IV.1. Introduction

Enzymes are proteins, which catalyse a variety of reactions in the biological systems. The multifacacious enzymes present in living cells can be isolated from the various biological active sites. Different techniques have been used to measure the enzyme of the salivary glands were detected using histochemical and calorimetric methods for enzymes viz, proteolytic, macerating and cellulolytic enzymes (Miles, 1972); invertase (Persuad and Davey, 1971); peroxidase, catalase and lipase (Saxena and Bhatnagar, 1980). In addition to the enzymes, precursors like free amino acids mainly fluctuated by pH, salt concentration and temperature, in general (Adedire, 1984; Barnad and Prosser, 1973; Ghilov, 1978) and temperature in particular (Boyer et al., 1960; Colourick and Kaplan, 1955, 1959; Gutfreund, 1965; Sebrell and Harris, 1954; Webbe, 1966). The digestive enzymes commonly found in the salivary secretions and regions of the digestive tract of various insects have been examined by many authors and were comprehensive reviewed by House (1965). Regional localization of various enzymes in the alimentary canal of Coleoptera (Adedire and Balorgun, 1995), and Reduviidae (Cohen, 1993) have been recorded earlier. Several researches were explained the digestive enzymes of heteroptera which includes proteinase, lipase, phospholipase, amylase, pectinase, invertase, hyaluronidase and nucleases (Miles, 1972; Cohen, 1998). In addition, Cohen (1993) found out the proteinases, trypsin, and esterase like enzymes in the saliva of Zelus renardi.
A wide range of digestive enzymes were recorded in the alimentary canal of insects (Chapman, 2000) including reduviids (Sahayaraj et al., 2007a). In spite of the ample amount of information available on the digestive enzymes in insects (House, 1965; Applebaum, 1985; Suzuki and Veda, 1987; Madhuras and Rao, 1989) there is a dearth of information on impact of any abiotic on reduviid especially *R. marginatus* and *R. fuscipes*. Environmental factors largely determines the metabolic system of insects which inturn decides it response to treated below and above optimum temperature. *R. marginatus* and *R. fuscipes* have been considered as important biological control agents of many agricultural pests. They have been used in the augmentative biological control programme, where they are stored at different temperature. Moreover, these redvuiids were distributed many topographic regions in India. Hence it is imperative to determine the impact of various constant and variable temperatures on the enzyme profiles of *R. maginatus* and *R. fuscipes* fore hindguts separately.

**IV.2. Materials and Methods**

*R. marginatus* and *R. fuscipes* maintained in different temperatures regimes for a month were selected for this study. Ten active predators were washed, and dissected in insect ringer solution (IRS - 1% NaCl). Entire gut was removed, forget and hindguts were pooled separately and homogenised in ice cold IRS in a mortar and pestle. The homogenate was centrifuged in cold for 20 minutes and the supernatant was used as enzyme source.

**IV.2.1. Quantitative enzyme bioassays**
IV. 2.2. Invertase

Invertase activity was estimated using 0.2% sucrose as a substrate in the reaction mixture, with 10mM phosphate buffer (pH 6.8) and measuring the glucose per minute at 30°C (Sumida et al., 1994). Moreover the glucose estimation was done using the Dinitro Salicylic (DNS) reagent with dextrose for this standard at 540 nm. Moreover standard graph was drawn for comparison.

IV. 2.3. Amylase

Amylase activity was measured using dinitro salicilic acid (DNS) procedure with soluble starch as a substrate (Bernfield, 1955 and Baker, 1991. The reaction mixtures contained 0.2% soluble starch, 10 Mm borate buffer (pH 9.2) and enzyme extract. It was incubated at 37°C for 30 minutes and the reaction was terminated with the addition of 500 µl of DNS reagent. The colour developed was read at 575 nm and composed with standard maltose hydrate. The result was expressed as µg maltose released / mg / min.

IV. 2.4. Proteases

Protease activity was assayed following the method of Eguchi and Iwamoto (1976) as outlined. 60 µl of enzyme sample was added with 200 µl aliquot of 1 % azocasein (in 0.2 m glycine – NaOH - pH 10.0) and incubated at 37°C for 30 mts. The reaction was terminated by the addition of 300 Aliquot of 5% trichloacetic acid. After centrifugation at 1500g for 10 mts, an equal volume of 1M NaOH was added to the supernatant and absorbance was measured at 450 nm. One proteinase
unit was defined as the amount of enzyme that increased the absorbance by 1.0 OD under the given assay conditions.

**IV. 2. 5. Esterases**

Activity levels of esterases were estimated according to method of Van Aspereen (1959). The total assay mixture (6 ml) contained 5.0 ml of substrate in phosphate buffer (pH 7.5) and 1.0 ml of tissue extract. The reaction mixture was incubated at room temperature at 30°C for 20 minutes. The reaction was arrested by the addition of one ml of chromogen solution containing 2 parts of 1% solution of fast blue B and 5 parts of 5% Sodium lauryl sulphate solution. The colour developed after the addition of fast blue B was read against the reagent blank at 3 ml. All the above said enzymes were always carried out in triplicate and the mean values were expressed in the results.

**IV. 3. Results**

**IV. 3.1. Protease**

Protease activity was noticed both in foregut and hindgut. But the enzyme concentration was very higher in hindgut then foregut. When the temperature increased, protease activity was also increased from 10 – 25°C (0.216 to 0.591 µg/ml). Peak protease activity was recorded at optimum temperature 25°C, and then it was decreased observed at 30 and 35°C. As observed in foregut, the protease activity was also maximum at 25°C (1.047) followed by 30°C (0.951 µg/ml) and at 35°C (0.946 µg/ml) (Figures 9a and 9b). Similarly in *R. fuscipes*, maximum
protease activity was recorder at 25°C (0.87 and 1.49 µg/ml for fore and hindgut respectively (Figures 10a and b).

IV. 3.2. Esterase

Esterase activity was higher both in fore and hindgut of *R. marginatus* at lower temperature (20°C). Then, esterase level was decreased when the temperature increased. This trend was similar both in the fore and hindgut of *R. marginatus*. In *R. fuscipes* foregut, esterase activity was almost higher as well as same both at 20 and 30°C. But in hindgut, the activity was high during the moderate temperature at 30°C. This enzyme level was slightly varied between fore and hindgut of these reduviids.

IV. 3.3. Amylase and Invertase

Figures 10 a, b and 9 a, b shows amylase and invertase activities of *R. marginatus* and *R. fuscipes*. Irrespective of the predator species and location of gut, in general, activities of both invertase and amylase gradually increased from 10°C to 35°C. While we compare the location, these enzymes activities were well pronounced at hindgut than the foregut.

IV.4. Discussion

Digestive enzymes play a major role in the body of insects by conversing complex food materials in to micromolecules necessary to provide energy and metabolites (Wigglesworth, 1972). Amylase, protease, invertase and esterase showed maximum activity in salivary and haemolymph protein of many insects
Amylase is one of the key enzymes involved in digestion and carbohydrate metabolism in insects. Chatterjee et al., (1989) reported the presence and two different forms of amylase in digestive fluid and haemolymph. Abraham et al., (1992) noticed that amylase activity of the digestive fluid was 40 fold higher than that of haemolymph.

The major function of the digestive enzymes in reduviid is “extra-oral digestion”. Enzymes mainly used to disintegrate prey tissue before ingestion after which further digestion and takes place. Cohen (1998) called this type of digestion as enzymatic tissue maceration and he observed the process in Zelus renardit. To appreciate the role of macerating enzymes, it is necessary to understand the internal organization of the prey added (Balogun and Fisher 1970; Balogun, 1972). During feeding, the reduviids not only feed the haemolymph but also the interior contents including the organs (cells, tissues) and their networking macro and micro molecular complex including proteoglycans, collagens elastics etc. The nutrient rich materials in the prey are packed in a basement membrane that is impermeable of digestive enzymes (Agusti and Cohen, 2000). The enzymes like trypsin and chymotrypsin are present in the reduviids (Cohen 1998) used for digesting these materials too.

Salkeld1(1961 and 1965) reported cathepsin the proteinase in the posterior midgut of Sinca spp. and Z. renardii could liquify and extract all of the nutrients of a prey nearly equal to its own body weight with in less than two hours Matsumara, 1988 (Both in R. marginatus and R. fuscipes hindgut, the protease level was increased in foregut. Although quite a good number of reports exist on esterase
pattern in insect tissues, no information was available for reduviid predators. Esterase mainly plays a lipolytic role in eggs. Our studies showed that esterase also has an important role in digestion too.

The enzyme activities of the hindgut showed higher when compared to foregut. Moreover, food protein stimulates the secretion of more amount of protease in hindgut (Ishaya, et al., 1971, Upadhyay and Misra, 1991 and 1994). This studies shows that in addition to the abiotic factors like prey (Sahayaraj, 2007a; Sahayaraj et al., 2007a), abiotic factor, temperature also influence the production of protease in R. marginatus and R. fuscipes.

Invertase (β - fructo furanosidase also termed as β- fructosidase, saccharase, or sucrase) are glycoside hydrolases that catalyse the cleavage of sucrose (α - D – Glucopyranosyl - β - D- fructofuranoside) in to the two monosaccharrides, glucose and fructose (Shen, 1986; Law et al., 1977). Carbohydrates ingested by heterotrophic organisms undergo several metabolic steps, in the first of which polymorphic carbohydrates were cleaved into their monomers, which can pass through membranes. Invertase, thus, appears to be particularly important enzyme for insects. Given this general importance, surprisingly few studies have tried to quantify invertase activity in reduviids (Sahayaraj et al., 2007; Cohen, 1993). Invertase usually is quantified via the release of glucose from sucrose. The effect of high and lower temperature may reflect a reduced enzymatic level. The activities of the organism are influence either directly or indirectly by the environment. The extreme (too low and high)
conditions of the environment may upset the physiological aspect of the insects. Enzyme is one of the most important determinants of physiological characters.

The present study suggested that factors(s) other than prey nutrients, temperature was also involved in the production of enzymes from both fore and hindgut of the reduviids. The higher amylase and invertase activities recorded at 35°C in the hindgut homogenate is expected since the predator shore more amount of carbohydrate as reservoir which is considered as important metabolic food of insects (Eguch, 1983). These enzymatic variations in the gut could also due to the presence in the microbes gut. Hence screening of microbes is essential to know better about enzymes secreted in the gut. Previously Kalaiselvam and Arulpandi (2006), stated that thousands of protease was present, but only least number of proteases have been recognised in the digestive action and regulate the physiological process. These variations might be due to the activity of various autochthonous microbes present in the alimentary canal. Basic informations about the bacterial flora of gut were highlighted in the next chapter.

IV. 5. Conclusion

Protease activity was higher at 25°C in fore and hindgut of both predators whereas amylase and invertase activity was maximum at 35°C. In *R. marginatus*, foregut and hindgut showed maximum esterase activity at 20°C. But in *R. fuscipes*, esterase activity was maximum and equal both in 30°C and room temperatures.