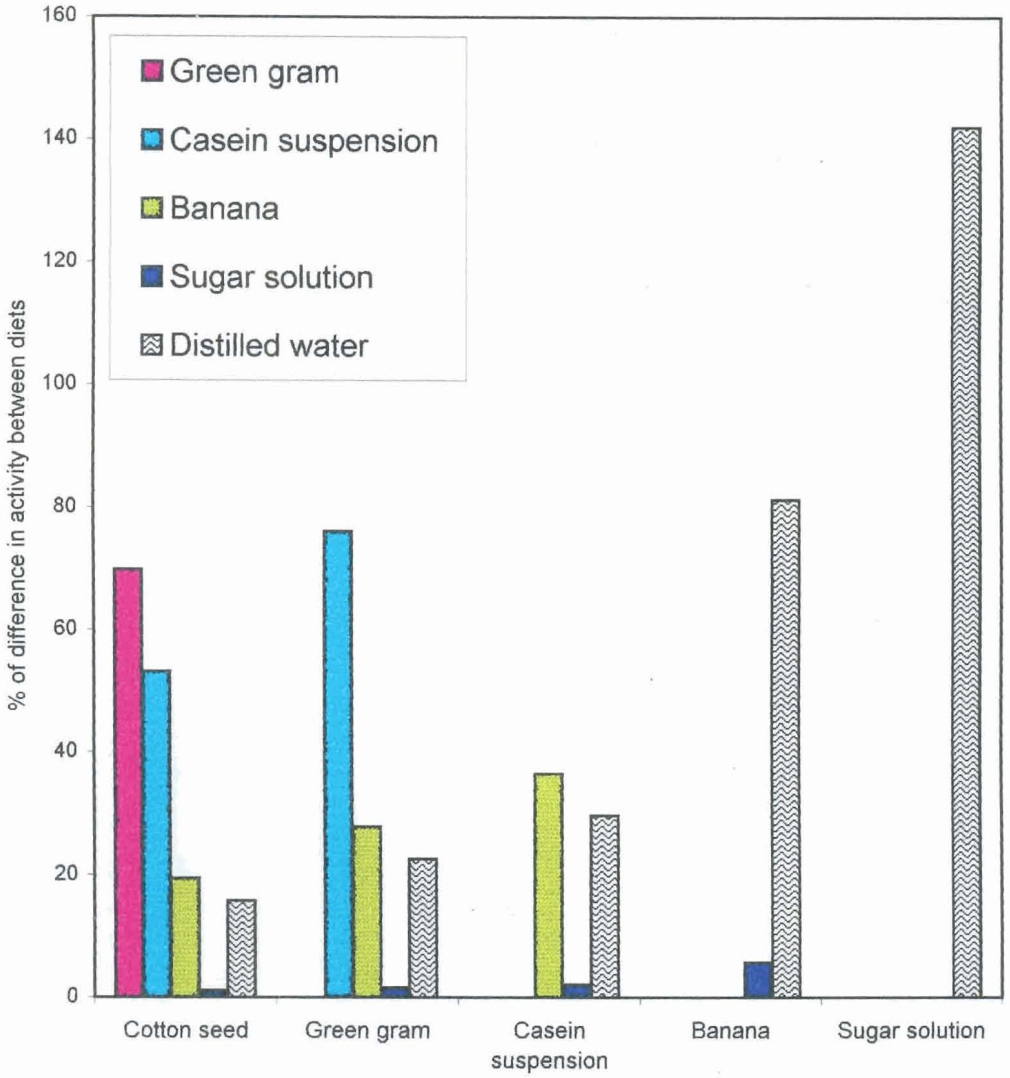


Figure 17. Percentage of hindgut protease activity exhibited by male insects fed with different diets represented by bars over to that of diets represented in the X axis

Table 18. Proteolytic activity of lumen content and gut tissue of hindgut *

	Female		Male	
Dietary regimen	Content	Tissue	Content	Tissue
Cotton seed	38.081 ± 5.62	9.956 ± 1.02	19.158 ± 11.82	4.154 ± 0.21
Green gram	31.34 ± 4.25	8.89 ± 1.54	9.905 ± 8.25	3.301 ± 1.02
Casein suspension	18.861 ± 1.25	5.245 ± 1.36	7.654 ± 6.28	1.242 ± 0.51
Banana	4.215 ± 1.11	2.354 ± 0.98	2.648 ± 2.29	0.958 ± 0.11
Sugar solution	1.213 ± 0.25	1.212 ± 0.56	0.3254 ± 0.13	0.096 ± 0.01
Distilled water	3.546 ± 0.28	1.356 ± 0.21	2.541 ± 1.86	1.012 ± 0.21

*The values are the means of six determinations with ± SE

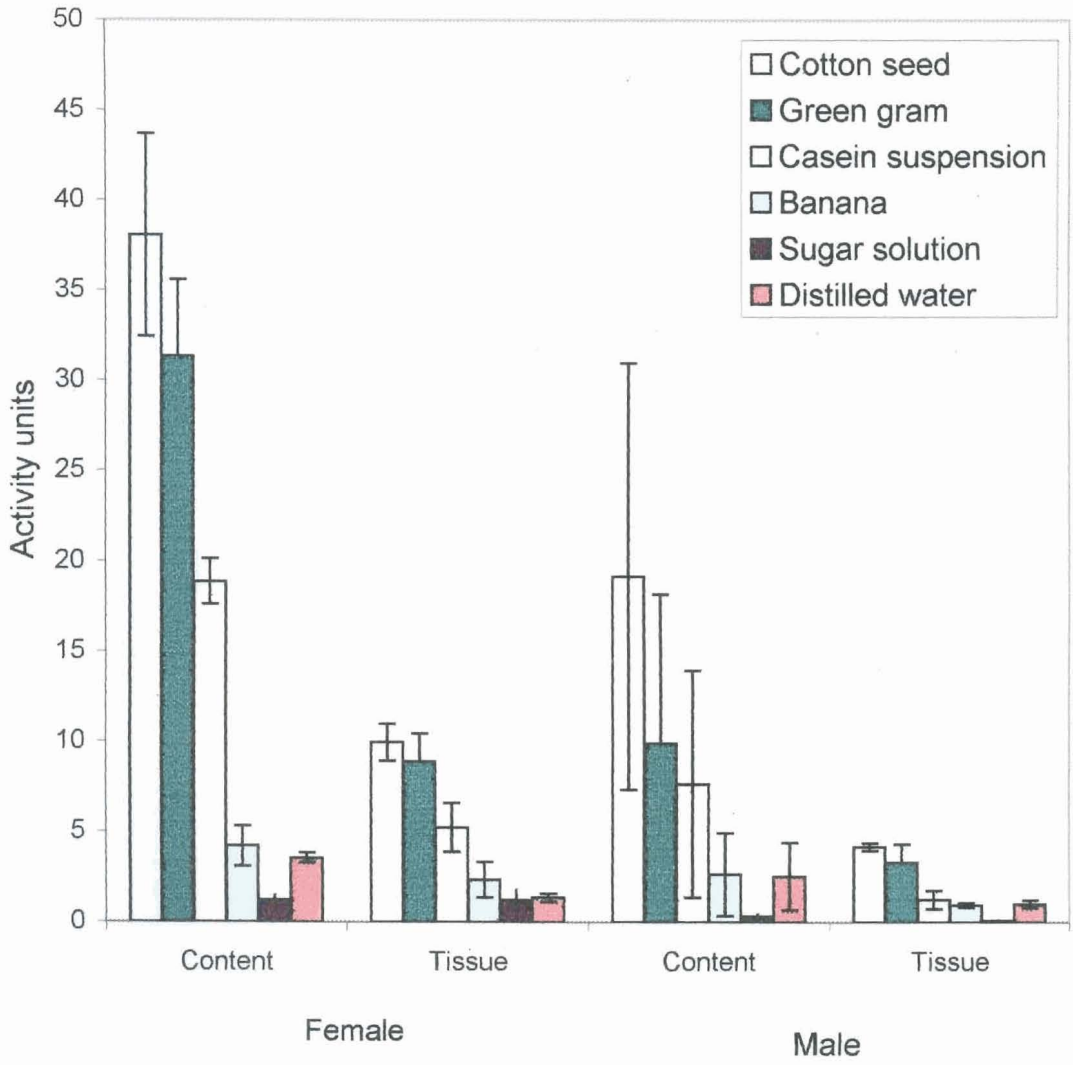


Figure18. Proteolytic activity of hindgut tissue and lumen content

60F

52

The relative protease activities of the lumen content and gut tissue of the hind gut of female and the lumen content of male insects maintained on different diets were: cottonseed > green gram > casein suspension > banana > distilled water> sugar solution. In the gut tissue of male insects the relative activities were cottonseed> green gram> casein suspension> distilled water> banana> sugar solution. The relative activities of the proteolytic enzyme of gut lumen content and gut tissue exhibited by insects fed on different diet are shown in table 19 and 20 and figure 19 and20.

Table 19 and 20
Figure 19 and 20

The gut lumen content and tissue of the female insects generally follow the same pattern of protease activity with reference to different diet of total gut protease activity. The difference between the proteolytic activities exhibited by insects fed with different diet was not significant in majority of cases.

Table 19. Relative activity of protease in the tissue and lumen content of the hindgut of female insects maintained on different diets

Dietary regimen		*Relative activity in lumen content	Significance#	*Relative activity in tissue	Significance#
A	B				
Cotton seed	Green gram	82.30	NS	89.29	NS
	Casein suspension	49.55	P<0.05	52.68	P<0.05
	Banana	11.07	P<0.02	23.64	P<0.02
	Sugar solution	3.19	P<0.001	12.17	P<0.02
	Distilled water	9.31	P<0.01	13.62	P<0.02
Green gram	Casein suspension	60.21	P<0.05	58.99	NS
	Banana	13.45	P<0.02	26.48	P<0.05
	Sugar solution	3.87	P<0.001	13.63	P<0.05
	Distilled water	11.31	P<0.01	15.25	P<0.05
Casein suspension	Banana	31.34	P<0.05	44.88	P<0.05
	Sugar solution	9.02	P<0.02	23.11	P<0.05
	Distilled water	26.37	P<0.05	25.85	P<0.05
Banana	Sugar solution	28.78	P<0.05	51.49	NS
	Distilled water	84.13	NS	57.60	NS
Sugar solution	Distilled water	292.33	P<0.05	111.88	NS

*The relative differences in protease activity in insects maintained in diets given in column A to that of column B are expressed as a percentage of activity in the latter over the former.

#Significance of difference between the activities exhibited by insects maintained on different diets given in the column.

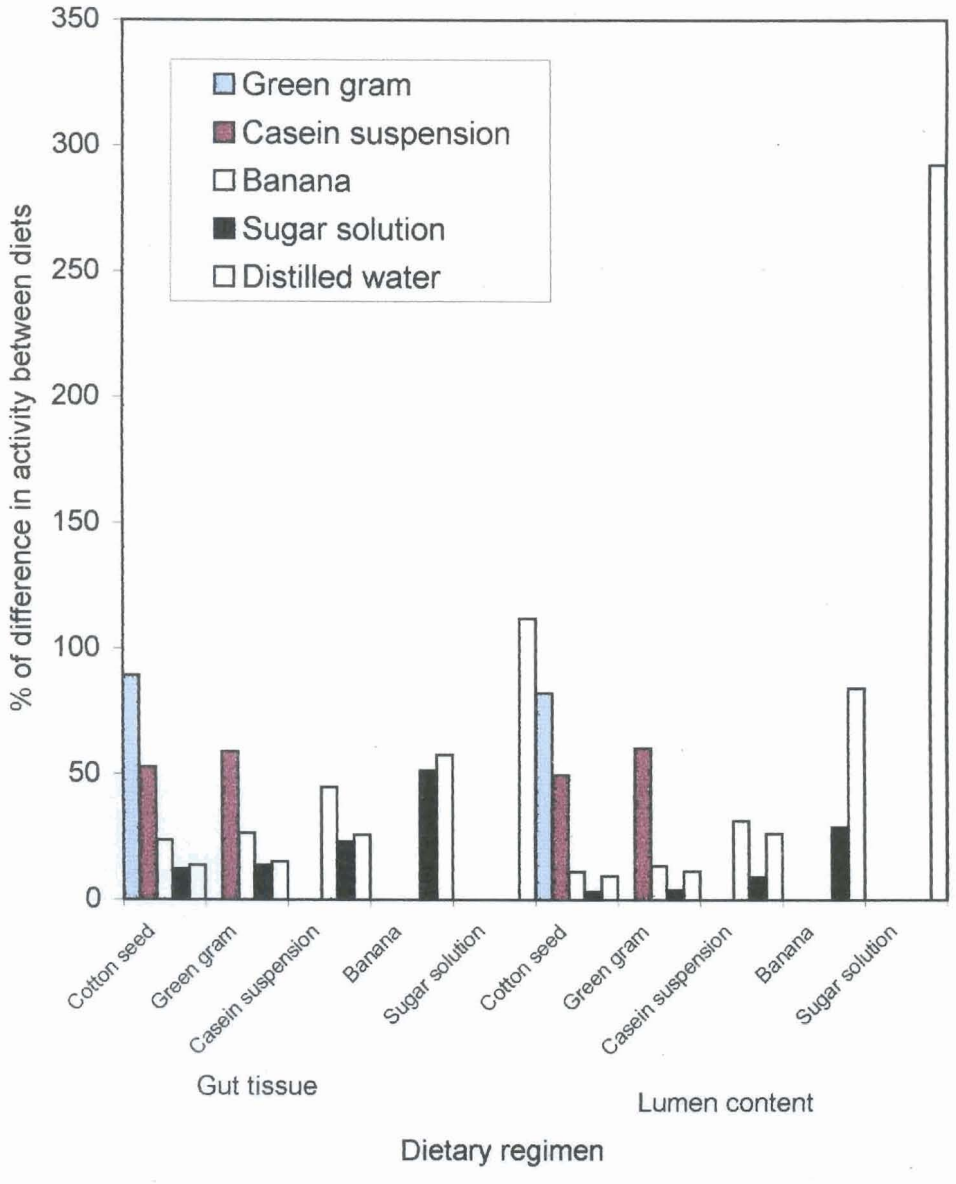


Figure 19 Difference in protease activity in the gut tissue and lumen content of the hindgut exhibitted by female insects fed on different diets.

Table 20. Relative protease activity in the tissue and lumen content of the hindgut region by male insects maintained on different diets.

Dietary regimen		*Relative activity in lumen content	Significance#	*Relative activity in tissue	Significance#
A	B				
Cotton seed	Green gram	51.70	NS	79.48	NS
	Casein suspension	39.95	NS	29.90	P<0.05
	Banana	13.82	P<0.05	23.07	P<0.05
	Sugar solution	1.70	P<0.02	2.32	P<0.001
	Distilled water	13.26	P<0.05	24.36	P<0.05
Green gram	Casein suspension	77.27	NS	53.58	NS
	Banana	26.73	NS	41.34	P<0.05
	Sugar solution	3.29	NS	4.15	P<0.02
	Distilled water	25.65	NS	43.66	NS
Casein suspension	Banana	77.17	NS	77.17	NS
	Sugar solution	7.754	P<0.05	7.76	P<0.05
	Distilled water	81.48	NS	81.48	NS
Banana	Sugar solution	12.29	NS	10.05	P<0.05
	Distilled water	95.96	NS	105.59	NS
Sugar solution	Distilled water	780.89	NS	1050.88	P<0.05

*The relative differences in protease activity in insects maintained in diets given in column A to that of column B are expressed as a percentage of activity in the latter over the former.

#Significance of difference between the activities exhibited by insects maintained on different diets given in the column.

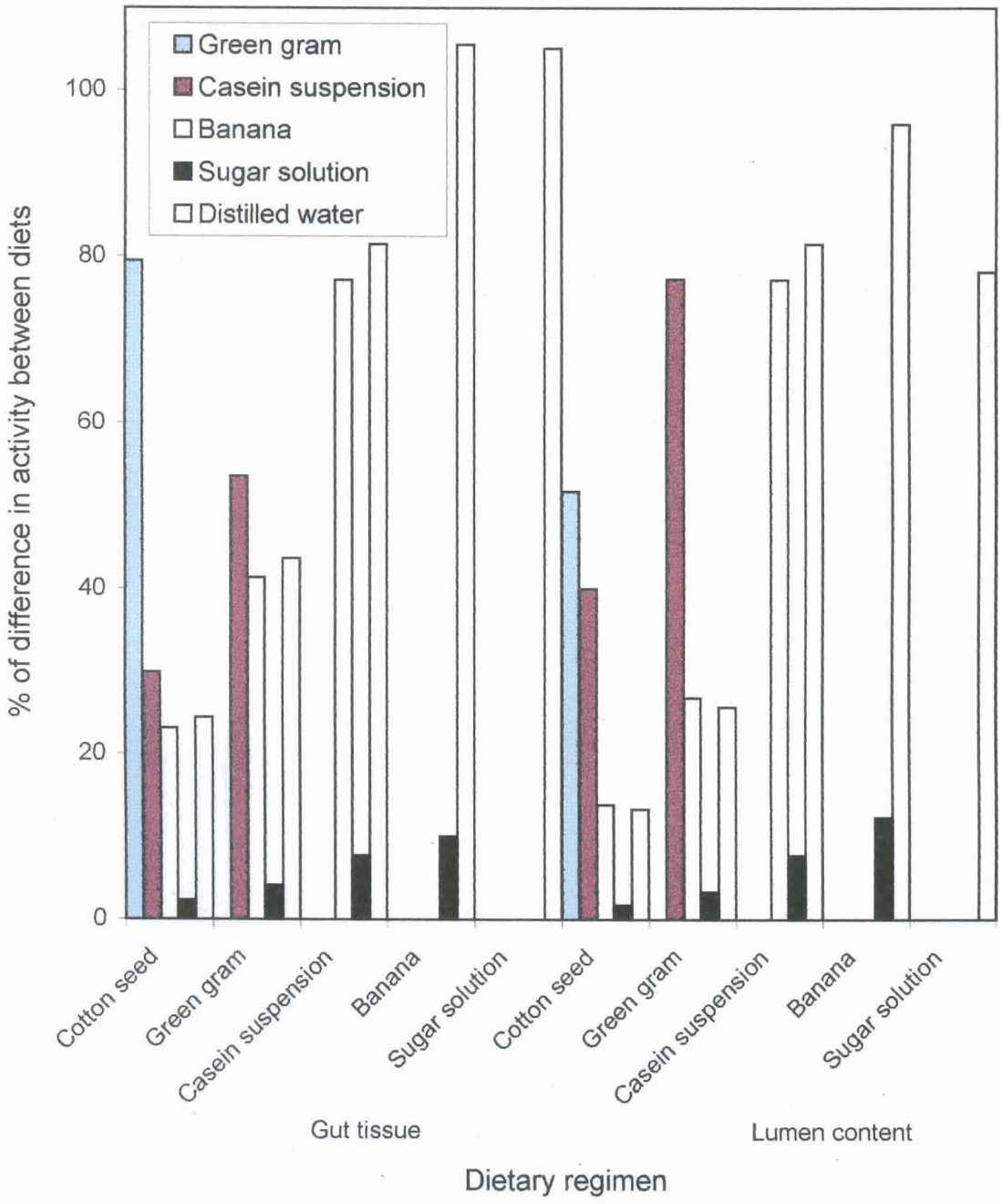


Figure 20. Difference in protease activity in the lumen content of the hindgut region by male insects fed on different diets.

DISCUSSION

K.S. Sajan “Studies on the protease activity in the digestive system of iphita limbata (Heteroptera : Pyrrhocoridae)” Thesis. Department of Zoology , University of Calicut, 2002

Discussion

DISCUSSION

General

I. limbata is a phytophagous bug feeding on plant sap, fruits and seeds. The greengram in which the bugs are maintained naturally contain more amount of protein when compared to that of other phytophagous insects feeding on leaf / stem. Since the digestive enzymes may have a direct relation to the concentration of the particular substrate in the feed material, it is expected that the proteolytic activity in *I. limbata* alimentary tract would be high when compared to other enzymatic activity as well as other phytophagous insects. A high protease activity observed in the present study that in the enzyme preparations from different parts of the alimentary canal (midgut and hindgut) of *I. limbata* was tune with the above. Protease activity of the midgut is reported in a number of other hemipteran insects like *D. cingulatus* (Muraleedharan and Prabhu, 1978), *D. fasciatus* (Geering-Sacher, 1972), *Cimex hemipterus* and *C. lectularius* (Houseman and Downe, 1982b), *L. disponsi* (Hori, 1970) and *L. rugulipennis* (Varis *et al.,* 1983, Colebatch *et al.,* 2001). In the present study, highest protease activity was observed in the lumen content of the adult female insects fed with cottonseed. In *L. rugulipennis* (Hori, 1973) midgut protease activity was only 5.6 µg of tyrosine/ gut at pH 7.7 and 30°C during 16 hours of incubation. However, the activity observed in *I. limbata* was far higher than that of *L. rugulipennis*. The midgut of the

larvae of *S. mauritia* exhibits a protease activity of 925±12 µg tyrosine/ gut/20 hour of incubation (Prema and Mohamed, 1980). The females of 7-day-old adults *B. mori* exhibited 1.52-µg tyrosine/ mg protein/min of protease activity (Jadhav and Kallapur, 1988). However, a low protease activity was reported in *Locusta* (Anstee and Charnley, 1977) and it was found to be only 60 µmoles of product formed/gut/min × 10^2. Protease enzyme activity was reported in a number of other insects. Protease activity was found in the midgut of *M. pustulata* (Srivastava and Srivastava, 1957). Three proteolytic enzymes have been separated by electrophoresis in *Musca* (Patel and Richards, 1960) and *Stomoxys* (Patterson and Fisk, 1958).

The low activity of protease using casein as a substrate at pH below 3.0 suggested the relatively low activity of pepsin-like enzyme in the digestive tract of adult *I. limbata.*

Though acidic, the optimum pH at 5.0 for proteases observed in the present study suggests the possible absence of pepsin in the gut of *I. limbata.* Pepsin has been qualitatively demonstrated in some lepidopterous larvae, *Gnorimoschema operculella* (Balyan, 1973) *Platyedra gossypiella* (Verma and Balyan, 1972) and *Sylepta derogata* (Verma *et al.,* 1977) and Orthoptera, *Gryllodes sigillatus* (Verma and Prasad, 1975) and Coleoptera, *M. pustulata* (Verma and Prasad, 1972).

The presence of increased hydrolytic activity at the acidic pH from 3.0 to 5.0 indicated the presence of a cathepsin like enzyme in *I. limbata* tissue preparation. Cathepsin is the most common intracellular autolytic enzyme in animal tissues (Baldwin, 1963), mostly acting at fairly acidic pH (Barnard and Prosser, 1973). However, this enzyme has been reported to function extracellularly in the digestive system of some invertebrates (Prosser and Brown, 1965). A protease similar to mammalian cathepsin, with respect to the optimum pH has been observed in the gut of *Laccotrephes maculatus* (pH 4.0; Khan, 1964), *R. prolixus* and *C. lectularius* (pH 5.0; Gooding, 1969), *Spodoptera eridania* (pH 4.0; Young, 1978) and in the salivary gland of *P. americana* (pH 4.0; Agarwal and Bahadur, 1981). The activation requirements and pH optimum of the protease from *R. prolixus* indicated cathepsin-B like enzymes (Houseman, 1978; Houseman and Downe, 1980). In spite of all these reports on the acidic pH optimum for this enzyme, Fruton (1960) described a cathepsin-C, which was active over the pH range 4.0 – 8.0. A number of cathepsins are known in insects the more important being cathepsin B and cathepsin-D. From *Schistosoma mansoni* a cathepsin-B like enzyme is partially characterized by Gotz and Klinkert (1993). The enzyme is reported in *G. morsitans morsitans* (Yan *et al.,* 2002). A cathepsin-D was characterized in colorado potato beetle, *L. decemlineata* (Brunelle *et al.,* 1999). A cathepsin L-like enzyme was expressed in the *D. melanogaster* (Tryselius and Hultmark, 1997).

Matsumoto *et al.*, (1997) identified and characterized a gene family comprising at least four genes encoding cathepsin L-like cysteine proteinases in *S. zeamais*.

Optimum conditions for activity

Hydrogen ion concentration

Maximum general proteases activity at pH 5.0 from the hindgut and midgut of *I. limbata* clearly suggests that an acidic gut lumen is suitable for this enzyme. Both sexes and both gut portions of the bug show the same optimal pH. The pH optimum for protease in many of the insects lies in a fairly acidic range (Liang *et. al*, 1991; Blanco-Labra *et al.*, 1996; Hernadez-Alvrez *et. at*, 2000; Wilhite *et al.*, 2000; Colebatch *et al.*, 2001). The pH-activity relations of the protease from several other hemipteran insects have been reported by several authors: The salivary gland of *Graphocroerus ventralis* exhibited a weak protease activity between pH 5.4 and 7.0 and the salivary protease of *Euacanthus interruptus* was active at weak acid and weak alkaline sides but not active at the neutral zone (Nuortera, 1956). The protease in the alimentary canal of *Laccotrephis maculatus* was active when pH values were between 4.0 and 10.0, with two optimum activities at pH 5.0 and 8.5 (Khan, 1964).

pH optima for different portions of the gut may vary in insects. In *N. cinerea* the anterior and posterior halves of midgut shows different pH optima (Elpidinia *et al.*, 2001). pH of gut lumen is much higher than that

of the gut wall in lepidopteran larvae (Waterhouse; 1949). In the present study, both portions of the gut (hindgut and midgut) show the same optimal pH. In *C. dilutus*, a hemipteran insect, the pH optimum of midgut extracts was acidic (pH 4.0), implying that acidic proteases predominate (Colebatch *et al.*, 2001). The maximal haemoglobinolytic activity in alfalfa weevil (*Hypera postica*) was at pH 3.5, but azocaesinolytic activity was maximal at pH 5.0 (Wilhite *et al.*, 2000). In *Diabrotica undecimpunctata howardi*, a coleopteran, midgut proteinase exhibits a slightly acidic optimum (Fabrick *et al.*, 2002). The salivary protease of *Oxycarenus hyalinipennis* was very active at pH 7.2, moderately active at pH 5.4 but not active at pH 3.0, while the proteases of the first and second ventriculi of the same insect were very active equally at pH 5.4 and 7.2 (Saxena and Bhatnagar, 1958). The pH inside the gut of non-blood fed female *Lutzomyia longipalpis* was measured with pH indicator dyes. The pH ranges obtained for crop, midgut, and hindgut were, higher than pH 6.0, pH 6.0 and lower than pH 6.0 respectively, (Gontizo *et al.*, 1998).

The activity of protease in *I. limbata*, though low at alkaline pH, points to an active proteinases at alkaline pH in the midgut. It is possible that substrates other than casein like synthetic substrates may be hydrolyzed at higher pH. Substantial evidences are available in insects where the same enzyme preparation hydrolysed different protein

substrates at different pH optima (Shino, 1930 a; Tatchell; 1958; Jany *et al.*, 1978 a).

Substrate concentration

The rate of casein hydrolysis by the homogenates of the midgut of *I. limbata* increased proportionally up to 1.5% casein in the *in vitro* test system. The K_m values calculated suggest a more affinity of midgut protease (low K_m) than hindgut protease (high K_m). Similarly, female insects showed more affinity than male insects. An appraisal of the literature on substrate concentration – activity relationships in insects indicate that a sex difference exists in the case of K_m values. The K_m values for *Pterostichus melanarius* gut protease, were 2.35 for males and 0.51 for females using denatured haemoglobin as substrates and 9.4 for males and 16.67 for females using bovine serum albumen as substrate (Gooding and Huang, 1969). The optimum casein concentration for midgut protease from *Tenebrio* and *Tribolium* (Birk *et al.*, 1962) and *Arthrodeis* (Rao and Rastogi, 1967) was 0.5% and from *B. mori* it was 0.4-0.5% (Eguchi and Iwamoto, 1976). K*m* value for trypsin like enzymes in *Osrinia nubialis* differs with different substrates (Bernardi., *et al.*, 1996).

Reaction temperature

As in the case of many other insects, the digestive proteases of *I. limbata* had a high temperature optimum. Temperature higher than 45°C resulted in rapid inactivation of the protease. The optimum temperature for

protease was 45°C in *M. domestica* (Lin and Richards, 1956), 44°C in *C. erythrocephalus* (Evans, 1958), 50°C in *S. calcitrans*, (Patterson and Fisk, 1958), 37°C in *T. castaneum*, *T. confusum* and *T. molitor* (Birk, *et al.*, 1962), 46 to 50°C in *A. aegypti* and *C. fatigans* (Gooding, 1966), 30°C in *Arthrodeis* (Rao and Rastogi, 1967), 47°C in *P. melanarius* (Gooding and Huang, 1969), 50°C in *S. littoralis* (Ishaaya *et al.*, 1971), 37°C in *L. disponsi* (Hori, 1973) and 55°C in *A. megatoma* (Baker, 1976). The trypsin like enzyme of European corn borer-larvae, *Ostrinia nubialis* shows a remarkable thermal stability of about 53°C (Bernardi *et al.*, 1996). Optimum temperature for an exocellular protease of *Serratia marcescens* was 50°C (Kaska *et al.*, 1976). Temperature optima for midgut proteases of *Heliotheri armigera* and *H. assulta* were 55°C and 60°C respectively (Xu *et al.*, 1994). The values obtained in the present study is comparable with those of the reported values.

Difference in protease activity between males and females

The protease activity of hindgut and midgut of *I. limbata* showed a sexual dimorphism. The females showed more proteolytic activity than the males. Insects fed with different diets also exhibited this difference. Similar high activity of digestive enzymes in female insects was reported in *B. mori* (Fujii and Kato, 1930), *L. migratoria* (Khan 1963), *D. melanogaser* (Doane, 1969b) and *Simulium venustum* (Yang and Davies, 1968b).

A lower level of digestive enzyme activity in the female than in the male was also reported in *Pterostichus melanarius* (Gooding and Huang, 1969). Increased enzyme activity in one or other stages of females over to that of males was found in few insects (Geering-Sacher, 1972; Das and Das 1982, Varis *et al.*, 1983; Baumann, 1990).

In *D. fasciatus* (Greening-Sacher, 1972), the females show a higher proteolytic activity than males which was correlated for the proteinase production and the maturation of eggs. In the fifth instar larva of *B. mori* sexual dimorphism in the midgut protease was evident, as female showed significantly higher protease activity than the male (Jadhav and Kallapur, 1988). According to the authors, the protein content of the female is always higher than male because of egg production.

A higher protease activity was also found in females of adult simulids and was interpreted in the context of its feeding habits (Yang and Davies, 1968). In this insect, the trypsin enzyme activity was same in both males and females when fed with sugar solution. However, when the insects were fed with blood-sucrose mixture the trypsin activity in females was increased but that of males remained the same. It may be noted that the female simulids are naturally bloodsuckers, but males are not.

In cockroaches, the proteolytic enzymatic activity of the caeca of males was twice higher than that of females (Baumann, 1990). In *L. rugulipennis* (Varis *et al.*, 1983) a significantly high activity was detected in

females than that of the males for 4 enzymes viz., amylase, polygalactouronase, proteinase and phosphatase. Sexual dimorphism in the levels of the activities and in the pattern of thermal acclimation of digestive enzymes was also observed in *P. americana* (Das and Das, 1982).

Early trypsin, a female–specific midgut protease in *A. aegypti* was isolated by Noriega *et al.*, (1996). This enzyme is present in the midgut during the first hours after ingestion of a blood meal and plays an essential role in the transcriptional activation of the late trypsin form, the major midgut endoprotease involved in the blood meal digestion.

Cycles of definite patterns of midgut protease activity are shown by female *D. cingulatus* and it can be comparable to vitellogenic activity (Muraleedharan and Prabhu, 1978). They concluded that there exists a close relationship between oogenesis and midgut protease activity in *D. cingulatus*. A high level of protease activity apparently leads to greater protein digestion and an increased availability of protease in the haemolymph for the synthesis of yolk protein during vitellogenesis. The high activity of protease exhibited by female *I. limbata* in the present study may be correlated with the vitellogenic activities of the female. A high level of protease activity presumably leads to an increased availability of amino acids in the haemolymph for the production of yolk proteins during vitellogenesis (Persaud and Davey, 1971). This type of coordination between the gonotrophic cycle and the secretion of the midgut digestive

enzymes is also noted in *N. cinerea* (Rao and Fisk, 1965) and *Aedes atropalpus* (Hudson, 1970). However, Persaud and Davey (1971) suggested that the level of midgut protease activity of the female *R. prolixus* is not closely coupled with oogenesis, for, sterilization of the females by a single dose of aminopterin fails to affect the cycle of protease activity. Sex specific midgut proteases were detected in *A. albimanus* by Lazares-Raga *et al.*, (1998). They also indicate that the possibility of sex dependent regulation of midgut proteins and protease production in this insect. However there are reports on the absence of sexual dimorphism in digestive enzymatic activity in a few insects. For example the digestive amylase of *B. mori* (Matsumura, 1934), the gut trypsin of *S. venustum*, (Yang and Davies, 1968b) and the amylase and protease activities of the salivary gland of *L. disponsi* (Hori, 1973) were practically the same for both sexes. Sarangi (1986) also got a similar result in fifth instar larvae of *B. mori.*

Relative activity of protease in different gut regions

Midgut

The high proteolytic activity in the gut tissue and in lumen content of the midgut of *I. limbata* indicate that midgut is the main site of synthesis, secretion and action of protease enzymes. The association of the major part of the enzyme in the lumen suggested that the digestion of proteins was extracellular; also, it satisfied the required acidity for optimum activity. It may, therefore, be inferred that these proteases are

synthesised in the midgut epithelium and are secreted into the lumen where they become optimally operational at the acidic pH prevailing in the lumen. The casein-hydrolysing activity found in the tissue homogenates may be due to the residual enzyme, which was not secreted into lumen. It may be due to the fact that a slight proteinase activity was magnified due to the action on the degradation products by the various peptidases which are concentrated in the epithelial tissue. It may also be noted that some of the proteinases have exopeptidases activity, in addition to the dominant endopeptidase activity (Desnuelle, 1960). The vast literature pertaining to midgut proteases establishes its universal occurrence in insects (Gatehouose *et al.*, 1985; Weiman and Neilsen, 1998; Kitch and Murdock , 1986; Campos *et al.*, 1989; Christellor *et al.*, 1989 ; Thie and Houseman, 1990 a, b; Mc Chie *et al.*, 1995 and Zhu *et al.*, 2000). Gooding and his colleagues have contributed much to the knowledge on the physiology of digestion of proteins in the midgut of insects (Gooding *et al.*, 1973). These workers found that trypsin and chymotrypsin are the main proteases responsible for blood digestion in insects. Proteolytic enzymes are localized in the midgut of both mosquito and stable fly (Champlain and Fisk, 1956). Thomas *et al.*, (1976), found that midgut of horse flies contains two trypsins with different molecular weights. Gooding (1969) demonstrated tryptic and chymotryptic activity in the midgut of *A. aegypti*, *C. fatigans*, *Melophagus ovinus* and *Pediculus humanus*. Aminopeptidase activity was

partially characterized from midguts of *A. stephensi.* by Billingsley (1990). Spirokern and Chen (1972) found that trypsin was the predominant enzyme in the larvae, pupae and adults of mosquitoes, *C. fatigans* and *C. pipens,* while chymotrypsin was found only in the larvae. Patel and Richard (1960) electrophoretically separated three trypsins, from the midgut of housefly with three different substrate specificities; the authors suggested the possibility of one of the three enzymes being elated to microflora of the midgut. A peptic protease has been obtained in the midgut of stable fly *S. calcitrans* (Lambremont *et al.,* 1959). Recently 24 Serine proteases has been identified by PCR of midgut cDNA library of tsetse fly, *S. calcitrans* (Lehane *et al.,* 1998).

A study of digestive proteinases in *Lasioderma serricorne* was performed to identify potential targets for proteinaceous bio-pesticides, such as proteinase inhibitors. Optimal casein hydrolysis by luminal proteases of *L. serricorne* was in pH 8.5- 9.0 buffers, although the pH of luminal contents was slightly acidic. Results from substrates and inhibitor analysis indicated that the primary digestive proteinase were serine proteinase (Oppert *et al.,* 2002)

The major proteinase activity in extracts of larval midguts from the southern corn rootworm, *D. undecimpunctata howardi* was identified as a cysteine proteinase (Fabrick *et al.,* 2002). A novel procarboxypeptidase was identified in the midgut of cotton pest *H. armigera* (Estebanez-Perpina *et*

al., 2001). Acidic proteases especially cysteine and serine proteases were located in the midgut of green mirid, *C. dilutus* (Colebatch *et al.*, 2001). Three isoforms of trypsins were identified in the midgut preparations from *L. migratoria* (Lam *et al.*, 2000). The above findings indicate that proteases are a group of enzymes that are seen in all insects and their expression is partially and temporarily regulated.

According to Zhu and Baker (1999) protein digestion in the grain borer, *R. dominica*, results from the action of a serine proteinases present in the midgut. Three proteolytic enzymes trypsin, chymotrypsin and amino peptidases – N were purified from laboratory – reared western spruce budworm, *C. occidentalis* larvae.

Hindgut

The hindgut protease activity in *I. limbata* using casein as a substrate was very low when compared with that of midgut. The activity inside the lumen may represent the unaltered midgut enzyme passed into the hindgut along with the food residues. Alternatively, it might have been elaborated by the hindgut tissue. The activity in the tissue may represent the biosynthetic activity of the hindgut cells, or it may be attributed to the reabsorption of enzyme from the lumen. Proteases were reported to be absent in the hindgut of insects, except for some isolated records (Agarwal, 1975).

The larvae of the *L. cuprina* excrete or secrete a chymotrypsin on to the skin of sheep to facilitate the establishment of the larval infestation. This protease is also a gut digestive protease. The chymotrypsin is synthesised primarily in the cardia, a small highly specialized organ located at the anterior end of the midgut and by midgut cells. Chymotrypsin is in hindgut but not in salivary gland (Casu *et al.*, 1996).

Effect of food on probable regulatory mechanism in protease of *I. limbata*

The results of the present study indicate that, the protease activity of *I. limbata* is significantly influenced by the diet. There was a remarkable increase in the proteolytic activity of the insects fed with more proteinaceous diet like cottonseeds, green gram and casein compared to low / non-proteinaceous diet like banana, sugar solution and distilled water. This difference is exhibited with a similar pattern in both sexes, different gut regions (hindgut and foregut) and lumen content and gut tissues. Casein fed insects showed lower proteolytic activity than insects fed with natural protein rich diet. Banana fed insects showed lower proteolytic activity, presumably because the food contains less protein content. It is interesting to note that the sugar solution fed insects showed significantly lower protease activity than distilled water fed insects (except in the activities of midgut lumen content and tissue of male insects, hindgut lumen content of male and hindgut tissue of female). Sugar

solution may have some inhibitory effect on protease activity or it may enhance the production carbohydrases than the amount of protease in the gut. The above findings indicate that the protein content in the diet has a stimulatory effect on the protease activity. Regulation of digestive enzyme by the food materials has been demonstrated in the cotton bug *D. cingulatus* (Muraleedharan and Prabhu, 1978), *G. morsitans* (Langley *et al.*, 1978), *L. rugulipennis* (Varis *et al.*, 1983), *B. mori* (Jadhav and Kallapur, 1988) and in *C. capitata* (Lemos *et al.*, 1992).

Applebaum (1985) has suggested that the enzyme production and secretion do not vary in continuous feeding insects while the enzymes are produced on demand in discontinuous feeders. Starved larvae of the black carpet beetle *Attagenus megatoma* (Baker, 1977) when fed with selected diets, an increase in proteolytic, trypsin and chymotrypsin activities has been observed and it has been correlated with the total midgut protein. At the same time total protease activity in these larvae was minimal when the larvae were fed with starch. Thus the control of proteolytic digestive enzymes in the larvae of *A. megatoma* was regulated by the amount of protein present in the midgut but not by the amount of food (Baker, 1977). When the insects are removed from the diet and starved, proteolytic activity in the gut is declined and came to a minimum within 92 hours after the removal from the diet (Baker, 1978). A similar observation was reported in *B. mori* (Jadhav and Kallapur, 1988) in which protease activity

of the midgut was significantly reduced as a result of starvation before they began to spin the cocoons. In *Locusta* starvation results in a reduction in the amount of enzyme activity (Anstee and Charnley, 1977). The high protease activity observed in the present study, when *I. limbata* are fed with casein solution is in tune with the above findings. Therefore, it was suggested that the component in the food or the type of food taken by the insect stimulate secretion of digestive enzyme. The nutritional superiority of a particular kind of food is determined by the components in the food ingested. Langley (1966) demonstrated that a meal of serum stimulates a rise in midgut proteinase in *G. morsitans,* while meals of saline or washed erythrocytes show no change in the enzyme activity. The results of the studies conducted on *G. morsitans* (Gooding, 1974a) and on *R. prolixus* (Gracia and Gracia, 1977) lend supports to this view.

The blood – sucrose mixture in the crop of female black flies stimulated steady trypsin activity, as the mixture was despatched slowly to the midgut for digestion (Yang and Davies, 1968). The increase in the amount of enzyme was independent of the concentration of blood in the mixture, but was related to the amount of mixture ingested. However, sucrose alone produced no increase in the enzyme activity. Trypsin activity was almost identical when the females of *S. venustum* fed on human, cow or duck whole blood or blood cells suspended in sucrose solution.

When *G. austeni* was fed with diluted sheep blood, haemoglobin or casein (Akov, 1972), it has been found that each has stimulated proteinase production to a different extent, which led him to conclude that any protein food stimulates protease. A statistically significant correlation was found between the amount of trypsin in the digestive part of the midgut of *G. morsitans* and the amount of protein in it (Gooding 1974 a, b, c and d). However, in unfed *G. morsitans* the amount of midgut trypsin is not correlated with the amount of protein in the digestive part of the midgut (Gooding, 1974 b). Although it remains to be established, Gooding (1975) is of the opinion that the trypsin levels in *G. morsitans* are probably regulated by a secretagogue mechanism. Similarly, a number of workers support the secretagogue mechanism of enzyme synthesis in blood feeding carnivorous (Foster, 1972 and Gooding, 1974a, b, c and d, 1975) and omnivorous insects (Engelmann, 1969). Gooding (1975) indicates that the level of digestive proteinase is influenced by some factor from outside the digestive tract. Therefore, it is evident that the secretion of the digestive enzyme in some insects is in response to the specific substrate in the food rather than quantity of intake.

There are some evidences that the occurrence of salivary enzymes of Hemiptera is dependent on the diet (Kretovick *et al.*, 1943; Adams and McAllan, 1958; Nuorteva and Laurema, 1961 and Adams and Dew, 1965). The results obtained from the studies on *R. prolixus* (Persaud and Davey,

1971) also suggested a local control of protease activity based on a secretagogue mechanism in the control of protease activity. They have also suggested that the blood meal or some other component of it, on coming into contact with the intestinal epithelium, chemically stimulates either the biosynthesis of protease or their activation. This would therefore bring *R.* into the line with *Aedes* (Fisk, 1950; Fisk and Shambaugh, 1952 and Shambaugh, 1954) and with *Blatta* (Gordon, 1968). In *Aedes* the protease activity was lowest in unfed insects and showed variation in activity for different insects fed with different diets. The highest protease activity was shown by the insect fed with a diet that contains only the erythrocytes and serum proteins. Their results suggest that some components in the blood plasma protein are responsible for the activation of protease. The insects fed with whole human blood showed a reduced activity than the previous case. The protease activity is still reduced in insects fed with erythrocytes and protein other than serum protein or non-proteins. The insects fed with erythrocytes and saline show a very low protease activity. The results of the present study that there is a decrease in protease activity in insects fed with low protein or non-protein diets are in tune with the above findings.

In *Melanoplus sanguinipes* (Hinks and Erlandson, 1995), the proteolytic activity in the midgut changed with different diets given to the insects. Digestion of food occurs in the order of wheat > oats > kochia. Oats showed higher tryptic activity than wheat. Chymotrypsin also showed

high activity when fed with oats. Lehane *et al.,* (1995) proposed that direct interaction of an element of meal with digestive enzyme producing cells resulting in increased rates of enzyme synthesis or secretion, should be referred to as prandial mechanism. Most studies suggest that paracrine or prandial mechanisms are the main factors controlling digestive enzyme synthesis and secretion in insects. Distinguishing between the two mechanisms is a significant challenge. In many insects, soluble proteins are potent stimulant of proteinase synthesis and secretion probably through the prandial/paracrine pathways. The details of the mechanisms involved are unknown. The feeding can affect the control of digestive enzyme synthesis at either transcriptional or translational level. Similar results are obtained by the studies in *S. calcitrans* (Moffat *et al.,* 1995 and Blackmore *et al.,* 1995).

Absence of a substrate specific stimulation of digestive enzymes is observed in a number of studies. Graf and Briegel (1989) found that trypsin synthesis is induced in a dose dependent manner by injection of either blood or sugar solutions into isolated midguts of *A. aegypti.* It is concluded that the stimulus for initial trypsin synthesis is mechanical and/or osmotic stress acting independently of the nervous system. The immediate induction of the initial trypsin was found to depend on the blood volume injected. Induction was also observed, although to a lower extent, when sucrose solution or 0.6% NaCl was applied. In summary, two

regulatory phases of trypsin synthesis can be recognized in *A. aegypti*, i. e., an immediate response due to mechanical/osmotic stimulation and a delayed response depend on the presence of protein. The latter was qualitative in nature and was previously referred to as secretagogue stimulation of tryptic activity (Fisk and Shambaugh, 1952; Gooding, 1973 and Briegel and Lea, 1975).

It is known that the amount of food ingested regardless of its composition stimulates enzyme production in insects. Such regulation may not be of secretagogue type. In *Locusta* (Anstee and Charnley, 1977), it has been observed that the amount of enzymes secreted by the midgut is related to the total quantity of food ingested and was thought to be due to the mechanism in which the information concerning the distension of the foregut is relayed to the brain and affects the release of a hormone, controlling enzyme secretion (Clarke and Langley, 1963). Dadd (1956) reported that in *T. molitor*, protease secretion was dependent on the quantity of food rather than on the composition of diet. The proteolytic activity of the larva did not change when the larvae were fed on flour, cellulose or water. In *C. crocale* (Christopher and Mathavan, 1985) food intake is thought to be a factor of paramount importance in the regulation of digestive enzyme secretion. Their resuls revealed that amylase and invertase activity showed a direct correlation to food consumption. The quantity of food ingested significantly affects the digestive enzyme activity.

Enzyme secretion is related to the quantity of food passing through the gut and/or to the total midgut area, i. e., the site of enzyme production. The secretion of digestive enzymes in some insects is influenced by the quantity of food consumed. In *B. germanica* and *P. americana,* Day and Powning (1949) observed that protease activity increased in the midgut whether the animals were fed on starch or gelatine. They have also studied changes in protease, amylase and invertase in the gut of starved *B.* fed with different diets and concluded that all enzymes were secreted regardless of the food. Similar results were obtained In *P. americana* (Agrawal, 1981) in which midgut protease activity is highly correlated to the quantity of the food consumed and not to the protein present in the food. He further demonstrated that the ingestion of water resulted in fluctuation in enzyme activity.

The correlation of food constituents to the digestive enzymes has been well established by Gooding (1969, 1973, 1975, 1977 a, b, c). In the gut of *A. aegypti,* the level of proteinase increased after a blood meal (Fisk, 1950; Fisk and Shamaugh, 1952). No significant activity of proteinase has been found in sugar fed male and female mosquitoes (Kunz, 1978a) while the activity of trypsin considerably increased in simulids when blood – sucrose mixture was fed (Yang and Davies, 1968 c). They found that the increase in enzyme was independent of the concentration of the blood in the mixture, but was related to the amount of mixture digested.

In the larvae of *Spodoptera litoralis*, fed on artificial diets exhibited much higher proteolytic activity than those reared on clover (Ishaya *et al.*, 1971). The increase was five-fold in the midgut wall and 30% in the lumen. This indicated a high secretion of protease, which is stimulated by the compounds in the artificial diets that contained high level of proteins than that of clover.

The results of the present study indicate that the secretion of protease enzyme in the midgut of *I. limbata* is controlled by the type of food consumed by the insect. In *A. aegypti*, midgut early trypsin is post-transcriptionally regulated by blood feeding (Noriega *et al.*, 1996). The early trypsin is a female specific protease present in the *A. aegypti* midgut during the first hours after ingestion of a blood meal. Despite the high levels of early trypsin RNA present in the midgut of unfed female, translation of the early trypsin RNA occurred only after a blood or a protein meal. Early trypsin RNA levels rapidly decreased during the first 24 hour after feeding but the steady- state level of the transcript rose again at the end of blood digestion cycle (60h) as the mosquito prepares for a second blood meal. The juvenile hormone controls early trypsin gene transcription in this insect (Noriega *et al.*, 1997). The induction of early trypsin transcription by JH is dose dependent and 'head–independent', suggesting that factors coming from the neuro–secretory axis are not required.

SUMMARY

K.S. Sajan “Studies on the protease activity in the digestive system of iphita limbata (Heteroptera : Pyrrhocoridae)” Thesis. Department of Zoology , University of Calicut, 2002

Summary

SUMMARY

- Protease in the midgut of *Iphita limbata* was active over a broad range of pH i.e., pH 3.0- 9.8. The optimum pH of the protease activity using casein as substrate was found to be pH 5.0. The activity was relatively high between pH 4.5 and 7.0 but declined sharply at alkaline conditions. The pH-enzyme activity relationship of hindgut protease showed a similar pattern of changes to that of midgut, but with a lower magnitude.
- A rectangular hyperbolic relationship was observed for reaction velocity of the enzyme against an increase in concentration from 0.25% to 2.5%. The K_m values for midgut protease using casein as a substrate were 0.67% and 1.07% for female and male insects respectively. The value for $V_{max,}$ with casein hydrolysis was 30.77 and 80.0 activity units in males and females respectively. A similar pattern was observed in hindgut protease activity but with low affinity, (high K_m value) and low $V_{max.}$
- The optimum temperature of protease activity was 47°C. Though the activity lowered in hindgut enzyme preparation, both male and female insects show the same optimal temperature as that of midgut enzyme preparations.
- Protease activity of *I. limbata* showed a sexual dimorphism. The female insects showed significantly more enzyme activity than male

insects in both midgut and hindgut enzyme preparations. Among the 3 types of enzyme preparations (gut with content, gut tissue, lumen content) the highest activity was exhibited by female, midgut lumen content enzyme preparations. The midgut lumen content showed a 4.7 fold and 1.7 fold increase in the enzyme activity compared to the gut tissue and gut with content respectively.

- All the three different types of midgut enzyme preparations from male and female insects showed significantly higher activity than that hindgut; the difference in the activity of protease was maximum in the lumen content of females.
- The protein content in the feed material has been found to be stimulatory on the protease activity. When the insects were fed with diets rich in proteins diets such as cottonseeds, green gram, casein suspension the enzyme activity was very high compared to those fed with low/ non-protein diets such as banana, sugar solution and distilled water.
- The effect of diet on protease exhibited a sexual dimorphism; the females showed more enzymatic activity when fed with all the different types of diet. However, in hindgut enzyme preparations the sugar solution fed insects did not show a significant sexual dimorphism.

- There was an increased midgut protease activity in female insects fed with distilled water than those fed with sugar solution. This difference was also seen in both sexes. It was argued that the carbohydrates in the diet might have a negative effect on the protease activity.

Bibliography

BIBLIOGRAPHY

Abbot, R. L. (1926) . Physiogly of digestion in Australian cockroach *Periplaneta australasiae* Fab. *J. Exp. Zool.* **44**, 219-253.

Abdul Jabbar, T. U. and Mohamed, U. V. K. (1989) . Some properties and distribution of maltase in the digestive system of *Orthaga exvinacea* Hampson (Pyralidae: Lepidoptera) . *J. Zool. Res.*, (**1&2**) , 47-55.

Adams, J. B. and Mc Allan, J. W. (1958) . Pectinase in certain insects. *Can. J. Zool.*, **36**, 305-308.

Adams, J. B. and Drew, M. E. (1965) . A cellulose hydrolysing factor in aphid saliva. *Can. J. Zool.*, **43**, 489-496.

Adedire, C. O. (1990) . Proteolytic activity of gut homogenate of the Kola weevil *Sophrorhinus insperatus* Faust. *Entomon.*, **15**, 171-177.

Agarwal, A. K. (1975) . Digestive enzymes in the excreta of the larvae of *Sesamia inferens* Walker and *Chilotraea infuscatellus* Snell. (Lepidoptera: Insecta) . *Experientia*, **31**, 816.

Agarwal, A. K. (1976 a) . Effect of various factors on the activity of trehalase from the larvae of *Sesamia inferens* Walker (Insecta) . *Experientia*, **32**, 1518-1520.

Agarwal, A. K. (1976 b) . Melezitase and maltase from the midgut of *Sesamia inferens* Walker (Lepidoptera: Insecta) . *Experientia*, **32**, 1033-1034.

Agrawal, O. P. (1981 a) . A study of the parameters of digestion in *Periplaneta americana* L. *Experientia.*, **37**, 840-841.

Agrawal, O. P. (1981 b) . Amylase activity in the alimentary tract and salivary glands of *Periplaneta americana L. Acta. Physiol*, **32** (**1**) , 29-36.

Agrawal, O. P. and Bahadur, J. (1978) . Role of salivary glands in the maintenance of midgut amylase activity in *Periplaneta americana* L. *Experientia.*, **34**, 1552.

Agrawal, O. P. and Bahadur, J. (1981) . A comparison between the salivary gland and gut protease activity with reference to pH optimum in *Periplaneta americana. Nat. Acad. Sci. Letters.*, **4**, 215-217.

Ahmad, Z., Saleemuddin, M. and Siddiqui, M. (1976) . Alkaline protease in the larvae of the army worm, *Spodoptera litura. Insect Biochem.*, **6**, 501-505.

Ahmad, Z., Saleemuddin, M. and Siddiqui, M. (1980) . Purification and characterization of three alkaline proteases from the gut of the larva of army worm, *Spodoptera litura. Insect Biochem.*, **10**, 667-673.

Akov, S. (1972) . *Protein digestion in haematophagous insects. In Insects and Mite Nutrition*, Edited by G. Rodriguez. 531-540. North Holland publishing company, Amsterdam.

Alarcon, F. J., Martinez, T. F., Barranco, P., Cabello, T., Diaz, M. and Moyano, F. J. (2002) . Digestive proteases during development of larvae of red palm weevil, *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Curculionidae) . *Insect Biochem. Mol. Biol.*, **32** (**3**) , 265-74.

Algimantas, P. Valaitis, Sylvie Augustin, Karenand, M. Clancy. (1999) . Purification and characterization of the western spruce budworm larval midgut proteinases and comparison of gut activities of laboratory-reared and field-collected insects. *Insect Biochem. Mol. Biol.*, (**29**) **5**, 405-415.

Anstee, J. H. and Charnley, A. K. (1977) . Effects of frontal ganglion removal and starvation on activity and distribution of six gut enzymes in *Locusta. J. Insect physiol.*, **23**, 965-974.

Applebaum, S. W. (1964) . The action pattern and physiological role of *Tenebrio* larval amylase. *J. Insect Physiol.*, **10**, 897 – 906.

Applebaum, S. W. (1985) . Biochemistry of digestion. In *Comprehensive Insect physiology Biochemistry and pharmacology.* Edited by Kerkut, G. A. and Gilbert, L. I., pergamon press, U. K. **4**, 279-311.

Applebaum, S. W. Harpaz, I and Bondi, A. (1964 a) . Amylase secretion in the larvae of *Prodenia litura* F. (Insecta) . *Comp. Biochem. Physiol.*, **13**, 107 – 111.

Applebaum, S. W., Birk, Y., Harpaz, and Bondi, A. (1964 b) . Comparative studies on the proteolytic enzymes of *Tenebrio molitor* L. *Comp. Biochem. Physiol.*, **11**, 85 – 103.

Applebaum, S. W., Jankovic, M. and Brik, Y. (1961) . Studies on the midgut amylase activity of *Tenebrio molitor* L. larvae. J. *Insect Physiol*, **7**, 100 – 108.

Baba, H., Katsuzaki, H., Komiya, T., Yamashita, O. and Imai, K. (2001) . Rapid degradation of the silkworm diapause hormone by trypsin and its suppression by VAP-map, a synthetic analog of the cuticular peptide of silkworm, Bm ACP-6. 7 (VAP-peptide) . *Biosci. Biotechnol. Biochem.*, **65** (**5**) , 1033-7.

Bai, C. and Sha, C. Y. (1989) . A study on the intestinal proteinases of *Mythimna separata* larvae. *Acta. Entomol. Sin.*, **32**, 22-25.

Bai, C. and Sha, C. Y. (1990) . Activities of digestive proteases of three species of Iepidopterous larvae. *Acta. Entomol. Sin.*, **33**, 296-300.

Baker, J. E. (1976) . Properties of midgut protease in larvae of *Attagenus megatoma. Insect Biochem.*, **6**, 143 – 148.

Baker, J. E. (1978) . Midgut clearance and digestive enzyme levels in larvae of *Attagenus megatoma* following removal from food. *J. Insect Physiol.*, **24**, 133 – 136.

Baker, J. E. and Fabrick, J. A. (2000) . Host hemolymph proteins and protein digestion in larval *Habrobracon hebetor* (Hymenoptera: braconidae) . *Insect. Biochem. Mol. Biol.*, **30 (10)** , 937-46.

Baker, J. K. (1977) . Substrate specifcity in the control of digestive enzymes in larvae of the black carpet beetle. *J. Insect physiol.*, **23**, 749-753.

Baldwin, E. (1963) . *Dynamic Aspects of Biochemistry.* University Press, Cambridge.

Ballentine, R. (1940) . Proteases of dragon fly nymphs. *Anat. Rec. Suppl.*, **78**, 44.

Balyan, B. S. (1973) . Hydrogen-ion concentration and digestive enzymes in the mature larva of *Gnorimoschema (Phthroimaea) operculella* Zeller (Gelechidae : Lepidoptera) *Indian J. Ent.*, **35**, 206 – 210.

Barnard, E. A. (1973) . Comparative biochemistry of digestive enzymes. In *Comparative Animal Physiology* (Prosser, C. L., Ed.) , pp. 139 – 146. Saunders, Philadelphia.

Barnard, E. A. and Prosser, C. L. (1973) . Comparative biochemistry and physiology of digestion. In *Comparative Animal Physiology* (Prosser, C. L., Ed.) . Saunders, Philadelphia.

Baumann, E. (1990) . Isolation and partial characterization of a chymotrypsin like endoproteinase from cockroach intestinal system. *Insect Biochem.*, **20**, 861-768.

Beazell, J. M. (1941) . A Re-examination of the role of the stomach in the digestion of carbohydrates and protein. *American Journal of Physiology.*, **132**, 42-50.

Beng, C., Hazel, H., Khoo, G. N., Chew, L. M., Wong, K. P. and Eweit, A. (1992) . Food ingestion and digestive enzymes in larval *Aedes aegypti* and *A. albopictus* (Diptera: Culicidae) . *J. Med. Entomol.*, **29**, 960-964.

Bernardi, R., Tedeschi, G., Ronchi, S., Palmieri, S. (1996) . Isolation and some molecular properties of a trypsin-like enzyme from larvae of

European corn borer *Ostrinia nubilalis Hubner* (Lepidoptera: Pyralidae) *Insect Biochem. Mol. Biol.*, **26 (8-9)** ,883-9.

Bernasconi, P. (1994) . Molecular cloning of a *Drosophila melanogaster* gene coding for an homologue of human carboxypeptidase E. *Arch. Insect. Biochem. Physiol.*, **27 (3)** , 169-78.

Billingsley, P. F. (1990) . Blood digestion in the mosquito, *Anopheles stephensi* Liston (Diptera: Culicidae) , partial characterization and post-feeding activity of midgut aminopeptidases. *Arch. Insect. Biochem. Physiol.*, **15 (3)** , 149-63.

Billingsley, P. F. and Downe, A. E. (1986) . The surface morphology of the midgut cells of *Rhodnius prolixus* Stal. (Hemiptera: Reduviidae) during blood digestion. Acta. Trop., **43 (4)** , 355-66.

Billingsley, P. F. and Downe, A. E. R. (1988) . Ultrastructural localisatiol of cathepsin B in the midgut of *Rhobnius prolixus* stal. (Hemiptera: Reduvidae) during blood digestion. *Int. J. Insect Morphol. Embryol.*, **17**, 295-302.

Billingsley, P. F. and Hecker, H. (1991) . Blood digestion in the Mosquito, *Anopheles stephensi* Liston (Diptera :Culicidae) :activity and distribution of trypsin aminopeptidase and alpha –glucosidase in the midgut. *J. Med. Enomol.*, **28 (6)** , 865-71.

Birk, Y., Harpaz, I., Ishaaya, I and Bondi, A. (1962) . Studies on the proteolytic activity of the beetles, *Tenebrio* and *Tribolium. J. Insect Physiol.*, **8**, 417 – 429.

Birk, Y., Sakel, E. and Applebaum, S. W. (1989) . Purification and characterization of trypsin from digestive tract of *Locusta migratoria. Int. J. Peptide protein Res.*, **34**, 498-505.

Blackmore, D., Williams, S. and Lehane, M. J. (1995) . Protein stimulation of trypsin secetion from the opaque zone of midgut cells of *Stomoxys Calcitrans. Comp. Biochem. Physiol.*, **11OB**, 301-307.

Blanco-Labra, A., Martinez-Gallardo, N. A., Sandoval-Cardoso, L. and Delano-Frier, (1996) . Purification and characterization of a digestive cathepsin D proteinase isolated from *Tribolium castaneum* larvae (Herbst) . *J. Insect. Biochem. Mol. Biol.*, **26 (1)** , 95-100.

Bongers, J. (1970) . Die carbohydrasen und esterasen in speicheldrusen und mitteldarm von *Oncopeltus fasciatus* Dall. (Heteroptera : Lygaeidae) . *Z. Vergl. Physiol.*, **70**, 382 – 400.

Bowles, B, M., Cainegie, P. R. and Sandeman, R. M. (1988) . Characterization of proteolytic and collagenolytic enzymes from the larvae of *Lucilia cuprina* the sheep blow fly. *Aust. J. Biol. Sci.*, **41**, 6269-6278.

Branca, D., Gugliucci, A., Bano D Brini, M. and Carafoli, E. (1999) . Expression, partial purification and functional properties of the muscle –specific xcalpain isoform p94. *Eur. j. Biochem.*, **265** (**2**) , 839-846.

Brey, P. T., Ahmed, A., Lee, W. J., Ashida, M. and Lehane, M. (1995) . Tyrosinase-type prophenoloxidase distribution in the alimentary canal of strains of *Anopheles gambiae* refractory and susceptible to *Plasmodium* infection. *J. Exp. Parasitol.*, **80** (**4**) , 654-64.

Briegel, H. (1975) . Excretion of proteolytic enzymes by *Aedes aegypti* after a blood meal. *J. Insect Physiol.*, **21**, 1681 – 1684.

Briegel, H. and Lea, A. O. (1975) . Relationship between protein and proteolytic activity in the midgut of mosquitoes. *J. Insect Physiol.*, **21**, 1597-1604.

Briegel, H. and Lea, A. O. (1979) . Influence of endocrine system on tryptic actibaty in female *Aedes aegypti. J. Insect physiol.*, **25**, 227-230.

Broady, R. M. (1989) . Characterization and ecological implication of midgut proteolytic activity in larval *Pieris rapae and Trichoplusia ni. J. Chem. Ecol.*, **15**, 2101-2114.

Brovsky, D. (1985) . Characterization of proteolytic enzymes of the midgut and excreta of the biting fly *Stomoxys Calcitrans. Arch. Insect Biochem. Physiol.*, **2**, 145-159.

Brunelle, F., Nguyen-Quoc, B., Cloutier, C. and Michaud, D. (1999) . Protein hydrolysis by colorado potato beetle, *Leptinotarsa decemlineata*, digestive proteases: the catalytic role of cathepsin D *Arch. Insect. Biochem. Physiol.*, **42** (**1**) , 88-98.

Campos, F. A. P., Xavier-Filho, J., Silva, C. P. and Ary, M. B. (1989) . Resolution and partial characterization of proteinases and α-amylases from midguts of larvae of bruchid beetle *Callosobruchus maculates. Biochem. Physiol.*, **92B**, 51-57.

Campos, I. T., Amino, R., Sampaio, C. A., Auerswald, E. A., Friedrich, T., Lemaire, H. G., Schenkman, S. and Tanaka, A. S. (2002) . Infestin, a thrombin inhibitor presents in *Triatoma infestans* midgut, a Chagas' disease vector: gene cloning, expression and characterization of the inhibitor. *Insect. Biochem. Mol. Biol.*,**32** (**9**) , 991-7.

Carlini, C. R., Oliveira, A. E., Azambuja, P., Xavier-Filho, J. and Wells, M. A. (1997) . Biological effects of canatoxin in different insect models: evidence for a proteolytic activation of the toxin by insect cathepsin like enzymes. *J. Econ. Entomol.*, **90** (**2**) , 340-8.

Casu, R.E., Eisemann, C.H., Vuocolo, T., Tellam, R.L., (1996) . The major excretory/secretory protease from *Lucilia cuprina* larvae is also a gut digestive protease. *Int. J.Parasitol.* **26 (6)** ,623-8.

Champlain, R. A. and Fisk, F. W. (1956) . The digestive enzymes of stable fly *Stomoxys calcitrans L. Ohio. J. Sci.*, **56**, 52 – 62.

Chapman, R. F. (1982) . Digestion and Absorption. In *The Incets Structure and Function* 3rd edition. 66-83. Edward Arnold (publishers) Ltd., London.

Chapman, R. F. (1985) . Coordination of digestion. In *comprehensive Insect Physiology Biochemistry and Pharmacology.* Edited by Kerkut, G. A. and Gilbert, L. I. **4**, 213-240. Pergamon press, Oxford.

Chen, X., Rosenfield, C. S. Roberts, R. M. and Green, J. A. (2001) . An aspartic proteinase expressed in the yolk sac and neonatal stomach of the mouse. *Biol. Reprod.* **65 (4)** , 1092-101.

Cheng, G. M., Pumpuni, C. B. and Beier, J. C. (1996) . Proteolytic enzyme activity and *Plasmodium falciparum* sporogonic development in three species of *Anopheles mosquitoes. J. parasitol.*, **82 (1)** , 11-6.

Chippendale, G. M. (1978) . The functions of carbohydrates in insect life processes. In *Biochemistry of Insects.* (Rockstein, M., Ed.) , pp. 1 – 42, Academic Press, New York.

Christeller, J. T., Laing, W. A., Markwick, N. P. and Burgess, E. P. J. (1992) . Midgut protease activities in 12 phytophagous lepidopteran larvae: Dietary and protease inhibitor interactions. *Insect Biochem. Mol. Biol.*, **22**, 735-746.

Christeller, J. T., Laing, W. A., Shaw, B. D. and Burgess, E. P. J. (1990) . Characterization and partial purification of the digestive protease of the black field cricket, *Teleogryllus commodus* (Walker) : Elastase is a major component. *Insect Biochem.*, **20**, 157-164.

Christeller, J. T., Shaw B. D., Gardiner, S. E and Dymock, J. (1989) . Partial purification and characterization of the major midgut proteases of grass grub larvae (*Costelytra zealandica*, Coleoptera: Scarabaeidae) . *Insect Biochem.*, **19**, 221-231.

Christopher, M. S. M. and Madhavan, S. (1985) . Regulation of digestive enzyme activity in the larvae of *Catopsilla crocale* (Lepidoptera) . *J. Insect physiol.*, **11**, 217-221.

Clarke, K. U. and Langley, P. A. (1963) . Studies on initiation of growth and moulting in *Locusta migratoria migratorioides* R. & F IV. The relationship between the stomatogastric nervous system and neurosecretion. *J. Insect physiol.*, **9**, 423-430.

Colebatch, G., East, P. and Cooper, P. (2001) . Preliminary characterization of digestive proteases of the green mirid, *Creontiades dilutus.* (Hemiptera: Miridae) .*Insect. Biochem. Mol. Biol.* **31 (4-5)** , 415-23.

Colebatch, G., Cooper, P. and East, P. (2002) . cDNA cloning of a salivary chymotrypsin-like protease and the identification of six additional cDNAs encoding putative digestive proteases from the green mirid, *Creontiades dilutus* (Hemiptera: Miridae) . *Insect. Biochem. Mol. Biol.*, **32 (9)** , 1065-75.

Collet, J. I. (1989) . Characterization of the peptidases of *Calliphora* many features allow the utilization of small peptides as an aminoacid reservoir. *Insect Biochem.*, **19**, 535-547.

Dadd, R. H. (1956) . Proteolytic activity of the midgut in relation to feeding in the beetles, *Tenebrio molitor* (L.) and *Dytiscus marginalis* (L.) . *J. Expl. Biol.*, **33**, 311-324.

Dadd, R. H. (1970) . Digestion in insects. In *Chemical Zoology.* Edited by Florkin, M. and Scheer, B. T. **5**, 117-145. Academic Press, NewYork.

Dadd, R. H. (1979) . Nucleotide, nucleoside and base nutritional requirements of the mosquito *Culex pipiens. J. Insect physiol.*, **25**, 353-359.

Dahlmann, B., Jany, K. D. and Pfleiderer, G. (1978) . The midgut endopeptidases of the honey bee (*Apis mellifica*) : Comparison of the enzymes in different ontogenic stages. *Insect Biochem.*, **8**, 203 – 211.

Das, A. K. and Das. A. B. (1982) . Compensations for temperature in the activities of digestive enzymes of *Periplaneta americana* (L.) . *Comp. Biochem. Physiol. A.* **71 (2)** , 255-63.

Day M. F. and Powning, R. F (1949) . A study of the processes of digestion in certain insects. *Aust. J. Sci. Res.* **2B**, 195-215.

Day, M. F. and Waterhouse, D. F. (1953) . The mechanism of digestion. *In Insect Physiology.* Edited by Roeder, K. D. Wiley, NewYork.

Dedet, J. P., Saf 'Janova, V. M, Desjeux, P., Emelyanova, L. P., Schnur, L. F. and Chance, M. L. (1982) . Ecology of a focus of cutaneous leishmaniasis in the Thies region (Senegal, West Africa) . Characterization and typing of the strains of *Leishmania* isolated, *Bull. Soc. Pathol. Exot. Filiales.*, **75 (5 Pt 2)** , 606-19.

Desnuelle, P. (1960) . Trypsin. In *The Enzymes* (Boyer, P. D., Lardy, H. and Myrback, K., Eds.) , **4**, 119 – 132. Academic Press, New York.

Dillon, R. J. and Lane, R. P. (1993) . Blood meal digestion in the midgut of *Phlebotomus papatasi* and *Phlebotomus langeroni. Med. Vet. Entomol.*, **7 (3)** , 225-32.

Doane, W. W. (1969b) . *Drosophila* amylases and problems in cellular differentiation. Problems in Biology : R. N. A. in Development. pp. 73 – 109.

Duspiva, F. (1936) . Die Proteolystischen Enzyme der Kleider-und Waschmottenraupen. *H. S. Zeitschr. Physiol. Chem.*, **241**, 177 – 200.

Eguchi, M. and Iwamoto, A (1976) . Alkaline proteases in the midgut tissue and digestive fluid of the silkworm *Bombyx mori. Insect Biochem.*, **6**, 491 – 496.

Eguchi, M. and Iwamoto, A. (1975 a) . Changes in protease, esterase and phosphatase in the alimentary canal of the silkworm during metamorphosis. *Insect Biochem.*, **5**, 495 – 507.

Eguchi, M. and Iwamoto, A. (1975 b) . Hydrolysis of solubilized fibrinoin and silk proteins in the midgut of the pharate adult of *Bombyx mori. J. Insect Physiol.*, **21**, 577 – 588.

Eguchi, M., Furukawa, S. and Iwamoto, A. (1972) . Proteolytic enzymes in the midgut of pharate adult of the silkworm, *Bombyx mori. J. Insect Physiol.*, **18**, 2457 – 2467.

Eisner, J. (1955) . The digestion and absorption of fats in the foregut of the cockroach, *Periplaneta americana (L.)* . *J. Exp. Zool.*, **130**, 159 – 181.

Elpidina, E. N., Vinokurov, K. S., Gromenko, V. A., Rudenskaya, Y. A., Dunaevsky, Y. E. and Zhuzhikov, D. P. (2001) . Compartmentalization of proteinases and amylases in *Nauphoeta cinerea* midgut. *Arch. Insect. Biochem. Physiol.*, **48 (4)** , 206-16.

Endege, W. O., Lonsdale-Eccles, J. D., Olembo, N. K., Mollo, S. K. and Moiyd-ole, O. K. (1988) . Purification and characterization of two fibrinolysins from the midgut of adult female *Glossina morsitans centralis. Comp. Biochem. Physion.* **92B**, 25-34.

Engelmann, F. and Wilkens, J.L. (1969) . *Nature.* (London) . **222**, 798.

Engelmann, F. (1969) . Food–stimulated synthesis of intestinal proteolytic enzymes in the cockroach *Leucophaea maderae. J. Insect physiol.*, **15**, 217-235.

Engelmann, F. and Geraerts, W. P. M. (1980) . The protease and the protease inhibitor in the midgut of *Leucophaea maderae. J. Insect physiol.*, **26**, 703-710.

Engelmann,F. and Wilkens, J.L. (1969) . *Nature* (London) . **222**, 798.

Estebanez-Perpina, E., Bayes, A., Vendrell, J., Jongsma, M. A., Bown, D. P., Gatehouse, J. A., Huber, R., Bode, W., Aviles, F. X. and Reverter, D. (2001) . Crystal structure of a novel mid-gut procarboxypeptidase

from the cotton pest *Helicoverpa armigera*. *J. Mol. Biol.*, **313** (**3**) , 629-38.

Evans, W. A. L. (1958) . Studies on the digestive enzymes of the blowfly *Calliphora erythrocephala* – II Kinetic constants of the larval gut proteinase *Exp. Parasitol.*, **7**, 69 – 81.

Fabric, J., Behnke. C., Czapla, T., Bala, K., Rao, A.G., Kramer, K.J.and Reeck, G.R.,. (2002) . Effects of a potato cysteine proteinase inhibitor on midgut proteolytic enzyme activity and growth of the southern corn rootworm, *Diabrotica undecimpunctata howardi* (Coleoptera: Chrysomelidae) . *Insect. Biochem. Mol. Biol.* **32** (**4**) ,405-15.

Felix, C. R., Betschart, B., Billingsley, P. F. and Freyvogal, T. A. (1991) . Post feeding induction of trypsin in the midgut of *Aedes aegypti* (Diptera: Culicidae) is separable in to 2 cellular phases. *Insect Biochem.*, **21**, 197-203.

Fisk, F. W. (1950) . Studies on proteolytic digestion in adult *Aedes aegypti* mosquitoes. *Ann. Ent. Soc. Am.*, **43**, 555-572.

Fisk, F. W. and Shambaugh, G. F. (1952) . Protease activity in adult *Aedes aegypti* mosquitoes and related to feeing. *Ohio J. Sci.*, **52**, 80 – 88.

Fletcher. F. and Haub, J. G. (1933) . Digestion in blowfly larvae. *Phormia regina* Meigen, used in the treatment of osteomyelitis. *Ohio. J. Sci.*, **33**, 101-109.

Foissac, X., Edwards, M. G., Du, J. P., Gatehouse, A. M. and Gatehouse, J. A. (2002) . Putative protein digestion in a sap-sucking homopteran plant pest (rice brown plant hopper; *Nilaparvata lugens*: Delphacidae) --identification of trypsin-like and cathepsin B-like proteases. *Insect. Biochem. Mol. Biol.*, **32** (**9**) , 967-78.

Ford, J. B. (1962) . Studies on the digestive process of *Dysdercus fasciatus* Sign. *Ann. Appl. Biol.*, **50**, 355.

Foster, W. A. (1972) . Influence of the medial neurosecretory cells on reproduction in female *G. austeni*. *Trans. R. Soc. Trop. Med. Hug.*, **66**, 322.

Fraenkel, G. (1940) . Utilization and digestion of carbohydrates by the adult blowfly. *J. Exptl. Biol.*, **17**, 18 – 29.

Francis, B. R. and Bulla, L. A. (1997) . Further characterization of BT-R1, the cadherin-like receptor for Cry1Ab toxin in tobacco hornworm (*Manduca sexta*) midguts. *Jr. Insect. Biochem. Mol. Biol.*, **27** (**6**) , 541-50.

Fraser, A., Ring, R. A. and Stewart, R. K. (1961) . Intestinal proteinases in an insect, *Calliphora comitria* L. *Nature*, **192**, 999 – 1000.

Fruton, J. S. (1960) . Cathepsins. In *The Enzymes.* (Boyer, P. D., Lardy, H- and Myrback, K., Eds.) , **4**, 233 – 241. Academic Press, New York.

Fuji, O. and Kato, K. (1930) . On the digestive enzymes of the silkworm, *Bombyx mori, Kumamoto Sanshi Hokoku,* **3**, 34 – 76.

Fuschs, M. S. and Fong, W. F. (1976) . Inhibition of blood digestion by α-ananitin and actinomycin- D and its effect on ovarian development in *Aedes aegypti. J. Insect physiol.*, **22**, 465-471.

Garner Karen, J., Shiv Hiremath, Kirsten Lehtoma and Algimantas, P. Valaitis, (1999) . Cloning and complete sequence characterization of two gypsy moth aminopeptidase-N cDNAs, including the receptor for *Bacillus thuringiensis* Cry1Ac toxin. *Insect Biochem. Mol. Biol.*, **29 (6)** , 527-535.

Gatehouse, A. M. R. Butter, K. J., Fenton, J. A. and Gatehouse, J. A. (1985) . Presence and partial characterization of a major proteolytic enzyme in the larval gut of *Callosobruchus maculates. Entomol. Exp. Appl.*, **39**, 279-386.

Gatehouse, L.N., Shannon, A. L., Burgess, E. P. and Christeller, J. T. (1997) . Characterization of major midgut proteinase cDNAs from *Helicoverpa armigera* larvae and changes in gene expression in response to four proteinase inhibitors in the diet. *Insect. Biochem. Mol. Biol.*, **27 (11)** , 929-44.

Geering-Sacher, K. (1972) . Studies on digestive proteinase activity in the midgut of adult *Dysderus fasciatus* (Hemiptera) . *J. Insect physiol.*, **18**, 2071-2076.

Giebel, W., Zwilling, R. and Pfleiderer, G. (1971) . The evolution of endopeptidases – XII. The proteolytic enzymes of the honey bee (*Apis mellifica*, L.) . *Comp. Biochem. Physiol.*, **38**, 197 – 210.

Gilbert, L. I. (1967) . Lipid metabolism and function in insects. *Adv. Insect Physiol.*, **4**, 69 – 211

Gillikin, J. W., Bevilacqa, S. and Graham, J. S. (1992) . Partial characterization of digestive tract proteinases from western corn root worm larvae- *Diabrotica virgifera. Arch. Insect Biochem. Physiol.*,, **19**, 285-298.

Gilmour, D. (1961) . Digestion. In *The Biochemistry of Insects.* 40-59. Academic Press, London.

Girard, C. and Jouanin, L., (1999) . Molecular cloning of cDNAs encoding a range of digestive enzymes from a phytophagous beetle, *Phaedon cochleariae. Insect. Biochem. Mol. Biol.*, **29 (12)** , 1129-42.

Gontijo, N. F., Almeida-Silva, S., Costa, F. F., Mares-Guia, M. L., Williams Pand Melo, M. N. (1998) . *Lutzomyia longipalpis* : pH in the gut, digestive glycosidases and some speculations upon *Leishmania* development. *Exp. Parasitol.*, **90 (3)** , 212-9.

Gooding, R. H. (1966) . *In vitro* properties of proteinases in the midgut of adult *Aedes aegypti* L. and *Culex fatigans* (Wiedemann) . *Comp. Biochem. Physiol.*, **17**, 115 – 127.

Gooding, R. H. (1969) . Studies on proteinases from some blood-sucking insects. *Proc. Ent. Soc. Ontario*, **100**, 139 – 145.

Gooding, R. H. (1972) . Digestive process of haemotophagous insects. I. A literature review. *Quest. Entomol.*, **8**, 5 – 60.

Gooding, R. H. (1973) . The digestive processes of haematophagous insects. IV. Secretion of trypsin by *Aedes aegypti* (Diptera: Culicidae) . *Can. Ent.*, **105**, 599-603.

Gooding, R. H. (1974 a) . Digestive proceeesses of haematophagous insects: control of trypsin secretion in *Glossina morsitans. J. Insect physiol.*, **20**, 957-964.

Gooding, R. H. (1974 b) . Digestive processes of haematophagous insects V. Inhibition of trypsin from *Glossina morsitans morsitans* (Diptera: Glossinidae) . *Can. Ent.*, **106**, 39-44.

Gooding, R. H. (1974 c) . Digestion of proteins by *Glossina morsitans. Third Int. Congress of Parasitology. Proc.* **2**, 946.

Gooding, R. H. (1974 d) . Digestive processes of haematophagous insects. VII. Comparison of animal fed and membrane fed adults of *Glossina morsitans*. West. (Diptera: Glossinidae) . *Bull. Ent. Res.* **64**, 175-181.

Gooding, R. H. (1975) . Digestive enzyme and their control in haematophagous arthropods. *Acta tropica.* **32**, 96-111.

Gooding, R. H. (1977 a) . Digestive process of haematophagous insects. XIV. Haemolytic activity in the midgut of *Glossina morsitans* Westwood (Diptera : Glossinidae) . *Can. J. Zool.*, **55**, 1899 – 1905.

Gooding, R. H. (1977 b) . Digestive process of haematophagous insects. XII. Secretion of trypsin and carboxypeptidase - B by *Glossina morsitans* Westood (Diptera : Glossinidae) . *Can. J. Zool.*, **55**, 215 – 222.

Gooding, R. H. (1977 c) . Digestive process of haematophagous insects. XIII. Evidence for the digestive function of midgut proteinases of *Glossina morsitans* Westwood (Diptera : Glossinidae) . *Can. J. Zool.*, **55**, 1557 – 1562.

Gooding, R. H. and Huang, C. T. (1969) . Trypsin and chymotrypsin from the beetle, *Pterostichus melanarius. Insect Physiol.*, **15**, 325 – 329.

Gooding, R. H., Cheung, A. C. and Rolseth, B. M. (1973) . The digestive processes of haematophagous insects. III. Inhibition of trypsin by honey and possible functions of the oesophageal diverticula of mosquitoes (Diptera) . *Can. Entomol.*, **105**, 433-436.

Gordon, R. (1968) . Observations on the effect of the neuroendocrine system *Blatta orientalis* L. on the midgut protease activity of the adult female and the level of infestation with nematode *Hammerschmidtiella diesingi* (Hammerschnidt, 1838) . *Gen. Comp. Endocrin.*, **11**, 284-291.

Gorman, M. J.andreeva, O. V. and Paskewitz, S. M. (2000) . Molecular characterization of five serine protease genes cloned from *Anopheles gambiae* heamolymph. *Insect. Biochem. Mol. Biol.*, **30 (1)** , 35-46.

Gotz, B. and Klinkert, M. Q. (1993) . Expression and partial charecterization of a cathepsin –B- like enzyme (Sm 31) and a proposed 'Heamoglobinase' (Sm32) from *Schistosoma mansoni. Biochem. J.*, **290 (pt 3)** , 801-6.

Gracia, E. des. and Gracia, M. L. M. (1977) . Control of protease secretion in the intestine of fifth instar larvae of *Rhodnius prolixus. J. Insect physiol.*, **15**, 217-235.

Graf, R. and Briegel, H. (1989) . The synthetic pathway of trypsin in mosquito A*edes aegypti* L (Diptera: Culicidae) and *in vitro* stimulation in isolated midguts. *Insect Biochem.*, **19**, 129-137.

Graf, R. and Briegel, H. (1985) . Isolation of trypsin isozymes from the mosquito *Aedes aegypti* (L.) . *Insect Biochem.*, **15**, 611-618.

Graf, R., Buechien, P. and Briegel, H. (1991) . Structural diversity of trypsin from different mosquito species on vertebrate blood. *Experientia.* **47**, 603-609.

Graf, R., Raikhel, A. S., Brown, M. R., Lea, A. O. and Brigel, H. (1986) . Mosquito trypsin: Immunocytochemycal localization in the midgut of blood fed *Aedes aegypti* (L.) . *Cell Tiss. Res.*, **245**, 19-27.

Greenberg, B. and Paretsky, D. (1955) . Proteolytic enzymes in the housefly *Musca domestica* (L.) *Ann. Ent. Soc. Am.*, **48**, 46 – 48.

Grogan, D. E. and Hunt, J. H. (1977) . Digestive proteases of two species of wasps of the genus *Vespula. Insect Biochem.*, **7**, 191 – 196.

Grogan, G. E. and Hunt, J. H. (1986) . Midgut endopeptidase activities of the hornet, *Vespa crabo germana* Christ. (Hymenoptera: Vespidae) . *Insects Soc.*, **33**, 486-489.

Hall, N. A. (1986) . Peptidases in *Drosophila melanogaster*. I. Characterization of dipeptidase andleucine aminopeptidase activities. *Biochem. Genet.*, **24 (9-10)** , 775-93.

Hamilton, J. V., Munks, R. J., Lehane, S. M. and Lehane, M. J. (2002) . Association of midgut defensin with a novel serine protease in the blood-sucking fly *Stomoxys calcitrans*. *Insect. Mol. Biol.*, **11 (3)** , 197-205.

Harshini, S., Nachman, R. J. and Sreekumar, S. (2002) . Inhibition of digestive enzyme release by neuropeptides in larvae of *Opisina arenosella* (Lepidoptera: Cryptophasidae) . *Comp. Biochem. Physiol., B. Biochem. Mol. Biol.*, **132 (2)** , 353-8.

Harsulkar, A. M., Giri, A. P., Gupta, V. S., Sainani, M. N., Deshpande, V. V., Patankar, A. G. and Ranjekar, P. K. (1998) . Characterization of *Helicoverpa armigera* gut proteinases and their interaction with proteinase inhibitors using gel X-ray film contact print technique. Electrophoresis, **19 (8-9)** , 1397-402.

Hatano, Y. and Hori, K. (1989) . Comparison of biochemical properties of proteinases from the midgut of two blood sucking insects, *Haematobia irritans* (L.) and *Stomoxys calcitrans* (*L.*) (Diptera: Muscidae) . *Appl. Entomol. Zool.*, **24**, 245-252.

Hernandez Alvarez, H.M., Mendiola Martinez, J., Fernandez-Calienes, A. and Valdez, M. (2000) . Identification of a neutral protease in the intestine of *Boophilus microplus* with electrophoresis in polyacrylamide gels copolymerized with gelatin. *Rev. Cubana. Med. Trop.*, **52 (3)** .165-9.

Hinks, C. F. and Erlandson, M. A. (1995) . The accumulation of haemolymph protein and activity of digestive proteinases of grass hoppers (*Melanoplus sanguinipes*) . *J. Insect physiol.*, **41**, 425-433.

Hiraizumi, K., Hourani, C. L., Zambarano, M. C. and Freeman, J. E. (1992) . Dipeptidase-C in *Drosophila melanogaster*: genetic, ontogeneticand tissue-specific variation. *Biochem Genet*, **30 (11-12)** , 603-24.

Hobson, R. P. (1931) . Studies on the nutrition of blowfly larvae. I. Structure and function of the alimentary tract. *J. Exp. Biol.*, **8**, 109 – 123.

Hoerler, E. and Breiegel, H. (1995) . Proteolytic enzymes of female *Anopheles*: Biphasic synthesis, regulation and multiple feeding. *Arch. Insect Biochem. Physiol.*, **28**, 189-205.

Holler, B., Ogami, Y., Zabel-Langhennig, A., Tillack, D., Engeland, K., Keim, V. and Mossner, J. (2001) . Role of prostaglandins in

regulation of pancreatic enzyme secretion by various diets. *Dig. Dis. Sci.*, **46 (2)** , 289-95.

Hori, K. (1970) . Some properties of proteases in the gut and in the salivary gland of *Lygus disponsi* Linn. (Hemiptera:Miridae) . *Res. Bull. Obihiro. Univ.*, **6**, 318-324.

Hori, K. (1973) . Studies on enzymes, especially amylase, in the digestive system of the bug *Lygus disponsi* and starch digestion in the system. *Res. Bull. Obihiro Univ.*, **8**, 173-260.

Hori, K. (1975) . Digestive carbohydrases in the salivary gland and midgut of several phytophagous bugs. *Comp. Biochem. Physiol.*, **50B**, 141-146.

Hori, K., Atalay, R. and Araki, S. (1981) . Digestive enzymes in the gut and salivary gland of the adult Hornfly *Haematobia irritans* (Diptera: Muscidae) . *Appl. Ent. Zool.* **16**, 16-23.

Horie, Y. (1959) . Physiological studies on the alimentary canal of the silkworm *Bombyx mori.* II. Carbohydrases in the digestive fluid and in the midgut tissues. *Bull. Seric. Exp. Stn. Japan*, **15**, 365-382.

Horie, Y., Tanaka, M. and Ito, T. (1963) . Proteolytic enzymes of digestive juice and midgut of the silkworm *Bombyx mori. J. Seric. Sci. Japan*, **32**, 8-15.

Hosbach, H.A., Egg, A. H. and Kubli, E. (1972) . *Rev. Suise. Zool.*, **79**, 1049.

House, H. L. (1973) . In the *Physiology of insecta*, (M. Rockstein, Ed. 2nd Ed. **5**, 63-117. Academic press, New York.

House, H. L. (1974) . Digestion in *The Physiology of Insecta.* 2nd edition Edited by Rockstein, M. Vol. **5**, 63-117. Academic Press, New York.

Houseman, J. G. (1978) . A thiol activated digestive proteinase from adults of *Rhodnius prolixus* Stal. (Hemiptera: Reduviidae) . *Can. J. Zool.*, **56**, 1140-1143.

Houseman, J. G. and Downe, A. E. R. (1980) . Endoproteinase activity in the posterior midgut of *Rhodnius prolixus* stal (Hemiptera: Reduviidae) . *Insect Biochem.*, **10**, 363-366.

Houseman, J. G. and Downe, A. E. R. (1981) . Exoproteinase activity in the posterior midgut of *Rhodnius prolixus* stal. (Hemiptera: Reduviidae) . *Insect Biochem.*, **11**, *579-582.*

Houseman, J. G. and Downe, A. E. R. (1982,a) . Characterization of an acidic proteinase from the posterior midgut of *Rhodnius prolixus* stal. (Hemiptera: Reduviidae) . *Insect Biochem.*, **2**, 651-655.

Houseman, J. G. and Downe, A. E. R. (1982,b) . Identification and partial characteriztion of digestive proteinases from two species of bed bug (Hemiptera: Cimicidae) . *Can. J. Zool.*, **60**, 1837-1840.

Houseman, J. G. and Downe, A. E. R. (1983) . Cathepsin-D like activity in the posterior midgut of hemipteran insects. *Comp. Biochem. Physiol.*, **75B**, 509-512.

Houseman, J. G., Campell, F. C. and Morrison, P. E. (1987) . A preliminary characterization of digestive proteases in the posterior midgut of the stable fly *Stomoxyx calcitrans* L. (Diptera: Muscidae) . *Insect Biochem.*, **17**, 213-218.

Houseman, J. G., Mac Naughton, W. K. and Downe, A. E. R. (1984) . Cathepsin B and aminopeptidase activity in the posterior midgut of *Euschitus euschitoides* (Hemiptera: Pentatomidae) . *Can. Entomol.*, **116**, 1393-1396.

Houseman, J. G., Philogene, B. J. R. and Downe, A. E. R. (1989) . Partial characterization of proteinase activity in the larval midgut of the European corn borer *Ostrinia nubialis* Huebner. (Lepidoptera: Pyralidae) . *Can. J. Zool.*, **67**, 864-868.

Hudson, A. (1970) . Factors affecting egg maturation and oviposition by autogenous *Aedes atropalpus* (Diptera: Culicidae) . *Can. Ent.*, **102**, 939-949.

Ishaaya, I., Moore, and Joseph, D. (1971) . Protease and amylase activity in larvae of Egyptian cotton worm *Spodoptera littoralis. J. Insect physiol.*, **17**, 945-953.

Ishaaya, I., Moore, I. and Joseph, D. (1971) . Protease and amylase activity in larvae of Egyptian cotton worm *Spodoptera littoralis. J. Insect Physiol.*, **17**, 945 – 953.

Ito, T., Mukaiyama, F. and Tanaka, M. (1962) . Some properties of amylase of digestive juice and blood of the silkworm, *Bombyx mori, L. J. Seric. Sci. Japan*, **32**, 228 – 234.

Jadhav, G. and Kallapur, V. L. (1988) . Influence of age, sex and feeding on the protease activity of certain tissue of fifth instar silkworm *Bombyx mori. Entomon.*, **13**, 289-293.

Jany, K. D., Haug, H. and Ishay, J. (1978 a) . Trypsin-like endopeptidases from the midgut of the larvae from the hornets of *Vespa orientalis* and *Vespa crabro. Insect Biochem.*, **8**, 221-230.

Jany, K. D., Haug, H. and Phfleiderer, G. (1977) . Characterization of the low molecular weight protease from the hornet *Vespa crabro. Hoppe-Seyjers Z. Physiol. Chem.*, **358**, 1225.

Jany, K. D., Haug, H., Phfleiderer, G. and Ishay, J. (1978 b) . Enzymatic and chemical properties of endopeptidase from the larva of the hornet, *Vespa crabro. Biochemistry*, **17**, 4675-4682.

Jimenez, D. R. and Gilliam, M. (1989) . Age related changes in midgut ultrastructure and trypsin activity in the honey bee, *Apis mellifera. Apidologie.*, **20**, 287-303.

Johnson, K. S. and Felton, G. W. (2000) . Digestive proteinase activity in corn earworm (*Helicoverpa zea*) after molting and in response to lowered redox potential. *Arch. Insect. Biochem. Physiol.*, **44 (4)** , 151-61.

Johnston, K. A., Lee, M. J., Gatehouse, J. A. and Anste, J. H. (1991) . The partial purification and characterization of serine protease activity in midgut of larval *Helicoverpa armigera. Insect Biochem.*, **21**, 389-397.

Kaplan, R. A., Zwiers, S. H. and Yan, G. (2001) . *Plasmodium gallinaceum*: ookinete formation and proteolytic enzyme dynamics in highly refractory *Aedes aegypti* populations. *Exp. Parasitol.*, **98 (3)** , 115-22.

Kaska, M., Lysenko, O. and Chaloupka, J. (1976) . Exocellular proteases of *Serratia marcescens* and their toxicity to larvae of *Galleria mellonella. Folia Microbiol. (Praha)* ,**21 (6)** , 465-73.

Kawamura, M. Wadano, A. and Miura, K. (1987) . Purification and characterization of Insect cathepsin D. *Insect Biochem.*, **17**, 77-83.

Khalaf, H, Neumann, U. and Rimpler, M. (1995) . Electrophoretic analysis on the proteolytic decomposition of FSH preparations. *D. T. W. Dtsch Tierarztl Wochenschr*, **102 (10)** , 396-9.

Khan, M. A. (1962) . The distribution of dipeptidase activity in the digestive system of *Locusta migratoria* L. and *Dysdercus fasciatus* Dallas. *Comp. Biochem. Physiol.*, **6**, 169-170.

Khan, M. A. (1963) . The distribution of proteinase, invertase and amylase activity in various parts of alimentary canal of *Locusta migratoria* L. *Indian J. Ent.*, **25**, 200-203.

Khan, M. A. (1964) . Proteolytic activity in the digestive tract of the water scropton *Laccotrepes maculatus* Fabr. (Nepidae : Hemiptera) . *Ent. Exp. Appl.*, **1**, 335-338.

Khan, M. A. and Ford, J. B. (1967) . The distribution and localization of digestive enzymes in the alimentary canal and salivart glands of the cotton stainer, *Dysdercus fasciatus. J. Insect physiol.*, **13**, 1619-1628.

Khatoon, N. (1965) . The distribution of dipeptidase activity in the digestive organs of the larvae of *Utetheisa pulchella* L. (Lepidoptera : Arctidae) *Ent. exp. Appl.*, **8**, 289-292.

Khatoon, N. (1967) . The digestive enzymes of the larva of *Utetheisa pulchella* Linnaeus. *Beitr. Ent. Bd.*, **17**, 349-356.

Kitch, L. W. and Murdock, L. L. (1986) . Partial characterization of a major gut thiol proteinase from larvae of *Callosobruchus maculates* F. *Arch. Insect Biochem. Physiol.*, **3**, 561-575.

Klowden, M. J. (1990) . The endogenous regulation of mosquito reproductive behavior. *Experientia*, **46** (**7**) , 660-70.

Knecht, M., Hagenmaier, H. E. and Zebe, E. (1974) . The protease in the gut of the locust, *Locusta migratoria. J. Insect Physiol.*, **20**, 461-470.

Koiwa, H., Shade, R. E., Zhu-Salzman, K, D'Urzo, M. P., Murdock, L. L., Bressan, R. A. and Hasegawa, P. M. (2000) . A plant defensive cystatin (soyacystatin) targets cathepsin L-like digestive cysteine proteinases (DvCALs) in the larval midgut of western corn rootworm (*Diabrotica virgifera virgifera*) . *FEBS Lett.*, **471** (**1**) , 67-70.

Konarev, A. V., Anisimova, I. N., Gavrilova, V. A., Vachrusheva, T. E., Konechnaya, G. Y., Lewis, M. and Shewry, P. R. (2002) . Serine proteinase inhibitors in the Compositae: distribution, polymorphism and properties. *Phytochemistry*, **59** (**3**) , 279-91.

Kotani, E., Niwa, T., Tokizane, M., Suga,K., Sugimura, Y., Oda, K., Mori Hand Furusawa T. (1999) . Cloning and sequence of a cDNA for a highly basic protease from the digestive juice of the silkworm, *Bombyx mori. Insect. Mol. Biol.*, **8** (**2**) , 299-304.

Kramer, K. J., Felsted, R. L. and Law, J. H. (1973) . Cocoonase Unstructural studies on an insect serine protease. *J. Biol. Chem.*, **248**, 3021-3028.

Kretovick, V. L., Bundel, A. A. and Pshenova, K. V. (1943) . Mechanism of wheat injury by *Eurygaster integriceps. Compt. Rend. Acad. Sci. URSS.* **39**, 31-33.

Kunz, P. A. (1978 a) Resolution and properties of the proteinases in adult *Aedes aegypti* (*L*) . *Insect Biochem.*, **8**, 169-175.

Kunz, P. A. (1978 b) . Resolution and properties of the proteinases in the larvae of the mosquito, *Aedes aegypti. Insect Biochem.*, **8**, 43-51.

Kuroda, M. (1933) . Biochemical genetics on the digestive amylase in the silworm. *Idengaku Mag.*, **29**, 8-12.

Lam, W., Coast, G. M. and Rayne, R. C. (2000) . Characterization of multiple trypsins from the midgut of *Locusta migratoria.* langeroni., *Insect. Biochem. Mol. Biol.*, **30 (1)** , 85-94.

Lambremont, E. N. Fisk, F. W. and Ashrafi, S. (1959) . Pepsin-like enzymes in larvae of stable flies. *Science*, **129**, 1484-1485.

Langley, P. A. (1966) . The control of digestion in the tsetse fly, *Glossina morsitans.* Enzyme activity in relation to the size and nature of the meal. *J. Insect. Physiol.*, **12 (4)** , 439-48.

Langley, P. A. (1967) . Experimental evidence for a hormonal control of digestion in the tsetse fly, *Glossina morsitans* Westwood: a study of the larva, pupa and teneral adult fly. *J. Insect. Physiol.*, **13 (12)** , 1921-31.

Lara, P., Ortego, F., Gonzalez-Hidalgo, E., Castanera, P., Carbonero, P. and Diaz, I. (2000) . Adaptation of *Spodoptera exigua* (Lepidoptera: Noctuidae) to barley trypsin inhibitor BTI-CMe expressed in transgenic tobacco. *Transgenic Res*, **9 (3)** , 169-78.

Law, J. H., Dunn, P. E. and Kramer, K. J. (1977) . Insect proteases and peptidases. *Adv. Enzymol.* **45**, 389-425.

Lehane, M. J. (1976) . Digestive enzyme secretion in *Stomoxys calcitrans* (Diptera: Muscidae) . *Cell. Tiss. Res.* **170**, 275-287.

Lehane, M. J. (1977) . A hypothesis of the mechanism controlling proteolytic digestive enzyme production levels in *Stomoxyxs calcitrans. J. Insect Physiol* **23,** 713-715.

Lehane, M. J. (1994) . Digestive enzymes, haemolysins and symbionts in the search for vaccines against blood-sucking insects. *Int. J. parasitol.*, **24 (1)** , 27-32.

Lehane, M. J., Blackmore, D., Williams, S. and Moffat, M. R. (1995) . Regulation of digestive enzymes levels in insects. *Comp. Biochem. Physiol.*, **110B**, 285-289.

Lehane, S. M., Assinder, S. J. and Lehane, M. J. (1998) . Cloning, sequencing, temporal expression and tissue-specificity of two serine proteases from the midgut of the blood-feeding fly *Stomoxys calcitrans. Eur. j. Biochem.*, **254 (2)** , 290-6.

Lemos, F. J. A., Zoculoto, F. S. and Terra, W. R. (1992) . Enzymological and excretory adaptations of *Ceratitis capitata* (Diptera: Tephritidae) larvae to high proteins and high salt diets. *Comp. Biochom Physiol.* **102A**, 775-779.

Lenz, C. J., Kang, J., Rice, W. C., McIntosh, A. H., Chippendale, G. M. and Schubert, K. R. (1991) . Digestive proteinases of larvae of the corn

earworm, *Heliothis zea* characterization, distribution and dietary relationships. *Arch. Insect. Biochem. Physiol.*, **16** (**3**) , 201-12.

Liang. C, Brookhart, G., Feng, G. H., Reeck, G. R. and Kramer, K. J. (1991) . Inhibition of digestive proteinases of stored grain Coleoptera by oryzacystatin, a cysteine proteinase inhibitor from rice seed. *FEBS Lett.*, **278** (**2**) , 139-42.

Lichtenstein, N. (1947) . Proteolytic enzymes of insects. 1. Proteases of the silkworm, *Bombyx mori* L. *Enzymologia*, **12**, 156-165.

Lim, Do Seon Moon, Myung Jin and Yoe, Sung Moon (1991) . Midgut estrase of the cabbage butterfly *Pieris rapae* L. *Korean J. Entomol.* ***21***, 61-70.

Lin, S. and Richards, A. G. (1956) . A comparison of two digestive enzymes in the housefly and American cockroach. *Ann. Ent. Soc. Am.*, **49**, 239-241.

Lopez-Ordonez, T., Rodriguez, M. H. and Hernandez-Hernandez, F. D. (2001) . Characterization of a cDNA encoding a cathepsin L-like protein of *Rhodnius prolixus. Insect. Mol. Biol.*, **10** (**5**) , 505-11.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) . Proteins measurement with the Folin-phenol reagent. *J. Biol. Chem.*, **193**, 265-275.

Mahmood, F. and Borovsky, D. (1992) . Biosynthesis of trypsin like and chymotrypsin like enzymes in immature *Lutzomyia anthophora* Diptera: Psychodidate. *J. Med. Entomol.*, **29**, 489-495.

Mahmood, F. and Borovsky, D. (1993) . Biosynthesis of serine proteases in *Lutzomiya anthophora* (Diptera: Psychodidae) . *J. Entomol.* **30**, 683-688.

Matsumoto, I., Emori, Y., Abe, K. and Arai, S., (1997) . Characterization of a gene family encoding cysteine proteinases of *Sitophilus zeamais* (maize weevil) and analysis of the protein distribution in various tissues including alimentary tract and germ cells. *J. Biochem.*, **121** (**3**) , 464-76.

Matsumura,S. (1930) .Action of the salivary amylase in the silk worm, *Bombyx mori. Sanshigak. Mag.*, **2**, 251.

Matsumura, S. (1934) . Genetical and physiological studies on the action of the digestive juice and blood amylase of the silkworm, *Bombyx mori. Nagano Sanshi Hokoku*, **28**, 1 – 124.

Mazumdar-Leighton, S. and Broadway, R. M. (2001) . Identification of six chymotrypsin cDNAs from larval midguts of *Helicoverpa zea* and

Agrotis ipsilon feeding on the soybean (Kunitz) trypsin inhibitor. *Insect. Biochem. Mol. Biol.*, **31 (6-7)** , 633-44.

Mc Chi, T. K., Christeller, J. T., Ford, R. and All Sopp, P. G. (1995) . The properties in the midgut of three Scarab white grub species. *Arch. Insect Biochem. Physiol.*, **28**, 351-363.

Miller, J. W., Kramer, K. J. and Law, J. W. (1974) . Isolation and partial characterization of the larval midgut trypsin from tobacco horn-worm, *Manduca sexta* Johanson. *Comp. Biochem. Physiol.*, **48**, 117 – 129.

Milne, R. and Kaplan, H. (1993) . Purification and characterization of a trypsin-like digestive enzyme from spruce budworm (*Choristoneura fumiferana*) responsible for the activation of delta-endotoxin from *Bacillus thuringiensis*. *Insect. Biochem. Mol. Biol.*, **23 (6)** , 663-73.

Moffat, M. R., Blackmore, D.,and Lehane, M. J. (1995) . Studies on the synthesis and secretion of trypsin in the midgut of *Stomoxys calcitrans*. *Comp. Biochem. Physiol.*, **110B**, 291-300.

Moffat, M. R. and Lehane, M. J. (1990) . Trypsin is stored as an inactive zymogen in the midgut of *Stomoxys calcitrans*. *Insect Biochem.*, **20**, 719-723.

Moon, R.P., Tyas, L., Certa,U., Rupp, K., Bur, D., Jacquet, C., Matile, H., Loetscher, H., Grueninger-Leitch, F., Kay, J., Dunn, B.M., Berry, C., Ridley, R.G. and Hoffmann-La Roche, (1997) . Expression and characterization of plasmepsin I from *Plasmodium falciparum*. *Eur. J. Biochem.* **244 (2)** , 552-60.

Morgan, M. R. J., (1975 a) . Relationship between gut cellobiase, latase, aryl- β - glucosidase, and aryl- β - galactosidase activities of *Locusta migratoria*. *Insect Biochem.*, **5**, 609 – 617.

Mori, M. (1930) . Enzymes of Silkworm, *Bull. Chem. Soc. Japan*, **5**, 159 – 163.

Muraleedharan, D. and Prabhu, V. K. K. (1978) . Food intake and midgut protease activity in the red cotton bug *Dysdercus cingulatus* Fabr. (Heteroptera: Pyrrhocoridae) . *Entomon*, **3**, 11-17.

Muraleedharan, D. and Prabhu, V. K. K. (1979 a) . Role of the median neurosecretory cells in secreation of protease and invertase in the red cotton bug *Dysdercus cingulatus*. *J. Insect Physiol.*, **25**, 237-240.

Muraleedharan, D. and Prabhu, V. K. K. (1979 b) . Effect of quality of food on midgut invertas activity in the red cotton bug *Dysdercus cingulatus* Fabr. *Ind. J. Exp. Biol.*, **17**, 1262-1263.

Muraleedharan, D. and Prabhu, V. K. K. (1981) . Hormonal influence on feeding and digestion in a plant bug *Dysdercus cingulatus* and a caterpillar *Hyblaea puera. Physiol. Ent.* **6**, 183-189.

Murray, R. D., *et. al.* (1990) . Comparative absorption of [13C] glucose and [13C] lactose by premature infants. *American Journal of Clinical Nutrition*, **51**, 59-66.

Muse, W. A. (1984) . Studies on proteolytic activity in the midgut homogenate of the blowfly (*Chrysomia chloropya* (*Weid*) Diptera: Calliphoridae) . M. Sc. Thesis, University of Ife, Ile, Ife, Nigeria.

Nachman, R. J., Teal, P. E., Radel, P. A., Holman, G. M. and Abernathy, R. L. (1996) . Potent pheromonotropic/myotropic activity of a carboranyl pseudotetrapeptide analogue of the insect pyrokinin/PBAN neuropeptide family administered *via* injection or topical application. *Peptides*, **17** (**5**) , 747-52.

Nagaraju, J. and Abraham, E. G. (1995) . Purification and characterization of digestive amylase from tasar silk worm *Antheraea mylitta* (Letidoptera: Saturnidae) . *Comp. Biochem. Physiol.*, **110B**, 201-209.

Nishide, K. and Kusano, T. (1976) . Carbohydrases of the digestive tract of the larvae of cabbage butterfly, *Pieris rapae crucivora* Biosduval. *J. Fac. Agric. Tottori. Univ.*, **11**, 12 – 22.

Nogueira de Melo, A. C., Giovanni-De-Simone, S., Branquinha, M. H. and Vermelho, A. B. (2001) . *Crithidia guilhermei*: purification and partial characterization of a 62-kDa extracellular metalloproteinase. *Exp. Parasitol.*, **97** (**1**) , 1-8.

Noriega, F. G., Colonna, A. E. and Wells, M. A. (1999) . Increase in the size of the aminoacid pool is sufficient to activate translation of early trypsin mRNA in *Aedes aegypti* midgut. *Insect. Biochem. Mol. Biol.*, **29** (**3**) , 243-7.

Noriega, F. G., Shah, D. K. and Wells, M. A. (1997) . Juvenile hormone controls early trypsin gene transcription in the midgut of *Aedes aegypti. Insect. Mol. Biol.*, **6** (**1**) , 63-6.

Noriega, F. G., Wang, X. Y., Penningto, N. J. E., Barillas Mury, C. V., Wells and M. A. (1996) . Eartly trypsin a female –specific midgut protease in *Aedes aegypti* : isolation, amino terminal sequence determination and cloning and sequencing of the gene. *Insect. Biochem. Mol. Biol.*, **26** (**2**) , 119-26.

Novillo, C., Castanera, P. and Ortego, F. (1999) . Isolation and characterization of two digestive trypsin-like proteinases from larvae of the stalk corn borer, *Sesamia nonagrioides. Insect. Biochem. Mol. Biol.*, **29** (**2**) , 177-84.

Nuorteva, P. (1956) . Notes on the anatomy of the salivary gland and on the occurrence of protease in these organs in some leaf hoppers (Hem : Auchenorrhyncha) . *Ann. Ent. Fenn.*, **22**, 103 – 108.

Nuorteva, P. and Laurema, S. (1961) . Observation on the activity of salivary proteases and amylases in *Dolycoris baccarum* (L.) (Heteroptera: Pentatomidae) . *Ann. Ent. Fen.* **27**, 93-97.

O'Keefe, S. J. D., *et. al.* (1991) . Milk-induced mal absorption in mal nourished African patients. *American Journal of Clinical Nutrition*, **54**, 130-135.

Oppert, B., Hartzer, K. and Zuercher, M. (2002) . Digestive proteinases in *Lasioderma serricorne* (Coleoptera: Anobiidae) . *Bull. Entomol. Res.*, **92 (4)** , 331-6.

Overney, S., Yelle, S. and Cloutier, C. (1998) . Occurrence of digestive cysteine proteases in *Perillus bioculatus*, a natural predator of the Colorado potato beetle. *Comp. Biochem. Physiol., B. Biochem. Mol. Biol.*, **120 (1)** , 191-5.

Owhashi, M., Harada, M., Suguri, S., Ohmae, H. and Ishii, A. (2001) . The role of saliva of *Anopheles stephensi* in inflammatory response: identification of a high molecular weight neutrophil chemotactic factor. *Parasitol. Res.*, **87 (5)** , 376-82.

Ozkizilcik, S. and Chu, F. L. (1996) . Preparation and characterization of a complex micro encapsulated diet for striped bass Morone *J. Microencapsul.*, **13 (3)** , 331-43.

Pant, N. C., Srivastava, P. D. and Ghai, S. (1959) . Physiology of digestion in the larvae of *Chilo zonellus* Swinhoe. *Indian J. Ent.*, **21**, 238 – 245.

Parker, G. F. and Roberts, D. B. (1996) . AGI, a previously unreported *D. melanogaster* alpha-glucosidase: partial purification, characterization and cytogenetic mapping. *Biochem. Genet.*, **34 (3-4)** , 117-31.

Patel, N. G. and Richards, A. G. (1960) . Proteolytic enzymes from the midgut of the housefly. *J. Insect Physiol.*, **4**, 146 – 153.

Patterson, R. A. and Fisk, F. W. (1958) . A study of the trypsin-like protease of the adult stablefly, *Stomoxys calcitrans* (L.) *Ohio J. Sci.*, **58**, 299 – 310.

Peaucellier, G. (1983) . Purification and characterization of proteases from the polychaete annelid *Sabellaria alveolata* (L.) . *Eur. j. Biochem.*, **136 (3)** , 435-45.

Pernas, M., Sanchez-Monge, R., Gomez, L. and Salcedo, G. A. (1998) . Chestnut seed cystatin differentially effective against cysteine

proteinases from closely related pests. Unidad de Bioquimica, E. T. S. Ingenieros Agronomos, Ciudad niversitaria, Madrid, Spain. *Plant. Mol. Biol.*, **38** (**6**) , 1235-42.

Persaud, C. E. and Davey, K. G. (1971) . The control of protease synthesis in the intestine of adults of *Rhodnius prolixus*. *Insect Physiol.*, **17**, 1429-1440.

Peterson, A. M., Fernando, G. J. and Wells, M. A. (1995) . Purification, characterization and cDNA sequence of an alkaline chymotrypsin from the midgut of *Manduca sexta*. *Insect. Biochem. Mol. Biol.*, **25** (**7**) , 765-74.

Plataue, F. (1874) . Digestion in insects. *Me. Acad. Roy. Belg.*, **41**, 1 – 124.

Plínio, T., Cristofoletti, Walter, R. and Terra (1999) . Specificity, anchoring and subsites in the active center of a microvillar aminopeptidase purified from *Tenebrio molitor* (Coleoptera) midgut cells. *Insect Biochem. Mol. Biol.*, (**29**) **9**, 807-819.

Powning, R. F. Day, and Irzykiewicz, H. (1951) . Studies on the digestion of wool by insects. II. The properties of some insect proteinases. *Aust. J. Sci. Res., B.*, **4**, 49 – 63.

Prema, A. K. and Mohamed, U. V. K. (1980) . Gut protease activity in *Spodoptera mauritia* Boisd. *Geobios*. **7**, 233-234.

Prosser, C. L. and Brown, F. A. Jr. (1965) . *Comparative Animal Physiology* (2nd Ed) . W. B. Saunders Company. Philadelphia and London.

Raga-Cazares, F. E., Sanchez-Contreras, M. E., Rodriguez, M. H. and Hernandez-Hernandez, F. C. (1998) . Sex specific proteins and proteases present in the midguts of *Anopheles albimanus* (Diptera: Culicidae) . *J. Med. Entomol.*, **35** (**2**) , 184-6.

Ramos, A., Mahowald, A. and Jacobs-Lorena, M. (1993) . Gut-specific genes from the black fly *Simulium vittatum* encoding trypsin-like and carboxypeptidase-like proteins. *Insect. Mol. Biol.*, **1** (**3**) , 149-63.

Rao, B. R. and Fisk, W. F. (1965) . Trypsin activity associated with reproductive development in the cockroach, *Nauphoeta cinerea* (Blattaria) . *J. Insect physiol.*, **11**, 961-971.

Rao, G. S. and Rastogi, S. C. (1967) . Digestive enzymes in the midgut of tenebrionid beetle, *Arthrodes*. *Proc. Zool. Soc. Calcutta*, **20**, 69 – 81.

Rastogi, S. C. (1962) . On the salivary enzymes of some phytophagous and predaceous Heteroptera. *Science and Culture*, **28**, 479-480.

Reddy, G. V., Quero, C. and Guerrero, A. (2002) . Activity of Octylthiotrifluoropropan-2-one, a Potent Esterase Inhibitor, on Growth, Development and Intraspecific Communication in

Spodoptera littoralis and *Sesamia nonagrioides J. Agric. Food Chem.* **50 (24)** , 7062-8.

Regel, R., Matioli, S. R. and Terra, W. R. (1998) . Molecular adaptation of *Drosophila melanogaster* lysozymes to a digestive function. *Insect. Biochem. Mol. Biol.*, **28 (5-6)** , 309-19.

Sakurai, H. (1968) . Physiological studies on the digestion of coccinellid beetles (Coleoptera : Coccinellidae) , with special reference to their food habits. *Appl. Ent. Zool.*, **3**, 130 – 136.

Sakurai, H. (1968) . Physiological studies on the digestion of coccinellids beetles (Coleoptera: Coccinellidae) , with special reference to their food habits. *Appl. Ent. Zool.* **3**, 130-138.

Salvador, M. T., Murillo, M. D., Rodriguez-Yoldi, M. C., Alcalde, A. I., Mesonero, J. E. and Rodriguez-Yoldi, M. J. (2000) . Effects of serotonin on the physiology of the rabbit small intestine. *Can. J. Physiol. Pharmacol.*, **78 (5)** , 359-66.

Sandeman, R. M., Feehal, J. P. and Bundoora. (1990) . Tryptic and chytpmotryptic proteases released by larvae of the bowfly *Lucilia cuprina. Int. J. parasitol.*, **20**, 1019-1023.

Sarangi, S. K. (1986) . Studies on midgut protease activity during fifth instar development of the silk worm *Bombyx mori* L. *Entomon.* **11**. 165-169.

Saxena, K. N. (1954 a) . Physiology of the alimentary canal of *Leptocosrisa varicornis* Fabr. (Hemiptera : Coreidae) . *J. Zool. Soc. India*, **6**, 111 – 112.

Saxena, K. N. (1954 b) . Feeding habits and physiology of digestion in certain leaf hoppers (Homoptera : Jassidae) . *Experientia.* **10**, 383 – 384.

Saxena, K. N. (1954 c) . Physiology of digestion in *Leptocorisa varicornis* Fabr., (Hemiptera : Coreidae) . *Curr. Sci.*, **23**, 132.

Saxena, K. N. (1955) . Studies on the passage of food, hydrogen-ion concentration and enzymes in the gut salivary glands of *Dysdercus koenigii* Fabr. *J. Zool. Soc. India*, **7**, 145 – 154.

Saxena, K. N. (1958) . Digestion and absorption of carbohydrates in the alimentary canal of the red cotton bug, *Dysdercus koenigii* Fabr. (Hemiptera : Pyrrhocoridae) . *Physiol. Zool.*, **31**, 129 –138.

Saxena, K. N. (1963) Mode of ingestion in the heteropterous insect *Dysdercus koenigii* (F.) (Pyrrhocoridae) . *J. Insect Physiol.*, **9**, 47-71.

Saxena, K. N. and Bhatnagar, P. (1958) . Physiological adaptations of dusky cotton bug *Oxycarenus hyalinipennis* (Costa) (Heteroptera :

Lygaeidae) to the host plant cotton. *Proc. nat. Inst. India. B*, **24**, 245-257.

Scheneider, F., Houseman, J. G. and Morrison, P. E. (1987) . Activity cycles and the regulation of digestive proteases in the posterior midgut of the *Stomoxyxs calcitrans* (L.) (Diptera: Muscidae) . *Insect Biochem.*, **17**, 859-862.

Schernthaner, J. P., Milne, R. E. and Kaplan, H. (2002) . Characterization of a novel insect digestive DNase with a highly alkaline pH optimum. *Insect. Biochem. Mol. Biol.*, Mar 1;**32 (3)** , 255-63.

Schlottke, E. (1937 a) . The digestive enzymes of insects. I. The distribution of enzymes in the intestinal canal of meat-eating Carabidae and alteration in their concentration during digestion. *Z. Veergleisoh. Physiol.*, **24**, 210-247.

Schlottke, E. (1937 b) . The digestive enzymes of insects. II. The enzymes of the leaf and hay grasshoppers and their dependence on the mode of life. *Z. Vergleisch. Physiol.*, **24**, 422-450.

Schlottke, E. (1937 c) . The digestive enzymes in insects. III. The dependence on the enzyme content and diet: studies on *Periplaneta americana L. Z. Vergleisch. Physiol.*, **24**, 463-490.

Sexena, K. N. (1955) . Studies on the passage of food hydrogen ion concentration and enzymes in the gut and salivary glands of *Dysdercus Koenigii* Fabr. *J. Zool. Soc. India.* **7**, 145-154.

Sexena, K. N. (1958) . Digestion and Absorption of Carbohydrates in the alimentary canal of the red cotton bug. *Dysdercus koenigii* Fabr. (Heteroptera: Pyrrhocoridae) . *Physiol. Zool.* **31**, 129-138.

Shamabaugh, G. F. (1954) . Protease stimulation by foods in adult *Agdes aegypti* Linn. *Ohio J. Sci.*, **54**, 151-160.

Sharma, B. R., Martin, M. M. and Shafer, J. A. (1984) . Alkaline protease from the gut fluids of detritus feeding larvae of the cranefly, *Tipula abdominalis* (Say.) (Diptera: Tipulidae) . *Insect Biochem.*, **14**, 37-44.

Shinoda, O. (1930 a) . Contribution to the knowledge of intestinal secretion in insects. III. On the digestive enzymes of the silkworm. *J. Biochem.*, **11**, 345-367.

Shinoda, O. (1930 b) . Contributions to the knowledge of intestinal secretion in insects. IV. Comparison of the pH optima of the digestive enzymes from different groups of insects. *Kyoto Imp. Univ. Anniversary Vol.* 9-24.

Shukle, R. H., Murdock, L. L. and Gallun, R. L. (1985) . Identification and partial characterization of a magor gut proteinase from larvae of the

Hessain fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae) . *Insect Biochem.*, **15**, 93-102.

Silva, C. P., Terra, W. R. and Lima, R. M. (2001) . Differences in midgut serine proteinases from larvae of the bruchid beetles *Callosobruchus maculatus* and *Zabrotes subfasciatus*. *Arch. Insect. Biochem. Physiol.*, **47** (**1**) , 18-28.

Simpson, S. J. and Bernays, E. A. (1983) . The regulation of feeding: locusts and blowflies are not so different from mammals. *Appetite*, **4** (**4**) , 313-46.

Sinha, M. (1975) . Pepsin like activity in the midgut of *Sarcophaga ruficornis* and *Musca domestics*. *Appl. Ent. Zool.*, **10**, 313-315.

Sinha, M. (1976) . Digestive enzymes in the gut and salivary glands of *Sacrophaga ruficornis* Fab. And *Musca domestica* L. (Diptera : Insecta) . *Appl. Ent. Zool.*, **11**, 260-262.

Smartt, C. T., Chiles, J., Lowenberger, C. and Christensen, B. M. (1998) . Biochemical analysis of a blood meal-induced *Aedes aegypti* glutamine synthetase gene. *Insect. Biochem. Mol. Biol.*, **28** (**12**) , 935-45.

Soo Hoo, C. F. and Dudzinski, A. (1967) . Digestion by the larvae of the pruinose Scarab, *Sericesthis geminata*. Ent. Exp. appl., **10**, 7-15.

Spinella, S., Levavasseur, E., Petek, F. and Rigothier, M. C. (1999) . Purification and biochemical characterization of a novel cysteine protease of *Entamoeba histolytica*. *Eur. j. Biochem.*, **266** (**1**) , 170-180.

Spiro-kern, A. and Chen, P. S. (1972) . Uber de Proteasen der Stechmick, *Culex pipiens*. *Rev. Susse. Zool.*, **79**, 1151-1159.

Srivastava, U. S. and Srivastava, P. D. (1957) . Observations on the feeding habits and digestion in *Mylavris phalerata* Pall., the blister beetle (Coleoptera: Meloidae) . *Proc nat. Acad. Sci. India*, **27** (**B**) , 144-149.

Stiles, J. K., Wallbanks, K. R. and Molynent, D. H. (1991) . The use of casein substrates gels for determining trypsin-like activity in the midgut of *Glossina palpalis* (Diptera: Glossinidae) . *J. Insect physiol.*, **37**, 247-254.

Stone, T. E., Li, J. P. and Bernasconi, P. (1994) . Purification and characterization of the *Manduca sexta* neuropeptide processing enzyme carboxypeptidase E. *Arch. Insect. Biochem. Physiol.*, **27** (**3**) , 193-203.

Sumenkova, V. V., Ermicheva, F. M. and Yazlovetskii, I. G. (1989) . Characterization of gut proteases of some species of *Chrysopa* with different feeding habits. *J. Evol. Biochem. Physiol.*, **25**, 301-306.

Swingle, H. S. (1925) . Digestive enzymes of an insect. *Ohio J. Sci.*, **25**, 209 – 218.

Takanona, T. and Hori, K. (1974) . Digestive enzymes in the salivary gland and midgut of the bug, *Stenotus binotatus. Comp. Biochem. Physiol.*, **47 A**, 521 – 528.

Tatchell, R. J. (1958) . The Physiology of digestion in the larvae of the horse bot-fly, *Gasterophilus intestinalis* (De Geer) . *Parasitology*, **48**, 448 – 458.

Teo, L. H. and Woodring, J. P. (1994) . Comparative total activities of digestive enzymes in different gut regions of the house cricket *Acheta domestica* L. (Orthoptera: Gryllidae) . *Ann. Entomol. Soc. Am.* **87**, 886-890.

Terra, W. R. (1988) . Physiology and biochemistry of insect digestion: an evolutionary perspective. *Braz. J. Med. Biol. Res.* **21**, 675-734.

Terra, W. R. and Ferreira, C. (1994) . Insect digestive enzymes: Properties, compartment –alization and function *Comp. Biochem. Physiol.*, **109B**, 1-62.

Terra, W. R. and Braz (1988) . Physiology and biochemistry of insect digestion: an evolutionary perspective. *J. Med. Biol. Res.*, **21 (4)** , 675-734.

Terra, W. R., Ferreira, C. and De Bianchi, A. G. (1979) . Distribution of the digestive enzymes among the endo-and ectoperitrophic spaces and midgut cells of *Rhynchosciara* and its physiological significance. *J. Insect Physiol.*, **25**, 487 – 494.

Terra, W. R., Ferreira, C. and Garcia, E. S. (1988) . Origin distribution properties and function of the major *Rhodnius prolixus* midgut hydrolases. *Insect Biochem.*, **18**, 423-434.

Thei, N. M. R. and Houseman, J. G. (1990 a) . Identification of cathepsin-B, D and H in the larval midgut of Colorado potato beetle *Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae) . *Insect Biochem.*, **20**, 313-318.

Thie, N. M. R. and Houseman, J. G. (1990 b) . Cysteine and Seriene proteolytic activities in larval midgut of yellow meal worm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) . *Insect Biochem.*, **20**, 741-744.

Thomas, A. W. and Gooding, R. H. (1976) . Digestive processes of haematophagous insects. VIII. Estimation of meal size and

demonstration of trypsin in horse flies and deer flies (Diptera: Tabanidae) . *J. Med. Ent.* **13**, 131-136.

Thomas, A. W., Rolseth, B. M. and Gooding, R. H. (1976) . Digestive process of haemotophagous insects. IX. Some properties of two trypsins from female house flies and deer flies (Deptera : Tabanidae) . *J. Med. Ent.,* **13**, 341 – 346.

Thomsen, E. and Moller, I. B. (1963) . *J. Exp.Biol.* **40**, 301.

Treherne, J. E. (1967) . Gut absorption. *A. Rev. Ent.,* **12**, 43 – 58.

Tryselius, Y. and Hultmark, D. (1997) . Cysteine proteinase 1 (CP1) , a cathepsin L-like enzyme expressed in the *Drosophila melanogaster* haemocyte cell line mbn-2. *Insect. Mol. Biol.*, **6 (2)** , 173-81.

Turunen, S. (1979) . Digestion and absorption of lipids in insects. *Comp. Biochem. Physiol.,* **63 A**. 445 – 460.

Uscian, J. M., Miller, J. S., Sarath, G. and Stanley Samuelson, D. W. (1995) . A digestive phosphatase A2 in the tiger beetle *Cicindela circumpicta. J. Insect physiol.*, **41**, 135-141.

Valaitis, A. P., Augustin, S. and Clancy, K. M. (1999) . Purification and characterization of the western spruce budworm larval midgut proteinases and comparison of gut activities of laboratory-reared and field-collected insects. *Insect Biochem. Mol. Biol.*, **29 (5)** , 405-15.

Varis, A. L., Laurema, S. and Miettinen, H. (1983) . Variation of enzyme activities in the salivary glands of *Lygus rugulipennis* (Hemiptera: Miridae) . *Ann. Ent. Fenn.* **49**, 1-10.

Verma, P. S. and Balyan, B. S. (1972) . Studies on the hydrogen-ion concentration and digestive enzymes in the mature larva of *Platyedra gossypiella* Saund. (Lepidoptera : Gelechidae) . *Indian J. Ent.*, **34**, 136 – 141.

Verma, P. S. and Prasad, M. (1972) . Studies on the hydrogeneion concentration and the digestive enzyme in *Mylabris pustulata* Thump. (Coleoptera : Meloidae) . *Indian J. Ent.*, **34**, 294 – 299.

Verma, P. S. and Prasad, M. (1975) . The digestive physiology of *Gryllodes sigillatus* Walker. (Orthoptera : Gryllidae) . *Indian J. Ent.*, **37**, 19 - 23.

Verma, P. S., Singh, B. and Agarwal, V. K. (1977) . Physiology of digestion in the mature larva of *Sylepta derogata* Fabr. *Indian J. Ent.* **39**, 132 – 138.

Vundla, W. R. M, Brossard, M., Pearson, D. J. and Labengo, V. L. (1992) . Characterization of aspartic proteinases from the gut of the tick,

Rhipicephalus appendiculatus Neuman. *Insect Biochem. Mol. Biol.*, **22**, 405-410.

Ward, C. W. (1972) . Diversity of proteases in the keratinolytic larvae of the webbing clothes moth, *Tineola bisselliella. Comp. Biochem. Physiol.*, **42 B**, 131 - 135.

Ward, C. W. (1975 a) . Resolution of proteases in the keratinolytic larvae of the webbing clothes moth. *Aust. J. Biol. Sci.*, **28**, 1 – 24.

Ward, C. W. (1975 b) . Aminopeptidases in webbing clothes moth larvae. Properties and specificities of the enzymes of intermediate electrophoretic mobility. *Biochem. Biophys. Acta,* **410**, 361 – 369.

Ward, C. W. (1975 c) . Aminopeptidases in webbing clothes moth larvae. Properties and specificities of the major enzymes of high electrophoretic mobility. *Aust. J. Biol. Sci.*, **28**, 447 – 455.

Waterhouse, D. F. (1949) . The hydrogen-ion concentration in the alimentary canal of larval and adult Lepidoptera. *Aust. J. Sci. Res. (Ser. B.)* ,. **2**, 428 – 437.

Waterhouse, D. F. (1957) . Digestion in insects. *A. Rev. Ent.*, **2**, 1 – 18.

Wharton, D. R. A. Wharton, M. L. and Lola, J. E. (1965) . Cellulose in the cockroach with special reference to *Periplaneta americana. J. Insect physiol.*, **11**, 947-959.

Wieman, K. F. and Nielsen, S. S. (1988) . Isolation and partial characterization of a major proteinase from larval *Acanthoscelides obtectus* Say, (Coleoptera: Bruchidae) . *Comp. Biochem. Physiol.*, **89B**, 419-426.

Wigglesworth, V, B. (1928) . Digestion in the cockroach III. The digestion of proteins and fats. *Biochem. J.* **22**, 150-161.

Wigglesworth, V. B. (1972) . Digestion and Nutrition in *The Principles of Insect Physiology.* 7th Edition, 427-495. Chapman and Hall, London.

Wijffels, G., Gough, J., Muharsini, S., Donaldson, A. and Eisemann, C. (1997) . Expression of angiotensin-converting enzyme-related carboxydipeptidases in the larvae of four species of fly. *Insect. Biochem. Mol. Biol*, **27** (**5**) , 451-60.

Wilhite, S.E. Elden, T.C., Brzin, J. and Smigocki, A.C. (2000) . Inhibition of cysteine and aspartyl proteinases in the alfalfa weevil midgut with biochemical and plant –derived proteinase inhibitors. *Insect Biochem. Mol. Biol.*, **30** (**12**) , 1181-8.

Winnie Lam Geoffrey, M., Coast Richard, C. and Rayne (1999) . Isolation and characterization of two chymotrypsins from the midgut of *Locusta migratoria, Insect Biochem. Mol. Biol.*, **29** (**7**) , 653-660.

Wolfson, J. L. (1987) . Cysteine digestive proteinases in Coleoptera. *Comp. Biochem. Physiol.*, **87B**, 783-787.

Woods, H. A. and Kingsolver, J. G. (1999) . Feeding rate and the structure of protein digestion and absorption in lepidopteran midguts. *Arch. Insect. Biochem. Physiol.*, **42 (1)** , 74-87.

Wyatt, G. R. (1967) . The biochemistry of sugars and polysaccharides in insects. *Adv. Insect Physiol.*, **4**, 287 – 360.

Xiong, B. and Jacobs-Lorena, M. (1995) . Gut-specific transcriptional regulatory elements of the carboxypeptidase gene are conserved between black flies and *Drosophila. Proc. Natl. Acad. Sci. U S A.*, **92 (20)** , 9313-7.

Xu, Gang, Qin and Junde (1994) . Extraction and charecterization of midgut proteases from *Heliothis armigera* and *H-assulta* (Lepidoptera: Noctuidea) and their inhibition by tannic acid. *J. econ. enomol.*, **87 (2)** , 334-338.

Yan, J., Cheng Q., Li, C. B. and Aksoy, S. (2002) . Molecular characterization of three gut genes from *Glossina morsitans morsitans*: cathepsin B, zinc-metalloprotease and zinc-carboxypeptidase. *Insect. Mol. Biol.*, **11 (1)** , 57-65.

Yan, J., Cheng, Q., Li, C. B. and Aksoy, S. (2001) . Molecular characterization of two serine proteases expressed in gut tissue of the African trypanosome vector, *Glossina morsitans morsitans. Insect. Mol. Biol.*, **10 (1)** , 47-56.

Yang, Y. J. and Davies, D. M. (1968 a) . Amylase activity in blackflies and mosquitoes (Diptera) . *J. Med. Ent.*, **5**, 9 – 13.

Yang, Y. J. and Davies, D. M. (1968 b) . Occurrence and nature of invertase activity in adult blackflies (Simulidae) . *J. Insect Physiol.*, **14**, 1221 – 1232.

Yang, Y. J. and Davies, D. M. (1968 c) . Digestion, emphasizing trypsin activity in adult simulids (Diptera) fed blood, blood-sucrose mixtures, and sucrose. *J. Insect Physiol.*, **14**, 205-222.

Yoe, S. M. and Kim, H. R. (1987) . Purification and immunological study of protease in the larval midgut of *Pieris rapae. Korean J. Entomol.* **17**, 123-128.

Yonemura, M., Kasatani, K., Asada, N. and Ohnishi, E. (1991) . Involvement of a serine protease in the activation of prophenol oxidase in *Drosophila melanogaster. Zool. Sci.* **8**, 865-867.

Young, R. G. (1978) . Activities of some midgut acid hydrolyses of the soutehern armyworm, *Spodoptera eridania.* Ann. Ent. Soc. Am., **71**, 95 – 98.

Zaidi, Z. S. (1985) . Proteolytic activity in the gut of red cotton bug, *Dysdercus Cingulatus* Fabr. And its predator, *Antilochus Ccocqueberti* (Fabr.) (Heteroptera: Pyrrhocoridae) . *Curr. Sci. India.* **54**, 252-253.

Zeng, F. and Cohen, A. C. (2000) . Comparison of alpha-amylase and protease activities of a zoophytophagous and two phytozoophagous Heteroptera. *Comp. Biochem. Physiol., A. Mol. Integr. Physiol.*, **126** (**1**) , 101-6.

Zerbo do Carmo, A., Silva de Moraes, R. L. and Brochetto-Braga, M. R. (2001) . Protein requirements in larvae and adults of *Scaptotrigona postica* (Hymenoptera: Apidae [correction of Apidia], Meliponinae) , midgut proteolytic activity and pollen digestion. *Comp. Biochem. Physiol., Biochem. Mol. Biol.*, **129** (**1**) , 139-47.

Zhu, Y. C. and Baker, J. E. (1999) . Characterization of midgut trypsin-like enzymes and three trypsinogen cDNAs from the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae) . *Insect. Biochem. Mol. Biol.*, **29 (12) ,** 1053-63.

Zhu, Y. C. and Baker, J. E. (2000) . Molecular cloning and characterization of a midgut chymotrypsin-like enzyme from the lesser grain borer, *Rhyzopertha dominica. Arch. Insect. Biochem. Physiol.*, **43** (**4**) , 173-84.

Zhu, Y. C., Kramer, K. J., Dowdy, A. K. and Baker, J. E. (2000) . Trypsinogen-like cDNAs and quantitative analysis of mRNA levels from the Indian meal moth, *Plodia interpunctella. Insect. Biochem. Mol. Biol.*, **3.**

Zindler, D. and Potzer, M. (1992) . Identification and characterization of the digestive proteinases from fire bat *Thermobia domestica. Comp. Biochem. Physiol.*, **103B**, 669-673.