Chapter I. GENERAL INTRODUCTION

I.1. Reduviid as an IPM Component

Biological control is a component of an Integrated Pest Management (IPM) strategy, where natural enemies like predators, parasitoides and pathogens placed an important role. Adoption of IPM strategies helps to reduce the use of insecticides. These tactics were ecologically sound, economically viable and socially acceptable method (DeBach and Hagen, 1991). Reduviids suppressed till more than 18 lepidopterans few coleopteran and hemipteran pests were reduced the most of the pest population both in laboratory and field situation (Ambrose, 1999; Sahayaraj 2003; 2006). Though reduviids were polyphagous predators being less specific in selecting prey and with a wide range could be possible serve to reduce the outbreak of many pest species and could be immense help in checking the damage of agricultural crops. Many scientists considered reduviids as a less specific in their choice of prey but most of the entomologists continued to stress that they could play a vital role in biological control programme (Sahayaraj, 2004, 2006). Unfortunately the biological potential of reduviids has not been investigated in the field situation and at a large scale release studies were not carried out by any one in any part of the world.

Reduviids played a major role in suppressing the pest population in India (Sahayaraj, 2002a, 2006) and they can be utilized as a biological control agent, where a variety of pests occured (Schaefer, 1988; Lakkundi, 1989; Sahayaraj, 2000; Sahayaraj and Martin, 2003; Sahayaraj, 2006). Hence there was a better
scope for utilising the reduviids in the biological control programme. Ragupathy and Sahayaraj (2002a) pointed out that among the Harpactorine reduviid species belongs to *Rhynocoris* genus mainly associated with the agricultural pests, they mainly present either in agricultural ecosystems or nearby ecosystem such as semiarid zones, scrubjungles and forests etc. Previously Navarajanpaul (2003) listed 18 reduviids, which were predominant in various agricultural fields. More than 65 reduviids were reported to be presented in various ecosystems such as cotton, soybean, rice, sugarcane, groundnut, wheat, sunflower and pigeon pea etc. (says Sahayaraj, 2007a).

1.2. Temperature and Predatory Insects

1.2.1. Hemipteran insects

Little information was available about the impact of cyclic conditions of temperature and humidity on development of entomophagous predators. Temperature influence on development and reproduction of heteropteran Pentatomidae (1992a,b, 1993,1994; James, 1992; Torres et al., 1998; Whittman et. al., 2002); Anthocoridae (Izumi and Ohto, 2001; Parajulee et al., 1995) were studied in details. Information about the effect of cold storage was available for eggs of a pentatomid predator, *Podisus maculiventris* (Say.) (Goryshin and Tuganova, 1989). Influence of temperature on the biology of hemipteran predators in general have been reported by many authors (Silva, 1985; Braga et al., 1998; Galvao et al., 1999; Rocha, et al.,2001; Almeida, et al., 2003; Izumi and Ohto, 2001). Monitoring methods for determining the effects of temperature on
oviposition in over wintering females of *Pseudocalpis pentagona* developed by Wigglesworth (1972); Mitsuyoshi (2004).

### I.2.2. Reduviid predators

*Rhynocoris marginatus* (Fab.) and *Rhynocoris fuscipes* (Fab.) were considered to be the most important predators of many pests. All the five nymphal instars and also the adults were obligatory entomophagous and potential predators of many economically important crop pests. It was previously reported that under laboratory conditions, the temperature essential for the eclosion and molting of reduviids were ranged either between 16-34°C (Gomez - Nunez and Fernandez, 1963) or 15°C and 35°C (Okasha, 1964,1968a,b).

Population dynamics of reduviid predators in relation to various biotic and abiotic factors were reported by Ambrose (1980); Vennison (1988); Sahayaraj (1991); Kumaraswamy (1991). The population density of a particular reduviids depends upon the biotic and abiotic factors (Goel, 1978; Haridass, 1987; Ambrose and Livingstone, 1989; Vennsion and Ambrose, 1990; Sahayaraj 1991; Kumaraswami and Ambrose, 1993 and 1994). Recently Dhanasing and Ambrose (2006) reported the seasonality on the reduviid predator’s population of Thoothukudi District, Tamil Nadu, India. The climatic abiotic factors indirectly govern the distribution and density of assassin bugs in any natural ecosystem as reported by Ambrose and Rani (1991), Ambrose and Rajan (1995). Sahayaraj (2007a) reported that the reduviid population has been observed abundant in dry areas, even in lower rainfall and relative humidity and moderate temperature.
I.3.1 *Rhynocoris marginatus* (Fab.) (Heteroptera: Reduviidae)

*Rhynocoris marginatus* (Fab.) was a polyphagous, multivoltine, entomosuccivores, polymorphic, crepuscular and alate bug predominantly found in the scrubjungles, semi arid zones, tropical rain forests and agroecosystem of south India (Livingstone and Ambrose, 1978; Sahayaraj, 1994 and 2002, 2007a). It was an selective biological control agent of many agricultural and forest insect pests like *Earias fraterna* (Fab.) (Ambrose, 1988), *Papilio demoleus* (L.) *Earias vittella* (Fab.) (Nayer et al., 1976), *Corcyra cephalonica* (Stainton) (Bhatnagar et al., 1983), *Helicoverpa armiger* (Hubner) (Ambrose 1987), *Mylabris indica* (Faust), *Mylabris pustulata* (Fab) (Imms, 1985 and Nayer et al., 1976), *Achea janata* (Linn.), *Oxycarenus hyalinipennis* (Costa) and *Approarea modicella* (Deventer) (Sahayaraj, 1995a,d; Sahayaraj et al., 2003) and *Spodoptera litura* (Fab.) and *Amsactta albistriga* (Walker.) (Sahayaraj, 2000).

Recently Sahayaraj (2007a) explained the biological control potential of *R. marginatus* on four groundnut pest under laboratory condition. George et al., (2002) observed the nutritional influence of prey on the biological and biochemistry of *R. marginatus*. There was a great effect of biopesticides on the incubation period and hatchaility of *R. marginatus* eggs (Sahayaraj and Paulraj, 1999). Previously impact of space (Vennison, 1988), mating behaviour (Ambrose and Livingstone, 1985), starvation (Ambrose et al.,1990a,b) prey influence (Ambrose and Claver,1996) on the biology of this bug was worked out. Bio-efficacy and prey size influence on the developmental period (Sahayaraj, 1995a,b,c) and predatory potential of this bug (Ambrose and Claver, 1996) recorded. Ecotypic
(Ambrose, 1987) and polymorphic diversity (Ambrose and Livingstone, 1978; Vennison and Ambrose, 1988) of this reduviid was also documented. Sahayaraj et al. (2003) observed the effect of two biopesticides on the eggs and nymphal instars of this predator and in the same year Sahayaraj and Martin (2003) found out that, augmented control in groundnut pests. Recently Sahayaraj et al. (2007) and Sahayaraj and Balasubramanian (2008) studied the prey influence on salivary gland and gut enzymes quality of this reduviid.

1.3.2. *Rhynocoris fuscipes* (Fabricius) (*Heteroptera: Reduviidae*)

*Rhynocoris fuscipes* (Fabricius) was a crepuscular, brightly coloured (black and red), entamophagous, harpactorine reduviid found in concealed habitats such as underneath the stones and cervices (Ambrose and Mayamuthu, 1994, Ambrose, 1987). When it present in an agroecosystem and it predate upon various insects like *Helicoverpa armigera* (Hubner), *Corcyra cephaionica* (Stainton), *Achea janata*, *Plutella xylostella* (L.) *S. litura* (Fab.) *Myzus Persica* (Sulz.), *Lygus hespes* (Fabricius). *Viginatiocta punctata* (Walker.), *Rhaphid opaipa* (Thunb.), *Foveicollis lucas* (Distant.), *Semiethisa pervolagata* (Walker.) (Singh, 1985), *Epilacrisia stigma* (Muls.) (David and Natrajan, 1989) *Cryptosilla pyranthes* (Linn.) (Hiremath and Thondarya, 1983), *Calocoris angustatus* (Leth.) (Ambrose, 1980); *Patanga succincta* (Linn.), *Dysdercus cingulatus* (Fab.), *Earias vitella* (Fab.) (Singh and Sing, 1987), *Earias insulana* (Boisduval) (Cherian, 1987), *Nezara viridulla* (Linn.) (Singh and Gangrade, 1975); *Perigrinus maidis* (Ashm.), (Ponnamma et al., 1919); *Spilosoma obliqua* (Walker.) (Cherian and Kylasam, 1939); *Myllocoris curvicornis* (Fab.), (Cherian and Brahmachari, 1941);
Aulacophosa foveicollis (Fab.) (Ambrose, 1995); Pleopidas mathias (Fab.), Clavigarata gibbosa (Spinda.), Clavigarata horrens (Distant.), Dolycoris indicus (Stal.) (Mohanadas, 1996). Pest suppression efficacy mass rearing and functional response of R. fuscipes on various crop pests (Singh and Gangrade, 1975; Ponnamma et al., 1919; Singh, 1985; Ambrose and Livingstone, 1986b; Singh and Singh 1985; Ambrose, 1995, and reproductive performance on three lepidopteran pests (Babu et al., 1995; Ambrose and claver, 1995; George and Ambrose, 1999a,b; George et al., 2000a, 2000b;) were also been studied.

I.4. **Augmentation**

Prey record of reduviids were large and diverse, conservation and augmentation of reduviid predator and their utilization in biological control of insect pests have been gaining momentum in India and other countries in recent years (Ambrose, 1995; Schaefer, 1988; Sahayaraj, 2007a). Though conservation and augmentation are two different theoretical phenomena, they can’t be separated. Since, augmentation usually produced effects were interrelate to each other (Rabb et al., 1976). Conservation and augmentation of reduviids can be achieved by manipulation of these natural enemies (De Bach and Hagen, 1964) with abiotic factors (Chapman, 2000) in order to make them more efficient in the management of pest population.

Augmentation (or) accelerated production of biological control agents at roughly one million time, the female progeny rate during the time required for the completion of one generation of biocontrol agents with economical procedure involving minimum labour was a pre requisite for any successful biocontrol
programme (Clark et al., 1978). Augmentation of reduviid predator was attempted by Edward in 1962. In Rhynocoris carmelita (Stal.) and Platymeris rhadamathar (Gerstalker) (Rhyckman and Rhyckman, 1996); Reduvius sensiles, (Faust) Reduvius vanduzueri Wygodizinsky and Usinger and Reduvius sonoraensis (Walker) further more Tawfik et al. (1983a,b) also recorded the augmentation behaviour of Allaeocranum biannulipes (Montr and Singh.)

In India, an exotic reduviid predator Platymeris laevicollis (Distant) was colonised laboratory released in large numbers on the crowns of the coconut at Pandalan in Kerala and Androth in Lakshadweep and Vital in Karnataka (Antony et al., 1979). They found that the establishment of this predator population and the control Orius rhinocerous beetle. Ambrose (1988, 1995); Schaefer (1988); Sahayaraj (2006) felt that the urgent need for evolving strategies to mass rear the potential reduviid predators, their subsequent large scale of release in to the pest infested agroecosystem and to assess their biological control potential.

1.5. Need for storage of Insects

Overview of storage of insects primarily relates to IPM programmes where insects and mites were to be mass reared and released to produce some beneficial results. It is a part of a multi-disciplinary pest control strategy. The purpose of maintaining or storing the natural enemies under laboratory or refrigerated condition for utilise them when and where the natural enemies were not available in natural condition and also integrate them in IPM programme (Leppla, 1984).
Mass rearing of natural enemies to control the agricultural pests is recorded in ancient Chinese history, and united states of America (USA) and it has been practiced for over 100 years (Ferguson, 1990). Over 60 years ago, storage of implementation coinciding with onset on reliable mechanical refrigeration (King, 1934; Schread and German, 1934). Subsequently, the use of low temperature has proved to be a valuable tool in mass rearing purpose. Plenty of information was available for many natural enemies related with temperature and insect development.

Lakkundi and Prashad (1987) explained the mass rearing of reduviid predators with freezed and immobilized larvae of *Corcyra cephalonica* (Stain.). Later Sahayaraj (1991) mass reared few reduviids on head crushed *C. cephalonica* by larval card method. This method prevents the entangling of reduviids in the web of larvae undergoing metamorphosis. Furthermore both alive and frozen larvae of *C. cephalonica* was used to mass rearing of *R. marginatus* (Sahayaraj and Jeyalakshmi, 2002). In addition, substrata alteration and prey or predator density alteration (Kumaraswami, 19991; Sahayaraj, 1995a,b,c; 2001,2002; Ambrose, 2001; George *et al.*, 2002; Sahayaraj *et al.*, 2003) and types of preys (Ambrose *et al.*, 1990; Sahayaraj and Martin, 2003) have been tested for the mass production of insects. Mass rearing of reduviid predator reduced the post embryonic developmental period, enhanced the adult longevity and female biased sex ratio of *R. marginatus* and *R. fuscipes* (Kumaraswamy, 1991; Sahayaraj, 1991, 2007a).

I.6. Storage of insects life stages
An adequate storage of the natural enemies of pests was essential to face the problems related to production, planning and the unpredictability of demand. Cold storage was a useful technique to ensure the availability of beneficial insects for further research or field release without maintaining or continuous rearing. Furthermore tolerance to cold may be considered as a desirable attribute for shipment procedure (Van lanteran and Woets, 1988; Howe, 1967). Ezequiel and Carlos (2007) recently assessed the optimal temperature and substrate for male. Effect of temperature on development of the heteropteran predators were studied by Izumi Ohto (2001) and Carlos et al. (2007). Very recently Caceres et al. (2007) identified the various protocols for storing and transporting the egg of various types of insects.

Information about the effect of cold storage was available for the eggs of a pentatomid predators, Podisus maculiventris (Say) (Goryshin and Tuganova, 1989; Usharani, 1992); eggs and adults of Podisus maculiventris (Say) and P. sagitta (Fab.) (De Clercq and Degheele, 1992b, 1993). In reduviids cold storage of R. marginatus and R. fuscipes eggs with various temperature was studied by Sahayaraj and Paulraj (1999).
I.7. Temperature impacts on Enzymology

The importance and relevance of digestive physiology to the control of insect have been recognised by Uvarov (1996), Ishaaya and Swirski, (1970). In spite of the ample amount of information available on the digestive enzyme of insects (Howe, 1974; Applebaum, 1985), dearth amount of information was available about the effect of temperature on digestive enzymes of heteropteran predators. Esterase constitutes a major group of hydrolytic enzymes have been reported (Augusti and Cohen, 2000) earlier. Amylase was one of the key enzymes involved in digestion and carbohydrate metabolism of insect (Horie and Watanabe, 1980).

I.8. Gut Microflora

Microorganisms play an important and often essential role in the growth and development of many insect. Endosymbionts contribute to insect reproduction, digestion, nutrition, and pheromone (Buchner, 1965). Symbiotic relationship between insects and their gut bacteria have been studied extensively in several insects (Houck, 1991; Chen and Purcel, 1997; Breznake and Bryne, 1982). The diversity of the insects were reflects in the large and varied microbial communities inhabiting in the gut (Dillon and Dillon, 2004). The indigenous (autochthonous) to gut bacteria was regarded as a valuable metabolic resources to the nutrition of the host by improving the ability to live on sub-optimal diets, improved digestion efficiency, acquisition of digestive enzymes and provision of vitamins (Douglas, 1992; Tanada and Kaya 1993; Biggs and Greego, 1994; Bignell et al., 1997). The contribution of gut microbiota to the nutrition and disease suppression was also

Gut microflora at different insect order like Orthoptera (Hunt and Charnley 1981); Diplura and Placoptera (Findley et al., 1986); Coleoptera (Lemke et al., 2003); Isoptera (Smith and Douglass, 1987); Blattaria (Santo et al., 1998; Donovan et al., 2004); Lepidoptera (Mc Killip et al., 1997; Pankaj et al., 2003, Sahayaraj and Mary Joseph 2003; Broderick et al., 2004); Heteroptera (Dasch et al., 1984; Fukatsu and Hosokawa, 2002; Sahayaraj 2007b) were available in the literature. However, no one has taken initiative to study the impact of various constant temperatures on reduviid predators THBP and gut enzymology.

I.9. **Macromolecule**

Polymerase chain reaction (PCR) was a name given by Ehrlich, 1989. Then it was called as People Choice Reaction (Das, 2005). He also explained a simple and rapid DNA extraction method from plant, animal and insects which were suitable for RAPD and other PCR analysis. RAPD profiling (Williams et al., 1990) was still the method of choice for many researchers looking to address a wide range of biological issues in an equality diverse array of organisms. RAPD data have enabled insights into population structure geographical origins and invasion routes of colonising species and conservation genetics (Mark and Jervis, 2005).

Owing to the recent progress in molecular biology, in particulars the development of PCR, more sensitive DNA technologies have become available,
such as Random Amplified polymorphic DNA (RAPD) and Amplified fragment length polymorphism (AFLP). The more extensive overview of molecular genetic technologies and applications in insects was given by Hoy (1994). Practical guides for DNA technologies were provided by Sambrook et al. (1989). Gozlan et al., (1997) attempted to use RAPD–PCR to distinguish between strains of three species of *Orius* spp used in augmentative release programmes. Species were readily distinguished, but a high degree of polymorphism prevented discrimination between strains (Says Mark and Jervis, 2005. Very recently Reza et al. (2008) reported factors affecting detect ability of prey DNA - PCR based methods applied in the gut contents of invertebrate predators.

It was well known that PCR-based detection from feacces or urine of phytophagous reduviid bugs, and blood samples from mammals was more efficient than the other techniques (Mosser et al., 1989; Brenier et al., 1992; Russomando et al., 1992; 1996; Brigitte and Simone, 1998; Carezza Booto et al., 2005). However, no information was available about PCR based techniques on polyphagus heteropteran predators including *R. marginatus*, and *R. fuscipes*.

Carbohydrate, protein and lipid are the important constituents of any cell. Any animals need these macromolecules in proposed concentrations. The content of these macromolecules could be altered by both biotic and abiotic factors. George and Ambrose (1999) reported biochemical modulations of reduviids on an insecticide. In this chapter, I analysed the impact of temperature on the macromolecular composition of these two reduviids. From the available literature
it was very clear that very little information was available about the impact of
temperature on reduviids including *R. marginatus* and *R. fuscipes*.

I.10. Usage of ELISA technique in reduviid predators

Laboratory optimisation is necessary to quantify the rate of antigen decay and identify the immune response developed by species specific antigen of the particular predators (Stuart and Greenstone, 1990). Recently ELISA is a series of controlled laboratory experiments that suggested that the indirect ELISA varies in sensitivity between predator species in gut content immunoassays and be attributed to a combination of uncontrollable abiotic and biotic factors (Eckert *et al.*, 1981). For instance, temperature variations, predator metabolic rate, quantity of prey consumed, and development stage of the prey consumed can all affect the quantitative outcome of a gut content immunoassay (Mclver, 1981; Hagler and Cohen, 1990). Initially researchers were not concerned with this variable sensitivity because predators gut content immunoassays are inherently qualitative in nature. All these factors can influence detection of prey material (Says Sunderland, 1996). Furthermore the most collection of arthropods gut-content analyses can yield false- positive data due to surface-level contamination with target prey or increases interaction between predators and prey due to inappropriate sampling protocols (Hardwood and Obrycki, 2005a,b).

In the early investigation, several authors opinioned (Hagler *et al.*, 1992; Hagler and Naranjo, 1994) and subsequently contributed to our understanding of the role of invertebrate predators in biological control long detection periods for prey antigens following them their consumption (Harwood *et al.*, 2001) compared
with the relating short ones for prey (Sheppard et al., 2004) can sometime make immunological techniques advantages in the field assessment of predation.

From the available literature it was very clear that no information was available about the impact of temperature on reduviid predators such as *R. marginatus* and *R. fuscipes*. Hence the present investigation was undertaken with the following objectives:

- Biology and biological control potential of *R. marginatus* and *R. fuscipes* on *Spotoptera litura* (Fab.) (Lepidoptera : Noctuidae) and *Dysdercus cingulatus* (Fab.) (Hemiptera : Pyrrochorridae) and *Corcyra cephalonica* (Stainton) (Lepidoptera:Noctuiidae) in relation to constant temperatures (10-35°C).

- Eggs and adult macromolecular (total carbohydrate, protein and lipid) profiles in relation to various temperatures.

- Influence of temperatures on the autochthonous gut bacterial populations of these reduviids and their hydrolytic enzyme activities.

- Nucleic acid (DNA) and antibody profiles of these reduviids in relation to constant temperature.