NATURAL, FACTITIOUS HOST AND OLIGIDIC DIETS ON BIOECOLOGY, BACTERIAL, MOLECULAR AND ANTIBODY PROFILES OF *RHYNOCORIS MARGINATUS* (FAB.)

Thesis submitted to

Manonmaniam Sundaranar University for

the Degree of Doctor of Philosophy in Zoology

By

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May 2008

CERTIFICATE

I certify that the thesis entitled **"Natural, Factitious host and Oligidic diets on Bioecology, Bacterial, Molecular and Antibody Profiles of *Rhynocoris marginatus* (Fab.)"** being submitted by **Mr. R. BALASUBRAMANIAN (Reg. No. 2138)** is a bonafide record of research work carried out by him independently at the Crop Protection Research Centre, Department of Advanced Zoology and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai under my guidance for the degree of Doctor of Philosophy in Zoology. The details furnished in the thesis is the original work of the candidate and has not been submitted elsewhere in part or full for any other degree, diploma, associateship or other similar titles. It is not the plagiarism of any other work either published or unpublished without acknowledgement.

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DECLARATION

I do here by declare that the thesis entitled **"Natural, Factitious host and Oligidic diets on Bioecology, Bacterial, Molecular and Antibody Profiles of *Rhynocoris marginatus* (Fab.)"** is the result of the original study carried out by me under the guidance of **Dr. K. Sahayaraj,** Director, Crop Protection Research Centre, Department of Advanced Zoology and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai for the degree of Doctor of Philosophy in Zoology. This work has not been submitted earlier in full or part for any other degree, diploma or associate ship elsewhere. I also assure that no part of the thesis is a reproduction from any other sources either published or unpublished without acknowledgement.

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ACKNOWLEDGEMENT

Words are not enough to thank my guide I take this opportunity to acknowledge the guidance and encouragement of my guide **Dr. K. Sahayaraj,** Department of Advanced Zoology and Biotechnology, St. Xavier's College, Palayamkottai. I have been inspired by his meticulousness, his attention to detail and her energetic application to any problem. I value his concern and support at all times, good and bad.

I whole heartedly thank to Principal **Rev. Dr. Alphones Manickam, S.J., (Present)** and **Rev. Dr. A. Antonysamy, S. J., (Former)** St. Xavier's College, Palayamkottai for providing the adequate institutional and laboratory facilities.

I am grateful to **Prof. M. Thomas Punithan,** Head, Department of Zoology, St. Xavier's College, Palayamkottai for his encouraging words and special care during my study period.

I am highly indebted to My father **S. Rathinam** for their motivation, guidance and prayer that enabled me to undertake my higher studies.

I acknowledge the Council for Scientific and Industrial Research, **Government of India** New Delhi, financially assisted me to carry out my research in the form of Senior Research Fellowship during the tenure of my work.

My warm thanks to **Dr. Prince Jeyaseelan and Dr. N. Felix,** Department of Aquaculture, Fisheries college and Research Institute, Thoothukudi for their keen interest, affection and care shown towards me during my research tenure. It is truly a wonderful place to study and work. Their interest and involvement in my research and concern for my welfare have greatly motivated me.

I sincere thanks to **Rev.Dr.V.S.Manickam,** Director, Centre for Biodiversity and biotechnology, St.Xavier's College, Palayamkottai, for providing laboratory facilities.

With great pleasure, I thank to the faculty members of the **Dr. Natarajan**, Botany Department, St. Xaviers College, Palayamkottai and **Mr. Saravanagandhi**, Department of Botany, Rani Anna college for women, Tirunelveli, for their help, for their timely help to make things available.

My heartiest thanks to my colleagues at Crop Protection Research Centre **Dr. G. Raju,** Pioneer Kumaraswamy Memorial College, Nagercoil**, Dr. S. Karthick,** Sathyabama Deemed university, Chennai. **Miss S. Sujatha, Mr. Francise Borgio** and **Mr. S. Muthukumar, Mr.A.Vinoth Kanna, Mr. P. Kombiah** (Ph.D., Scholars) and M.Phil Scholars for their tremendous support, cooperation and selfless help extended by them during various stages of my work.

I have been privileged to have stayed with **Dr. M. Maridass** and **Dr. N. Siva** during the course of my thesis. I have learnt several things from them and highly indebted to them. Their continuous support, encouragement and appreciation have been extremely valuable to me.

I am beholden to **Dr. A. Benniamin, Scientist 'C'** Botanical Survey of India, Arunachal pradesh and **Mr. B. Wilson, Mr. A. Justin**, Sathyabama Deemed university, Chennai. **Mr. K. Thangavel**, SPK College, Alwarkurichi for their taught molecular techniques. **Mr. U. Ramesh,** AHRU, **Dr. Rilbert Janarthanan,** Lecturer, Dept. of Tamil, St. Xaviers college**,** Palayamkottai, **Miss.S.Sumathi, Mr.Suresh** for their encouraging words and better care.

I sincerely thank **Prof.Patrick De Clercq,** University of Gent, Belgium., **Dr.Ferkovich and Dr.Jeffray** USDA-ARS, USA, **Mr.K.Nagarajan,** ERU, SXC, for providing their reprints and encouragements.

I am always grateful to **Dr. Roy** and **Mr. Shanmugarajan,** Annai Velankanni Hospital Technician, Tirunelveli, for their technical assistance ELISA reader and timely help.

I thank **Mr. T. Sakthivel, Mr. J. Jegathesan** (Mannarpuram) and **Mr. K. Selvaraj** (Brother in law) (Sivakasi) his family for the making available the groundnut field facilities.

I have no words to express my gratitude for my Sister **Mrs. S. Rajeshwari** and my Brother **Mr.R.Arumugasamy** without whose love, patience, support and encouragement throughout, this thesis wouldn't be possible.

I have no words to express my gratitude for my **family members** and my relatives for their love, prayer, support, care and encouraging words that certainly acted as a paddle and propelled me to have a smooth sail in my academics.

I acknowledge the several friends and well wishers, whom I have not mentioned above and whose best wishes have always encouraged me.

Above all I thank the **Almighty God** who is being with me and showers his blessings and grace towards me in all walks of my life.

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DECLARATION

I do here by declare that the thesis entitled **"Temperature–Dependent Biology, Bio-efficacy and its impacts on Bacterial growth, Macro molecular and Antibody profiles of two Reduviid predators"** is the result of the original study carried out by me under the guidance of **Dr. K. Sahayaraj,** Director, Crop Protection Research Centre, Department of Advanced Zoology and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai for the degree of Doctor of Philosophy in Zoology. This work has not been submitted earlier in full or part for any other degree, diploma or associateship elsewhere. I also assure that no part of the thesis is a reproduction from any other sources either published or unpublished without acknowledgement.

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ACKNOWLEDGEMENTS

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I take this opportunity to acknowledge with always pleasure for the guidance and encouragement, meaningful motivation including much hard work of my guide **Dr. K. Sahayaraj**. I have been inspired by his meticulousness, his attention to detail and his energetic application to any problem. I value his concern and support at all times, good and bad.

I acknowledge with gratitude the guidance and support of **Rev. Dr. Alphones Manickam**, **Rev. Dr. Antony Leo Tagore**, S.J. (Secretary), and **Rev. Dr. A. Antonysamy**, S. J., St. Xavier's College (Autonomous), Palayamkottai for providing the adequate institutional and laboratory facilities. Their interest and involvement in my work and concern for my welfare have greatly motivated me.

I am grateful to **Prof. M. Thomas Punithan**, Head, Department of Zoology, St. Xavier's College (Autonomous), Palayamkottai for his encouraging words and special care during my study period. I also express my sincere gratitude to all staff members from the Department of Advanced Zoology and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai for their keen interest in my studies.

I thank for **Mr. R. Balasubramanian** and **Mr. J. Francis borgio,** their support and encouragement and patience in reviewing the drafts of my thesis. I have learnt

several things from them and am highly indebted to them. Their continuous support and appreciation have been extremely valuable to me.

I thank my seniors and friends **Dr. G. Raju, Dr. S. Karthick Raja Namachivayam, Mss. Jeyaparvathi, Mr. S. Muthu kumar, Mr. A. Vinoth Kanna, Mr. Kalidass,** and **Mr P. Kombiah** for their tremendous support, cooperation and selfless help

I am thankful to with great pleasure, I thank **Dr. Natarajan** Head of the Department of Botany and **Mr. Saravanaganthi** the faculty members of the Botany Department, St. Xavier's College (Autonomous), Palayamkottai for their kind help and their instrumentation facilities.

I thank **Dr. R. K**. **Jacob,** Principal, Chief Warden and Sub-warden Prof. **Mrs. S. Rajakumari**, **Miss. G. Suga** St. John's College, Sharon Hostel, Palayamkottai, for their ready to accommodate and special care in the hostel when ever necessary through my research periods.

I acknowledge **Miss. Sumathi, Miss Krishna Veni** and her family members for their unassuming friendship and willingness to help at all times.

I enjoyed the company of my friends and labmates **S.Thangarani, S. Ezhil, G. Marshia, S. Sumi, D. Josephine Geetha, J. Josephine Vinotha, A. Jasmine Priya, Y. Mary jeeva and Mary Sujin**.

I am grateful to **Dr. A. Benny**, Scientist 'C', Ministry of Environment and Forest, Kolkata, Mr. A. Justin, Sathyabama Deemed University, Chennai. **Dr. M. Maridass**, Young Scientist, Dept. of Science and Technology, New Delhi, **Mr. U. Ramesh** and,

AHRU, St. Xavier's College (Autonomous), Palayamkottai for their timely help to make things available.

I must make a special mention of **Dr. Ray,** and **Shanmugarajan**, Annai velankanni hospital for his cheerful company and enthusiastic help ELISA demonstration studies.

I have been privileged to have encourage to me **S. Jeno, A. Minil, M. Eugin Arputharaj,** and **M. Albert** during the course of this thesis. Their continuous support, encouragement and appreciation have been extremely valuable to me.

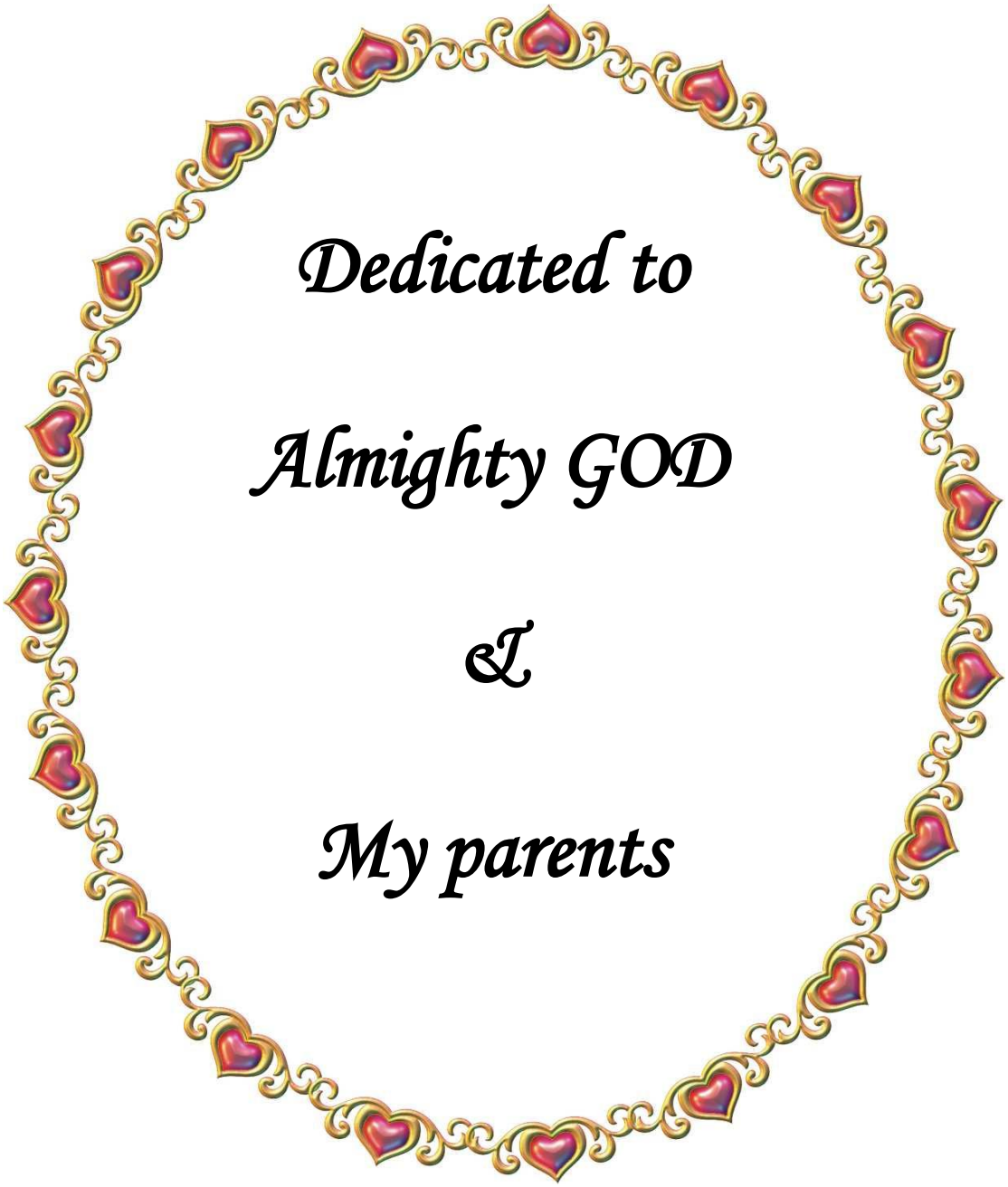
I am thankful to JD'S Computer centre, for their DTP works and necessary facilities regarding printing.

I acknowledge the several friends and well wishers, whom I have not mentioned above and whose best wishes have always encouraged me.

I must make a mention of my father's elder brother **Dr. C. Rev. Amalraj** and grandparents their steadfast faith in my capability has always spurred me to go ahead, especially in difficult times.

I have no words to express my gratitude for **my parents** and **my sister** without whose love, patience, support and encouragement throughout, this thesis wouldn't be possible. I am highly indebted to My father for their motivation, guidance and prayer that enabled me to undertake my higher studies.

Miss S. Sujatha


Dedicated to
Almighty GOD
&
My parents

Dedicated to

My

beloved Mother

ABSTRACT

Hunter reduviids have been distributed both in tropical and temperate regions in India. *Rhynocoris marginatus* (Fab.) and *Rhynocoris fuscipes* (Fab.) (Insecta: Hemiptera: Reduviidae) have been distributed in semiarid zones, scrub jungles, agro-ecosystem and tropical forests and hence, they have a lot of possibilities of facing environmental crisis. Moreover since these two reduviids were considered as a biological control agents of many economically important pests, augmentative release is an imperative one. The present investigation was under taken in the laboratory to find out the impact of constant temperatures (10, 15, 20, 25, 30 and 30^0C) and fluctuation temperatures (29 + 1.5^0C) on eggs and nymphal development and survival rate, sex ratio fecundity and hatchability at lower and higher threshold temperatures, morphometry and morphogenesis, autochthonous gut bacteria and their enzyme production and gut enzyme profile of crude whole animal carbohydrate, protein and lipid content, and immunogenic activity of whole predator DNA content and its polymorphism. Irrespective of the predators and exposure periods, constant temperatures gradually decreased when the egg hatching periods from lower temperature to higher temperature. However, the egg survival ability was diminished between 10 to 20^0C, 10 - 15^0C, and 35^0C were not suitable for *R. marginatus* and *R. fuscipes* development. However, the nymphal developmental periods of these two reduviids were significantly diminished from 20^0C to room temperature. It was also attributed in the survival rate of nymphal instars that constant temperatures were not have any impact on the

sex ratio of the two reduviids. All the tested temperatures were always in favour of female biased sex ratio. Constant temperatures reduced the pre- oviposition period, oviposition period, post-oviposition period, fecundity and hatchability, also size (length and weight) of these two reduviids. Interestingly when freshly moulted *R. marginatus* adults were subjected to serious of constant temperature, these are laid maximum number of 198 eggs/ female at 30^0C with 95% hatchability. Linear model analysis shows that *R. marginatus* needs 24.31 and 32.31% as lower and higher threshold. It was slightly increased to *R. fuscipes* (25.01 and 34.28% for lower and higher threshold temperatures). Morphological data reveals that total body length was gradually as well as significantly increased from 20^0C (1.30 cm) to room temperature (2.08 cm). It was also recorded many morphogenesis effects both in nymphs and adults of *R. marginatus* and *R. fuscipes* at 15, 20, and 35^0C.

The biological control potential studies reveals that the stage preference of *R. marginatus* fifth nymphal instars and adults were more successful in encounter the large sized preys. All the nymphal instars and adults of *R. fuscipes* mainly preferred second to fourth instar larvae of *S. litura* and second to fifth instars of *D. cingulatus.* Both *R. marginatus* and *R. fuscipes* approached their preys quickly at higher temperatures and handled more time finally the weight gain was also maximum. From this result did not observed much variation between the temperatures at 25 and 30^0C.

In *R. fuscipes,* esterase activity was maximum and equal both in 30^oC and room temperature. In *R. marginatus* foregut and hindgut showed maximum esterase activity at 20^0C. Protease activity was higher at 25^0C in fore and hindgut of both predators where as amylase and invertase activity maximum at 35^0C. The

total heterotrophic bacterial population (THBP) of *R. marginatus* and *R. fuscipes* whole gut was gradually increased from 10 to 30^0C. Between these two reduviids, *R. fuscipes* has maximum THBP with more number of bacterial species (13) then *R. marginatus* (11) speices. Temperature specific bacterial species were also recorded in these reduviids. However *Micrococcus variance* was the predominant species both in *R. marginatus* (40.56%) and *R. fuscipes* (47.22%). All the recorded bacterial species involved in the production of hydrolytic enzymes like amylase, protease, invertase and esterase. The whole body macromolecular contents like total carbohydrate, protein and lipid was higher in *R. marginatus* than *R. fuscipes*. Similarly, the protein content of *R. marginatus* alimentary canal was higher than *R. fuscipes.*

SDS-PAGE of *R. marginatus* gut protein polypeptides was ranged from 6.5 kDa to 500 kDa. 405 kDa polypeptide was specific for *R. marginatus* at 20^0C, another unique band (500 kDa) recorded at 10^0C. Whereas *R. fuscipes* possessed lowest range of polypeptides such as 10.0, 12.0, 14.0, 14.3 and 16.0 kDa. All these polypeptides were common from 10 to 25^0C. 20.0 kDa polypeptide was uniformly present in all the temperatures except at 30^0C.

The results of PCR amplified products such as 400 and 600bp were common irrespective of the primer in *R. marginatus.* Such a similarity was not observed when *R. fuscipes* whole body DNA was amplified with OPE-8, KTG-3 and KTG-5 primers. Interestingly OPE-8, KTG-3 and KTG-5 produced a unique amplified products of 1200, 150 and 50bp in *R. marginatus.* Similar these three primers produced 950, 200 and 300bp and 100bp in *R. fuscipes.*

Both constant and fluctuated temperatures reared reduviids fed with three pests such as *C. cephalonica* (Stainton) *D. cingulatus*, (Fab.) and *S. litura* (Fab.). Their gut and haemolymph was subjected to Indirect Enzyme Linked Immunosorbent assay (ELISA). *S. litura* fed gut and haemolymph were more immunogenic than other pests in both reduviids. Between the gut and haemolymph, it was more immune responsive that former from 25 to 30^0C.

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. Adoptation 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).

I.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 maculiventirs* (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 ecloson and moulting of reduviids were ranged either between 16-34^{o}C (Gomez - Nunez and Fernandez, 1963) or 15^{o}C and 35^{o}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 armigera* (Hubner) (Ambrose 1987), *Mylabris indica* (Faust), *Mylabris pustulata* (Fab) (Imms, 1985 and Nayer *et al.*, 1976), *Achea janata* (Linn.), *Oxycarenus hyalinipennis* (Costa) and *Approarema 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-efficay 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 redvuiid 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 maidiis (*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 reporductive 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); *Reduviius sensiles*, (Faust) *Reduviius vanduzueri* Wygodizinsky and Usinger and *Reduviius 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.

I.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 prooved 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

studied by Hagen, (1966); Dillon and Charnely (1986, 1988, and 1996). Gut microflora act as a reflection of the environments and incidence of entomophathogens (Lysenko, 1985).

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 fiagment 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 progrmmes. 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 : Noctuiidae) and *Dysdercus cingulatus* (Fab.) (Hemiptera : Pyrrochorridae) and *Corcyra cephalonica* (Stainton) (Lepidoptera:Noctuiidae) in relation to constant temperatures (10-35^{o}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.

Chapter II. BIOLOGY

II. 1. Introduction

Adequate storage of natural enemies for pest management is an essential to face the problems related to production, planning and unpredictability of demand storage is an useful techniques to ensure the availability of beneficial insects for further necessary response (or) field release without maintaining a continuous rearing. Further more, tolerance to low temperature may be considered as a desirable attribute in shipment procedures (Vanlenteren and Woets, 1999). The hunter reduviids *Rhynocoris marginatus* (Fab.) and *Rhynocoris fuscipes* (Fab.) are important polyphagous predators widely distributed throughout India (Imms, 1985; Nayar, *et al.,* 1976; Singh, 1985; Pawar *et al.,* 1986; Ambrose, 1995; Sahayaraj, 1995a, 2007a). These reduviids have been found in the agroecosystems such as groundnut, cotton, sugarcane, soyabean and also in semi-aridzone and scrubjuncles (Sahayaraj, 2002, 2006). Both *R. marginatus* and *R. fuscipes* preferably attack small, medium and large size agricultural pests such as aphids, bugs, grubs and larvae (Ambrose, 1999; Sahayaraj, 2002, 2006; Sahayaraj and Raju, 2006).

The development of forecasting system for the use of reduviid predators in an IPM programme largely depends on the understanding of the relationship between temperatures and development of the species of interest. Several studies have been addressed the effect of temperature on the biology of reduviids such as *Allaeocranum quadrisignatum* (Fab.) (Tawfik *et al.,* 1983a and 1983b), *Pristhesancus plagipennis* Walker (James, 1992), and egg hatching of *R.*.

marginatus and *R.. fuscipes* (Sahayaraj and Paulraj, 2001a,b). It was also reported by Galliard (1935) and Okasha (1964) that in reduviids the development was arrested during 15 and 35^{o}C. To our knowledge however no attempt has been made to determine the development, survival and fecundity of *R. marginatus* and *R..fuscipes* in relation to temperature. Therefore a study was undertaken to study the effects of constant temperature on development and reproduction of *R. marginatus* and *R. fuscipes.* Moreover, we are going to predict the optimum temperature for rearing these reduviids that could be used for further experimental study of these species.

II.2. Materials And Methods

R. marginatus and *R. fuscipes* life stages were collected from Sivanthipatti, Kongarayakurichi, Killikulam, Tirunelveli District, Tamil Nadu, India. They were maintained in the laboratory at 29 $\pm$ 1.5^{o}C, 75 $\pm$ 5% RH and 11L: 13D photoperiod on *C. cephalonica* larvae. The eggs laid by the predator were maintained in a small plastic container (60ml capacity). Newly hatched first instar nymphs were used for this experiment.

Development, survival and reproduction of both *R. marginatus* and *R. fuscipes* were studied using environmental chambers (Remi, Mumbai) at six constant temperatures *viz.,* 10, 15, 20, 25, 30 and 35^{o}C. The photoperiods for all experiments were 13D: 11L and relative humidity was maintained in 65 $\pm$ 5%. Individual egg batches were placed on filter paper in small plastic vials (60ml volume) with a perforated lid and humidity provided by a piece of wet cotton swabs. The experiment started with egg batches (approximately 100 to 153 in *R. marginatus* and 58 to 90 eggs for *R. fuscipes*) which contain 34, 45, 58 and 62

eggs/batch were subjected to each treatment regimes and replicated thrice and the vials were kept in the above mentioned temperatures. Control categories were maintained at room temperature (29 $\pm$ 1.5^{o}C). Egg development was monitored twice daily and their hatching percentage was recorded at each temperature separately. Upon emergence, the nymphs were transferred in to plastic boxes (12cm width and 4cm length) and maintained up to third instar. Then they were transferred to larger plastic boxes (15cm width and 7cm length) and maintained up to their death on *C. cephalonica* larvae in *ad libitum*. Weights of the newly hatched nymphs were recorded using Monopan balance (Dhona, Mumbai, India). (0.1 mg accuracy). Upon emergence the sex ratio of the adults were determined (female / female + male). Both weight gain and weight loss of the predators was determined by using the following formula adopted by Arnold (1959).

$$\text{Weight gain/loss} = \frac{\text{Final weight - Initial weight of the treatments}}{\text{Final weight - Initial weight of the control}}$$

Two males and two females were maintained separately in large plastic vials (15 cm width and 17 cm length) separately till their dealth with *C. cephalonica* in *ad labium.* Pre-oviposition, oviposition and post-oviposition periods, number of eggs laid, maximum and minimum number of eggs in a batch, oviposition index (total number of eggs laid/ oviposition period) and hatching percentage of the eggs were recorded. Water supply was checked in the environmental chambers daily. In another study, newly emerged adults (>6 hrs old) at 2:1 ratio (male: female) were also subjected in to the environmental chamber daily and all the above said parameters were recorded.

II.2.1. Statistical analysis

Nymphal developmental period of the control category was compared with other temperatures both in *R. marginatus* and *R. fuscipes* separately using student's't' test and their significance was expressed at 5% level. Sex ratio was subjected to chi- square test for each temperature separately. Reciprocals of the observed nymphal developmental duration (in days) were considered as developmental rates of each stage. Raw data from the developmental rate was regressed against six experimental temperatures by linear regression analysis. Further to examine *R. fuscipes* and *R. marginatus* instar – specific thermal developmental unit requirements, developmental models were constructed and degree- day calculations were performed after Braman *et al.* (1992).

Lower developmental threshold temperatures (LDT) were calculated by extrapolating the linear regression line to the x-axis of a graph with the reciprocal of developmental time on the Y-axis and increasing temperature on the X- axis (Arnold, 1959). Upper developmental threshold (UDT) therefore the temperature above, which the developmental rate decreased, was estimated directly from the data. Only the development times of *R. fuscipes* and *R. marginatus* which attained upto adult stage were used to estimate the lower developmental threshold (To) and upper development threshold (Tb). The mean number of degree-days (DD) required for developmental of each life stage was calculated using the following equation as suggested by Price (1984).

$$DD = D (T-t)$$

Where D was the developmental duration (days) 'T' was the temperature in ^{o}C during developmental and `t` is the lower developmental threshold in ^{o}C.

II.3. Result

II.3.1. Developmental period

Both *R. marginatus* and *R. fuscipes* completed their nymphal development when they were reared between 20 to 30^{o}C. However the total nymphal developmental period of both reduviids decreased with increasing temperature from 20 to 30^{o}C. At 35^{o}C the nymphs were developed faster and died during the beginning of the third nymphal instar. Among the different temperature tested, the total nymphal development period of *R. marginatus* was maximum at 20^{o}C (87.26 days), reduced at 25^{o}C (49.8 days) and further reduction was recorded at 30^{o}C (48.06 days) (Table 1). It was slightly decreased when *R. marginatus* was maintained under room temperature (46.05 day). Comparison between 20 and 25^{o}C (P=0.0739), 25 and 30^{o}C (P=0.0713) 20 and 30^{o}C (P=0.0049), room temperatures with 20^{o}C (P=0.0069) and 30^{o}C (P=0.0608) were significant. Similar trend was also observed in *R. fuscipes*. For instance, at 20^{o}C, the total nymphal developmental period was prolonged 37 days compared with room temperature (Table 2). When these predators were maintained at 10^{o}C and 15^{o}C, they were not reached up to adult.

Fluctuation of the temperature above 35^{o}C and below 10^{o}C and 15^{o}C, none of the eggs or nymphs hatched or eclosed successfully in both the predators. Because during moulting at high temperature (35^{o}C) and also at lower temperatures (10^{o}C and 15^{o}C) nymphal stages were unable to shed their exuviae

and the insects eventually died. Although statistical significant was recorded between 20 and 25^{o}C (P=0.0084), control to 20^{o}C (P=0.0077); and between control to 30^{O}C (P=0.0851), the comparison between 20 to 30^{O}C was insignificant (P = 0.0131) at 5% level.

II.3.2. Survival rate

At temperature between 10 to 20^{o}C, in *R. marginatus,* hatching or egg survival was ranged from 68.2 to 100% (Table 3). In *R. fuscipes* egg survival was maximum at 30^{o}C (100%) and 10^{o}C showed lowest survival rate (67.2%) followed by 25^{o}C (98.6%) (Table 4). At a higher temperature (35^{o}C), nymphal survival rate was arrested after fourth and third nymphal instars for *R. marginatus* and *R. fuscipes* respectively. Between the two predators, *R. fuscipes* nymphal survival rate was higher (83.25%) than *R. marginatus* (88.3%). When the eggs nymphs and adult were subjected to six temperatures, cannibalism was occurs both in nymphal stages and adults at 35^{o}C. Maximum and minimum nymphal total survival rate was recorded in room temperature and 20^{o}C respectively for both in *R. marginatus* and *R. fuscipes* (Tables 3 and 4).

II.3.3. Longevity

When freshly moulted adults of *R. marginatus* was subjected to different temperature regimes longest longevity was observed at 20^{o}C 7.66 and 17.26 days for male and female respectively (figure 1a). Similarly in *R. fuscipes*, the longest longevity was observed at 20^{o}C for male (73.33) and female (90.73days) followed by 15^{o}C and 10^{o}C and significantly shortest longevity was observed at 35^{o}C (Figure 1b).

Longest and shortest longevity was 28.66 and 69.75 days respectively for males and females of *R. marginatus* at 20^{o}C. But in *R. fuscipes* longest longevity (35.25 and 32.5 days for male and female respectively) was recorded at 25^{o}C. Longevity of the predators recorded both at 25 and 30^{o}C indicated similar life span when compared to room temperature for *R. marginatus*. When ever temperature increased longevity was also affected or decreased in both predators. Of these two tested predators, *R. fuscipes* had high tolerant capacity at lower temperatures of 15 and 20^{o}C (Figures 2a and b).

II.3.4. Sex ratio

In *R. marginatus* sex ratio was female biased (0.86) at RT. It was gradually diminished from 30^{O}C to 20^{O}C (0.82, 0.78 and 0.69 for 30, 25 and 20^{O}C respectively). Whereas in *R. fuscipes,* sex ratio was maximum at 30^{O}C (0.98) followed by RT (0.82) and 25^{O}C (0.68).

II.3.5. Oviposition periods

Tables 5 to 8 showed the oviposition pattern of *R. marginatus* and *R. fuscipes* in relation to different temperatures. It was very clear that in *R. margiantus* the pre-oviposition period was 12.9 days at room temperature. But it was prolonged upto two months (58.1± 1.9days) when freshly moulted adults was subjected at 20^{o}C (Table 5). In general preoviposition period was diminished when the temperatures was increased. Oviposition period was lasted for two months (57.2±5.0 days) at room temperature. It was gradually and significantly diminished when the temperature was decreased from 20 to 35^{o}C (table 5). Statistical comparison between the preoviposition and post-oviposition periods

(20-25^{o}C) were highly significant (P=0.0010) and also with in the oviposition periods between 25-30^{o}C was significant (P< 0.05). However the comparison of oviposition period between 25^{o}C to control category was insignificant.

In *R. fuscipes*, preoviposition period was same both at 30^{o}C and room temperature (21.33±4.54 days). However, it was prolonged more than 2 weeks when *R. fuscipes* was maintained at 25^{o}C. Oviposition period was shorter at 30^{o+}C followed by room temperature and 25^{o}C in *R. fuscipes*. Postoviposition period was gradually increased from RT to 30 and 25^{o} C (table 6). Oviposition period was lasted for 57.1 days at room temperature; it was highly reduced at 35^{o}C, followed by 30, 25 and 20^{o}C in *R. marginatus.* Statistical analyses for both pre and post-oviposition periods between 20-30^{o}C and 25 to 30^{o}C were significant (P<0.05). In *R. fuscipes* the oviposition period was shorter than the preoviposition period except at 25^{o}C and 30^{o}C. Statistical comparison between pre-oviposition and post - oviposition between 25-30^{o}C (P=0.0089) and 20-25^{o}C (P=0.0014) were significant. Within the post-oviposition the temperatures between 30 to 25^{o}C (p= 0.0987) and 30^{o}C and control (P=0.0102) were also significant.

II.3.6. Fecundity

R. marginatus laid a mean of 119.6 eggs/female. It was enhanced when *R. marginatus* was subjected at 30^{O}C. Minimum egg production was recorded in 25^{O}C. However when a freshly emerged *R. marginatus* was subjected to different temperature regimes, the egg production was decreased from the control category to 25 and 30^{O}C. When *R. marginatus* was maintained with different temperature from the first instar to adult the egg production was gradually decreased from 30 to 20^{O}C. Similar trend was also observed in *R. fuscipes.* Statistical comparison

between control and 20^{O}C (P 0.0418), 20-25^{O}C (P > 0.0737), 20- 30^{O}C (P = 0.049),25-30^{o}C (P = 0.0713) were significant in *R.*. *marginatus*. Comparison between control to 20^{o}C (P=0.0007), 25^{O}C (P=0.0611), 30^{O}C (P=0.0851) and also between 20 to 25^{O}C (P=0.0084) were significant at 5% level of paired sample't' test. Both minimum and maximum number of egg/ batch/ female was also recorded (see table 7 and 8). *R. marginatus* laid maximum number of egg at 30^{o}C (175.0 eggs /batch /female) when adults were subjected to this temperature. It was significantly reduced at 25^{o}C (154.31 egg/female) followed by 35^{o}C (147.0 egg/female) and 20^{o}C (112.0 egg/female).

II.3.7. Incubation period and Hatchability

R. marginatus egg hatched within 7.58 days having 95.5% hatchability. Irrespective of the temperature the incubation period was prolonged in *R. marginatus* adults maintained at different temperatures. However, the hatching percentage of the eggs were reduced at 20^{o}C and statistically insignificant at 25^{o}C and significantly (P = 0.0220) enhanced at 30^{o}C.

R. fuscipes egg hatched with 7.44 days having nearly 100% hatching in control category. It was insignificantly (P = 0.1030) reduced both at 25^{o}C and 30^{o}C. In contrast to the above observation *R. fuscipes* eggs production was maximum observed when the adults were subjected to 25 and 30^{o}C even. Similar kind of observation was also recorded for both maximum and minimum number of number of eggs/batch, incubation periods and hatchability. When compared with other temperature regimes, incubation periods were significantly enhanced at 20^{o}C. Egg hatching percentage was increased from 35 to 30^{o}C and 25^{o}C. Minimum egg

hatchability was recorded at 20^{o}C. Similar trend was also observed in *R. fuscipes* when the adults were subjected at different temperature regimes.

II.3.8. Developmental Models – Linear Model

Both linear and non-linear models were used to describe the relationship between development rates (L/d), and temperatures. To select the most appropriate model for both predators development, prediction, a comparative analysis model was performed here.

The over all coefficient for *R. marginatus* and *R. fuscipes* were 0.949 and 0.967 respectively. The linear model adequately described the lower temperature threshold (t_b) and degree day required for development. The linear regression was applied to know the relationship between development rates and temperatures. It was linear for all stages except in first and second nymphal instars of *R. marginatus*

and *R fuscipes* respectively. The estimated lower and higher temperatures thresholds (t_b) for eggs were 19.51 and 20.53 respectively. It was enhance for the nymphal stages at lower (24.1) and higher temperature (32.1) for *R. marginatus.* It was enhance to 25.41 and 34.28 for lower and higher temperature thresholds for *R. fuscipes*. When we consider the individual nymphal instars, lower and higher thresholds were recorded for fifth and first nymphal instars of *R. fuscipes* (Tables 9b and 10b).

II.3.9. Weight gain and weight loss

Impact of temperature on the total body weight gain and loss of *R. marginatus* and *R. fuscipes* is presented in figures 5 and 6. Both at higher 35^{o}C

and lower temperatures (10, 15°C), remarkable range of weight loss was noted. Optimum temperature like 25°C and 30°C, appreciable weight gain was observed in both predators. Maximum weight loss was recorded at 35°C throughout the experimental periods.

II.3.10. Morphometric analysis

Quantitative measurements of various body parts such as head, thorax and abdomen and their appendages of the two predators at different temperature are given in Tables 11 and 12. Lower temperature decreased the size of all the three legs, thorax, wings and rostrum of *R. marginatus*. But temperature has positive influence on antennal size (Table 11). However in *R. fuscipes* invariably size of all the body parts were higher at 20°C (see table 12).

II.3.11. Morphogenesis

Deformities were observed both in nymphal instar and adult of *R. marginatus* and *R. fuscipes* (Plates 1 and 2). Predators were reared at 15°C, were moulted incompletely and immediately after few hours these adults were died. Fifth nymphal instars had visibly longer hind legs and tibial portions were shorter than normal nymphs. At 35°C developed predators possessed fore and hind wings were reduced with fully burned appearance. Both the pairs of wings incomplete and irregular in shape (20°C).

Adults of *R. fuscipes* (Plate 2) consisted permanently attached moulted skin towards the posterior parts untill those predator death. At 20°C fore and hind wings were very thin plate 2(b) change the original position also whole wings were replaced from that surface. When the nymphs three turned to whole body

colouration pale brick to dark red, colour noticed on both predators. Since old exuvia was unable to remove from the body properly, Most of the fifth nymphal instars of (plate c) *R. marginatus* unable to moulted into adults. In *R. fuscipes* the exuvia was attached on the posterior abdominal region (Plate 2c and 2d). However in *R. marginatus* the moulted skin attached with the legs (e).

II. 4. Discussion

These two reduviids were distributed in rice, cotton, bendhi, chilli, groundnut, soybean, pumpkin, sugarcane, pigeon pea (Sahayaraj, 2006) and also their adjacent ecosystems like semiarid zone, scrub jungle and forests on throught India. These shows that there is lot of possibilities for facing a wide range of temperature between 20-35^{O}C. We also selected 10 and 15^{O}C inorder to find out the possibilities for storing these reduviids or augmentative release programme. Hence we used constant temperature treatments ranged from 10-35^{o}C, with 5^{O}C stepwise intervals. Moreover, very little informations is currently available on the effect of temperatures on development and survival of reduviid predators.

Reduviidae are to well known generalist predators feeding on a wide variety of prey (Miller, 1971). Their value as a regulator of insect pest populations has rarely been investigated. However recent interest in the extent to which generalist predators that lack of density dependent tracking may limitted prey (Murdoch *et al.,* 1985). Very little research work has been available about the effect of temperature on nymphal development and survival, reproduction of reduviids (Sheppard *et al.,*1982; Tawfik *et al.,* 1983a,b,c; James, 1992; Sahayaraj, 2007a).

Development of reduviid predator's *viz., R. marginatus* and *R. fuscipes* clearly showes that it increased as temperature decreased. In contrast, it was reported that for generalist predators, developmental period of *Orius laevigatus* (Rodlf *et al.,* 1993); *Lytocoris campestris* (Parajulee *et al.,* 1995); *Hypoaspis miles* (Acari) *Podisus maculiventris* (De Clercq and Degheele, 1992a, b) increased in accordance with temperatures. Developmental threshold of both predators nymphal instars and other performance shared progressively improved above 15^{o}C (15-20, 20-25 and 25-30^{o}C). Present results were confirmed the reports of other hemipteran predators (Jones and Copland, 1963; Oeting and Yonke, 1971; Dunbar and Bacon, 1972; Awan, 1983; Parajulee and Phillips, 1992; Ito and Nakata, 2000; Nakamura, 2003; Sayaka *et al.,* 2007; Sarah *et al.,* 2007). Nymphal development was generally studied at constant temperature between 25 and 30^{o}C and about 70% humidity on unspecified conditions of ambient temperature and humidity (Nishi and Takahasi, 2002). However temperature and humidity in insect habitats may be differ considerably and vary according to circadian and seasonal patterns (Melanby, 1954; Howe and Howe, 1965; Zachaniarren, 1985; Lee, 1991; Shinmizu and Kawaski, 2001; Logen *et al.,* 2006, 2007). Several authors have reported that acceleration of temperatures caused retardation of developmental periods both at fluctuated temperatures and also at constant temperatures (Mark and Jervis, 2005). Overall, early instar of reduviids required less time to develop than later instars and\are consistent with deata reported by Neal and Dougleas (1988) and Roy *et al.,* (2002) in Tingid, *Stephanitis pyrioides,* Braman *et al.* (1992) in *Corythucha cydoniae;* Usha Rani (1992) in *Eocnthecona furcellatea.* Which has been attributed to the higher metabolic activities noticed in the predator (Christian *et al.,* 1999). A

similar kind of susceptibility and survival was observed in another reduviid predator, *Allaeocranum biannulipes* (Tawfik and Awadallah, 1983).

The results revealed that the survival rate was decreased with increased temperature, which was attributed to absence of brisk activities in the freshly moulted nymphal stages. Similar temperature dependent immature survival and development was also observed from the coleopteran predator (Obrycki and Tauber, 1982; Evans, 1987; Wang and Wang, 1990; Ponsonby and Copland 1996; Schultz,1998; Neven, 2000). In both reduviids, highest mortality was occurred at the time of moulting. Stage specific mortality decreased for successively later stages, demonstrating that early instars of *R. fuscipes* and *R. marginatus* were more vulnerable to thermal tress than later life stages. Therefore, slow development of these reduviids in cool environment may be due to differences in moulting. that is during moulting time or incomplete or unable to shed the outer covering (exuviae), finally it leads to died. So survival rate decreased each instar in accord with temperature.

The percentage mortality curve versus the whole range temperatures resembled like inverted ∩ pattern observed for all the immature stages of *R. fuscipes* and *R. marginatus*. Previously it was similar kind result reported that U shaped curve of mortality versus temperature for the immature stags of coleopterans (Stern *et al.,* 1990; Herrera *et al.,* 2005). Survival of both *R. marginatus* and *R. fuscipes* had been remarkably negative effects at higher temperature. While we maintain the reduviids at 35^{o}C, none of nymphs reached in to adult. Similarly lower temperatures such as 10 and 15^{o}C were not suitable for the development of these reduviids. These findings were consistent with pervious

reports observed in other polyphagous predators (Mukerjii and Leroux, 1965; Valsova *et al.*, 1980; De Clercq and Degheele, 1992; Bramen and Pandley, 1993). But Diodonet *et al.* (1996) reported that the temperature at shown development of predators were threshold of 10 to 35^{o}C.

Nymphal instar of both *R. marginatus* and *R. fuscipes* obviously been initiated their development, but fail to proceeds after second instar at 10 and 35^{o}C and third instar at 15^{o}C (only *R. marginatus*) as observed in other heteropteran predators in general by Christian *et al.* (1999) and pentatomid bug in particular by Usharani (1992). However some thrived individuals were unable to leave the old exuvia and died during the moulting (Plate 1c). This was most prominent when reduviid was reared at 10 to 15^{o}C. *R. marginatus* and *R. fuscipes* were could not able to moult first to second instar at 10^{o}C and second to third and fourth instar at 15^{o}C. Similar kind of observation was also recorded in other reduviid predators (Buxton, 1930; Christian *et al.*, 1999). Both *R. marginatus* and *R. fuscipes* nymphal predators were sometimes able to survive for very long period without undergone into moulting at 15^{o}C (Luz *et al.*, 1998). Denlinger, 1991 summarised that the survival was higher for all species of predator at temperature between 25-30^{o}C. It was further reported that favoured nymphal stages also suffered by mortality at higher and lower temperature (Chirstian *et al.*, 1999). Moreover, the nymphal development and growth parameters were significantly affected by day temperatures (Howe, 1950; Evans, 1972; Becket *et al.*, 1998; Becket and Marton, 2003) may be a reason for the predator has not successfully invaded in different abiotic stresses.

Sex ratios of *R. fuscipes and R. marginatus* at higher temperatures was found to be femle biased. An opposite trend was observed by De Clercq and Degheele (1992) in *P. sagitta* and *P. maculiventris*. Sex ratio of *R. marginatus* was reported to be female biased under normal laboratory conditions (Sahayaraj *et al*., 2004). Field collection also shows that both the reduviids were female biased under field situation too. Unfavorable environmental conditions (extreme temperatures, food shortage) may alter the sex ratio of insects (Sarah *et al.*, 2007). The observed results indicated that even the reduviids present in the unfavorable temperature, it will not alter the sex ratio.

Mean while recently Sahayaraj (2007a) evidentially reported that there was a possibility for storing reduviid eggs in the refrigerator for a long period and subsequent used as a biological control agent would be a great benefit to the biological control programme. In this study, introduction of *R. fuscipes* and *R. marignatus* at 20^{o}C prolonged the incubation period for 10 and 12 days respectively. Results show that it can be feasible, if these predator eggs used in IPM when mass production is required. Hence it was concluded that *R. marginatus* and *R. fuscipes* can be stored upto 2 weeks with minimum reduction in hatchability at 20^{o}C.

R. marginatus and *R. fuscipes* eggs were collected from laboratory between Decembers to mid January. These eggs were failed to hatched, but all the eggs are changed to brownish colour, similar result was also observed by Lees (1955, 1991) and Hagerty *et al.* (2001). However most of the eggs fail to hatched, when exposed to either lower or higher temperatures with humidities for shorter and longer periods. The extension of embryonic developmental duration at lower temperature

results form the depression of egg metabolism due to water loss with low humidities noted in some insect eggs (Ferro and Chapman, 1979; Frazer and Gregor, 1992). This study showed that when the eggs storage at 35^{o}C most of the eggs were failed to hatched out, although eyespot were appear and colour changed with shrinked appearance also noted. This indicate that same embryonic development had occurred initially and it was not proceeds further (Salt,1953, 1965; Clark, 1996).

Usha rani, (1992) reported that low humidity increase water loss through the chorion, and resistance to desiccation depends on the ability of the eggs to retain water, though physical and physiological processes against Dalton's law (Wigglesworth, 1974). In the present investigation, results of both reduviids showed that low egg hatcghing was recorded at 20^{o}C. In addition Usha rani, (1992) defined as a direct response to a limiting factor such as temperature and moisture in which development immediately resumes upon restoration of that factor. In the case of *R. marginatus* and *R. fuscipes* showed result suggest that temperature is the limiting factor preventing embryo development and eclosion following oviposition. The present result also showed that the heavier adult predators reared at 25-30^{o}C laid more number of eggs. Similar phenomenon was reported for number of other insect species (Markkula and Roivaines, 1961; Shreeve,1986). It was also reveals that reproductive activities of these two reduviid predators were reduced as the temperature increased which corroborate with the reports of); Torres *et al.* (1998); Usharani, (1992). Similar trend was also observed in generalist heteropteran predators too (Kohno and Kashio, 1998; Nagai and Yano, 1999; Kino *et al.*, 1999; Ito and Nakata, 2000). Lower temperature has a negative effective on the incubation period of the eggs and nymphal survival of the predators studied.

Similarly reduction in the incubation period at high temperature was observed earlier in another reduviid predator *A. biannulipes* (Tawfik and Awadallah, 1983; Torres *et al.,* 1998; Readdio, 1926) and coleopteran predators too (Kishore *et al.,* 1994; Bakthavatsalem *et al.,* 1995; Omkar and Pervez,2002; Herrera *et al.,* 2003;). Furthermore, the incubation of reduviid eggs required fewer amounts of moisture, prologned dryness and optimal abiotic factors and the egg development (Vennison and Ambrose, 1990). Sahayaraj and Paulraj, (2003) suggested that when *R. marginatus* eggs were enable to tolerate that was too cold temperatures than the *R. fuscipes.*

II. 5. Conclusion

Both *R. marginatus* and *R. fuscipes* completed their nymphal development when they were reared between 20 to 30^{o}C. At 10 and 15^{o}C none of the nymphs were developed up to adults. However, the total nymphal developmental period of both reduviids decreased with increasing temperature from 20 (87.27 days) to 30^{o}C (46.06 days). It was slightly decreased when *R. marginatus* was maintained under room temperature (46.03 days). Irrespective of the temperatures, both reduviids had a female biased sex ratio. Reproductive characteristic of both predators on the tested temperatures revealed that both the predators laid viable eggs except at 10^{o}C and 15^{o}C. Egg laying capacity and hatchability was also higher at 30^{o}C in both predators. However, *R. fuscipes* did not succeed in egg production when stored below 20^{o}C. Deformities were recorded on the legs; wings, as well as incomplete moulting were also observed when the predators were maintained at 10, 15, 20 and 35^{o}C. Low and high threshold temperature were minimum and maximum for *R. marginatus* and *R. fuscipes* respectively.

Chapter III. BIO LOGICAL CONTROL POTENTIAL

III.1. Introduction

Insect pests are proof for delimiting factors in the ecosystem and in agriculture they oftenly causing total crop loss. Their problem has apparently been aggravated in the world by indiscriminating pesticide application including aerial spraying which has adversely affected natural bioresources. Because of high cost of protecting crops from insect pests with chemical insecticides, the increasing concern over residues in food and gradual depletion of natural resources, there is growing interest in the Integrated Pest Management (IPM) where the predators are an important constituent (Sahayaraj, 1994).

Four species of *Rhynocoris* and other predators were reported as potential biological control agents of *D. cingulatus* (Sitaramaiah *et al.*, 1976, Ambrose, 1988; Lakkundi, 1989; Sahayaraj and Ambrose, 1993a). Howeever, none of them have been used either under field cage or field evaluation. Use of entomophagous insects as biological control agents implies the availability of adequate techniques enabling large production at low costs. Moreover the effect of temperature on the rate of development and survivorship of predators are the fundamental importance to understand insect phenology and abundance (Sahayaraj, 2007a). The usefulness of a predator in the management of pests may relate in a part; to its ability to perform adequately under a range of environmental conditions.

D. cingulatus is a serious pest of cotton (Ambrose and Kumaraswamy, 1990). Cotton ecosystem has variety of natural enemies like Pentatomids, Redvuiids, Anthocorids, Spiders, Ants and Coleopteran predators. *Spodoptera litura* (Fab.) is also the serious pest of groundnut and cotton (Sahayaraj, 2007a). More than 20 reduviid species were known to predate upon *S. litura* both in the field and laboratory conditions (Sahayaraj, 1999, 2006). Among them *R. fuscipes* and *R. marginatus* are the common predator feeds primarily on larval forms of lepidopteran pests and young ones and adults of Hemipteran, Coleopteran and Isopteran pests, although it accept the prey from other insect orders (Ambrose and Livingstone, 1986a, b). Pest suppression efficacy of *R. fuscipes* on various crop pests (Ponnamma *et al.*, 1919; Ambrose 1995; Babu *et al.*, 1995; Sahayaraj, 2006) has been studied. Though cotton ecosystem has variety of natural enemies, each varying importance at different times is based upon its specificity (Sahayaraj, 2007a). However, even a single well-adapted species like assassin bug is capable of reducing even the well established pest population like red cotton bug (Sahayaraj, 2003). This is clearly indicated by the success that has been achieved by the introduction and the wide range of the reduviids for the control of red cotton bug. The investigation of bio-efficacy of reduviids in the field eage shows non-tibialorate reduviid, *R. marginatus* and *R. fuscipes* (Kumaraswami, 1991; George *et al.*, 2000a) suppressed more than 50% of both the fifth instar nymphs and adult of *D. cingulatus*.

III. 2. Materials and Methods

The laboratory emerged *R. marginatus* and *R. fuscipes* third, fourth, fifth nymphal instars and adult predators were incubated at six constant temperature (10, 15, 20, 25, 30 and 35^{o}C) these predators were used for evaluating their biological control potential.

III.2.1. Stage Preference

Stage preference study was conducted on all the life stages (except first and second instar) of *R. marginatus* with different life stages of *D. cingulatus* and *S. litura* separately by choice experiment. To study the stage preference, *R. marginatus* third instar was introduced in to a petridish (14cm height and 9cm width) containing fresh cotton leaves and *D. cingulatus* second, third, fourth, fifth nymphal instars and adults (each two) were released separately and the predatory behaviour was observed consecutively by visual observation for 6hrs. Successfully captured, killed and consumed prey stage was recorded as preferred stage of the reduviid. Similar procedure was followed for other life stages of these predators. In another study *R. marginatus* and *R. fuscipes* were provided with all five larval stages of *S. litura* separately and preferred pest stage was recorded. Fifteen replications were maintained for each life stages of the predators separately. Once stage preferred was known, biological control potential evaluation studies were conducted using the following procedures.

III.2.2 Biological control potential evaluation

Red cotton bug, *D. cingulatus* and *S. litura* life stages were collected from cotton fields, Tirunelveli District, Tamil Nadu, India and maintained on young, potted cotton plant under laboratory conditions. Laboratory emerged life stages of these pests were used for the experiments. The studies were conducted in two steps: first preferred stages of the predators were determined and then by using the preferred stages, biological control potential of the reduviids was observed.

Preferred stages of *D. cingulatus* (5 preys/ container) were introduced in to the container containing cotton twig (*Gossyppium hirsutum*) and it was allowed to acclimatise for 1hr. Then life stages of *R. marginatus* and *R. fuscipes* were introduced in to the same container separately and the feeding events like approaching time, handling time, weight gain were recorded continuously for 6 hrs with visual observation. After 24hrs, weight gained by the predator and number of prey consumed and / killed by a predator was also recorded and considered as predatory rate. Fifteen replicates were maintained for each life stages of both predators separately. Similar procedure was followed for *S. litura* too.

III.2.3. Statistical Analysis

Paired sample‘t’ test was used to determine the significance between third, fourth, fifth instar and adult predators separately compared with its six temperatures on both predators separately. It was applied for approaching time, consuming time, weight gain of the two predators on both cotton pests.

III.3. Results

III.3.1. Stage preference

R. marginatus third, fourth and fifth nymphal instars and adults preferred second, fourth and fifth nymphal instars of *D. cingulatus* respectively. The preference was different while *R. marginatus* provided with life stages *S. litura* (second, third, four and fifth instar larva). *D. cingulatus* second, third, and fourth nymphal instar were preferred by third, fourth and fifth nymphal instar and adults of *R. fuscipes* respectively. Both fifth nymphal instar and adults of *R. fuscipes* preferred fourth instar larva of *S. litura* where as third and fourth nymphal instar preferred second and third instar larva.

III.3.2. Temperature impacts on bioefficacy of *R. marginatus*

Table 13 to 14 shows the bioefficacy of *R. marginatus* on *D. cingulatus* and *S. litura* life stages. Results reveal that based upon the temperature variation both the predatory behaviour and bioefficacy varies. For instance at room temperature, *R.marginatus* third, fourth, fifth nymphal instars and adults consumed 3.33, 3.18, 2.44 and 3.93 *D. cingulatus* respectively. While we subject the reduviids in different temperature regimes, the predatory rate was reduced (figures 5 and 6) except in fifth instar *R. margingtus* at 25 and 30^{O}C. However, it was statistically insignificant (df = 4, P = 0.339; df = 7, P = 0.165) when compared with control category (p = 0.05). Eventhough the predatory rate was decreased than the control, the weight gain of *R.marginatus* (third, fourth and fifth nymphal instars) was increased at 30^{o}C and also at 25^{o}C in fourth instar. Approaching and handling

times were gradually decreased and increased from 10 to 35 respectively. Then it was slightly decreased at 35^{o}C. These predators were preferred ventral side of *D. cingulatus* and abdominal region on *S. litura*.

Another important parameters concern in the bioefficacy was handling time. Handling time was gradually increased from 10 to 30^{o}C and then decreased at 35^{o}C. This concept was common in all the life stages of both the reduviids whether the prey as *D. cingulatus* or *S. litura.* Statistical comparison between control to 15^{O}C of third instar, 20 and 30^{o}C in fourth instar and 10 and 30, 35^{o}C on shows insignificant (df-6; P=0.578; df = 7; P=0.572). Other temperature categories were significantly influence handling and approaching time (df =6; P = 0.009) fifth instar (df = 7; P = 0.002). In *R. marginatus* handling time was gradually increased from third instar to fourth instar and then gradually decreased to fifth nymphal instar and also to adults when *D. cingulatus* was offered a prey (Table 13). While we offer *S. litura* as a prey, handling time was higher in fourth instar nymphs of *R. marginatus* followed by adults, third and fifth instar (Table 14).

III.3.3. Temperature impacts on bioefficay of *R. fuscipes*

Handling time was gradually increased up to 25 and 30^{o}C when *D. cingulatus* was provided with third and fifth nymphal instars and fourth nymphal instar and adults of *R. fuscipes* respectively (Table 15). From the table 16, it was vary clear that nymphal instars and adults of *R. fuscipes* were took maximum time at 30^{o}C and 35^{o}C respectively to approach *S litura* nymphal instar and adults respectively. Similar trend was also observed for handling time too. However, irrespective of *R. fuscipes* life stages, it consumed maximum

D. cingulatus at 30^{o}C (Table 15). Life stages of *R. marginatus* consumed maximum amount of *D. cingulatus* at 30^{o}C except in fourth nymphal instars (see table 15), especially in the fourth instar *R. marginatus.* All other life stages were consumed maximum adult on *S. litura* at 30^{o}C (Table 14). Weight gain ranged from 0.44 mg at 10^{o}C to 2.41 mg at 30^{o}C on third instar, when the temperature attained moderately, weight gain was increased. But lower (10^{O}C) and higher temperatures (15^{O}C) showed minimum weight gain. Among the two cotton pests, the biocontrol efficiency of both the reduviids were maximum on *S. litura*. Another findings related the temperature reveals that the six temperature regimes, *R. fuscipes* more actively feeding at 30^{o}C. When *R. marginatus* provided with *S. litura* third and fourth instars between 30 and 25^{o}C, approaching time was significant (df = 4; P=0.016; df = 7; P=0.019). For the weight gain comparison between the six temperatures and control were highly significant by paired sample `t` test (df = 7, P= 0.000; df = 5, P=0.002; df=5, P=0.062; df = 7, P=0.025 for third, fourth and fifth nymphal instars and adults of *R. fuscipes* respectively on *D. cingulatus* at control). When *R. fuscipes* fed with *S.litura*, predator weight gain [(P=0.000) for 10 to 20^{O}C followed by 25 to 35^{O}C df = 3, P=0.008 and df = 5,P=0.002; df = 7; P=0.008], approaching time (P= < 0.082 and > 0.022) were statistically significant except for 25 with 30^{O}C. Irrespective of the *R. marginatus* life stages, the predatory rate was higher at 30 ^{O}C on both pests. Among the life stages, the predatory rate was higher on adults followed by fifth, third, fourth and third nymphal instars of *R. marginatus* (Figures 5a and 5b). As observed for *R. marginatus*, the predatory rate of *R. fuscipes* was also maximum at 30^{o}C

(figures 6a & 6b). However, the predatory rate of *R. fuscipes* fifth nymphal instar, and adult were differed on *D. cingulatus* to *S. litura.*

III.4. Discussion

The results of the current study demonstrate the searching and handling ability of two reduviid predators and also suggest their biological control potential of life stages of *S. litura* and *D. cingulatus*. Among the two pets tested, larvae of the cotton leaf worm *S.litura* were less aggressive than *D. cingulatus.* Hence *D. cingulatus* easily escape from the predators by vigorous thrashing movements. So the predators delayed to approach *D. cingulatus.* Results reveal that temperatures have no influence on the prey stage preference. However, from the results, it was vary clean that younger nymphal instars of these predators preferred younger (small) nymphal instar of *D. cingulatus* and larva of *S. litura*. Similar observation was observed in *R. fuscipes* on *Wezara viridula* Linn. (Singh and Singh, 1987), *R. lapidicola*, *R. nysiphagous* and *Coranus* sp., on *Nysius inconspicuous* Distant (Joseph, 1959), *R. fuscipes*, *R. kumarii* and *R. marginatus* on *H. armigera* (Kumaraswami, 1991), *Acanthaspis pedestris, Catamiarus brevipennis* and *Ectomocoris tibialis* on *H. armigera* (Sahayaraj, 1991), *R. kumarii* and *R. marginatus* on *S. litura* (Sahayaraj, 1994), *Plattymeris rhadamanthus* Gerst on *Oryctes monoceros* (Olive). Generally all the prey stages were attacked by all the life stages of the predators. It was also recorded that, *R. fuscipes* and *R. marginatus* did not attempt to prey *D. cingulatus* and *S. litura* which are smaller when compared to their own body. A similar hypotheses was also made by Ambrose (1999) and Sahayaraj (2007a).

The approaching time (or attack rate) and handling time were the parameters used to determine the magnitude of biological control efficiency / bioefficacy of any natural enemies. Table 13-16 show that the values of attack rate and handling times differed significantly among various temperatures, indicating that the predatory reduviids respond differently to pests tested. *S. litura* larva were to escaped quickly at moderate temperatures (25^{o}C, 30^{o}C), than the lower temperatures like 10^{o}C and 15^{o}C. Generally reduviids captured their preys which move very faster then the slow moving preys. This similar observation was recorded in other reduviids (Maran, 1999, Haridass and Ananthakrishnan, 1981; Maran *et al.*, 2002) and also in Pentatomid bugs (De Clercq and Degheele, 1992a; 1993). At 25 and 35^{o}C *R. marginatus* and *R. fuscipes* were very aggressive and they approached immediately repress and handle more prey, as well as they having higher consuming ability. The results implies that the predator will spend a large amount of time with non-searching activities (eg : resting or moving here and there without feeding) at low temperatures, while positive searching and preying activities would be expected at higher temperature. In Tirunelveli distinct in particular and Tamil Nadu, India in general, both *D. cingulatus* and *S. litura* populations in cotton is maximum during May to July (our personal observations), when temperature is usually < 30^{o}C, and the population of both reduviids were also approaching its annual peak. Therefore, a significant natural control effect of the reduviid on *D. cingulatus* and *S. litura* could be expected at that time.

Our laboratory tests revealed that the biocontrol efficacy of both predators were often similar in terms of handling and approaching time, even though, few

specific activities like of weight gain in accordance with the temperatures (higher and lower). This result corroborate the observations recorded in generalist arthropod predators observed by Campbell *et al.*(1974); Thompson (1978). They postulated that predation of the generalist predator was mainly influenced by physical factors such as temperatures. From the result it was very clearly that among the six temperatures, the bioefficacy of *R. marginatus* and *R. fuscipes* maximum at 25^{o}C and 30^{o}C. This kind of similar findings recorded by De Clercq and Degheele (1992a,b). They demonstrate that temperature was not only reduces the predatory activity but other factors like bioefficacy performance mentioned as above.

Capturing success of prey would be greatly depend on the relative size and strength of the prey and predator also favoured the temperature variation as reported in pyrochorrid predator (Shrewsburry, 1996; Lie *et al*., 2005) but it was not varied when the prey size was common (Sahayaraj and Ambrose 1994; Sahayaraj 2001, 2003). It was a well known fact that the predator required more time to search a prey at low temperature and it was spent more time for non-searching activities.

Several potential direct effects of temperature on predators (or) prey may observe prey vulnerability and exposure to predators (Anderson *et al.,* 2001). Direct temperature effects may influence prey detection of natural enemies, alarm signaling, escape behaviours and / or defense (Gilchrist, 1995), as well as predator foraging (Morgan, 1985), prey handling (Thompson, 1978) and metabolism (Schultz *et al.,* 1992). Markkula and Roivaines (1961) suggested that altering

abiotic factors, such as increasing light exposure and for temperature on decreasing ambient moisture levels might discourage natural enemies. From this point of view, we found out that temperature differences between environmental condition and the experimental level mainly influence the biological control efficiency was higher than room temperature. Same view was also already revealed in other predators by Shrewsburry (1996).

R. fuscipes and *R. marginatus* bio-efficacy on both pests such as *D. cingulatus* and *S. litura* were higher at 30^{o}C. Moreover, reduviids prey consumption has been found to increase as temperature increase. Which shows that these reduviids exhibited thermoregulatory behaviours or posses broad or plastic operational temperature ranges way be able to forage in varying thermal environments. Similar trend was recorded in *Chrysoperla carnea* (El – Walkil, 2003)

Aging has been considered as a declining change from maturity to senescence and it widely studied in insects and particularly in reduviids (reviewed by Ambrose, 1999 and Sahayaraj, (2006). Sahayaraj and Ambrose (1995); Sahayaraj (1995, 2004,); Ambrose and Sahayaraj (1996) reported that a linear relationship between reduviid predators age and their bioefficacy where as Sahayaraj (1994); Sahayaraj and Ambrose (1994) suggested prey age influence the reduviid bioefficacy. In earlier studies, it was suggested that biocontrol potential of the generalist predators were varied with their age (Luck *et al.*,1988; Rudolf *et al.*,1993; Islam and Chapman, 2001). Bioefficacy studies in small laboratory areas have been criticised (O'Neil and Widenmann, 1990; Wieddenmann and O'Neil

1990), since factors such as large searching areas, host plants, and weather under field conditions may influence the effectiveness of predators. But in this study, we placed cotton leaves in the experimental areas and we maintained constant temperature. Among the nymphal stages tested, fifth instar nymphs invariably took more time for consuming and subsequently sucked more amount of prey; it leads to increased weight gain than adults.

Studies are needed for evaluating more reduviids because the availability of additional reduviid predators of *D. cingulatus* and *S. litura* would lead to be an increased in successful biological control of these pests under various situations. Under natural conditions many factors are known to influence the biological control potential of predators (Islam and Chapman, 2001; Sahayaraj *et al.*, 2004). Among these, temperatures have a profound influence, as this governs the rate of growth and development and prey consumption (Crocker *et al.,* 1975; Pearson *et al.,* 1987; Shrewsburry, 1996; Anderson *et al*., 2001; Islam and Chapman, 2001). These have been no systematic evaluations of the influence of temperature on prey consumption by Reduviid predator. Such information's could be useful for predicting the potential of these predators under varying environmental conditions.

The preference of prey was mainly influenced by the nutrients such as carbohydrate, protein and lipid of the prey and prey defense (venom, saliva emission) and predator response and age. While we subject the predators in different temperature regimes, it not only alters the predatory rate, but also influences the enzyme, microbial combination and DNA content. Furthermore,

what kind of factors which influence the prey consumption is more imperative one and I highlight these points in the forth coming chapters.

III.5. Conclusion

Stage preference studies of *R. marginatus* to the life stages of *S. litura* and *D. cingulatus* showed that both fifth nymphal instar and adult predators were more successful in encountering the large sized preys. Though different nymphal instars of *R. marginatus* preferred life stages of lepidopteran larvae, second and third instar reduviid preferred second, third and fourth instar *D. cingulatus* and the remaining life stages of this reduviids often preferred *D. cingulatus* adult. All the nymphal instars and adults of *R. fuscipes* mainly preferred second to fourth instar larvae of *S.litura* and second to fifth instars *D.cingulatus* nymphs. Generally the larger size predator preferred larger prey and smaller size predator preferred smaller preys. The foraging behaviour of reduviid predator is greatly determined by temperatures. The variation in biological control with temperature could be described by extended model indicating that temperature influences the attack rate; handling time; predatory rate and weight gain in adults and nymphs. Biological control potential of *R. marginatus* and *R. fuscipes* on *S.litura, D. cingulatus* showed that predators approached their preys quickly at higher temperatures and handled more time. The result can be attributed to the fact that the reduviids are more active and have greater reproductive rates (see chapter 1) at 30^{o}C.

Chapter IV. ENZYMOLOGY

IV.1. Introduction

Enzymes are proteins, which catalyse a variety of reactions in the biological systems. The multifacious 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). Inspite 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^{o}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^{o}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^{o}C for 30 mts. The reaction was terminated by the addition of 300 Aliquot of 5% trichlroacetic 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^{o}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^{o}C (0.216 to 0.591 µg/ ml). Peak protease activity was recorded at optimum temperature 25^{o}C, and then it was decreased observed at 30 and 35^{o}C. As observed in foregut, the protease activity was also maximum at 25^{o}C (1.047) followed by 30^{o}C (0.951 µg/ml) and at 35^{o}C (0.946 µg/ml) (Figures 9a and 9b). Similarly in *R. fuscipes*, maximum

protease activity was recorder at 25^{o}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^{o}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^{o}C. But in hindgut, the activity was high during the moderate temperature at 30^{o}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^{o}C to 35^{o}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

(Chapman, 2000). 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^{o}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^{o}C in fore and hindgut of both predators whereas amylase and invertase activity was maximum at 35^{o}C. In *R. marginatus,* foregut and hindgut showed maximum esterase activity at 20^{o}C. But in *R. fuscipes,* esterase activity was maximum and equal both in 30^{o}C and room temperatures.

Chapter V. BACTERIAL DIVERSITY IN REDUVIIDS GUT

V.1. Introduction

Microorganism plays an important and essential role in the growth and development of many insects. Numerous investigations were available about the bacterial flora of insect guts appears to be fortuitous contamination of the insect's surroundings and its food (Hunt and Charnley, 1981). The indigenous gut bacteria is regarded as a valuable metabolic resource to the nutrition of the host by improving the ability to live on sub-optimal diets and digestion efficiency, acquisition of digestive enzymes and provision of vitamins (Douglas, 1992; Tanada and Kaya, 1993; Biggs and Mc Grego, 1996; Bignell *et al.,* 1997; Chen and Purcell, 1997; Braumen *et al.,* 2001; Broderick *et al.,* 2004).

In many groups of the Heteroptera, the posterior end of the midgut is characterised by the presence of sac-like appendages which opens in to the hindgut. These evaginations, called caeca (or) crypts, vary considerable in number and arrangement in different taxonomic groups and almost always contain specific bacteria (Miyamoto, 1961; Goodchild, 1966). Although several bacteria have been isolated from the gut of some heteropterans, the microbiological nature of symbiotic bacteria has been poorly understood. Meanwhile till today no works and hypotheses were reported related to this proposed work. Among the haemolymph sucking groups of the Heteroptera, the family Reduviidae shows the most remarkable behavioural and anatomical arrangement for transmission of the

symbiont (Gobalakrishnan, 2001). Many studies were reported about the number of intestinal microflora which closely associated with the feeding habits of insects (Jones, 1984).

Very little is known about the autochthonous gut bacterial populations associated with reduviids (Sahayaraj and Mary Joseph, 2003). Recently Sahayaraj (2006, 2007b, 2008) first isolated and identified the gut bacteria of three reduviid predators such as *Acanthaspis pedestris* (Stal.) *Haematorrhophus nigrovidaceous* (Reuter) and *Cattamierus brevipennis* (Distant). However, no information was available about the impact of temperatures on gut bacterial autochthonous bacterial population and their hydrolytic enzyme activities of *R. marginatus* and *R. fuscipes.*

V.2. Methodology

Laboratory emerged *R. marginatus* and *R. fuscipes* adults which maintained at different temperatures were used for this study. The stock categories were cultured under laboratory conditions (29 ± 1^{o}C and 80% RM) on *C. cephalonica* following the methods of Sahayaraj (2002).

V.2.1. Dissection of insects

Ten *R. marginatus* and *R. fuscipes* each were selected randomly from all temperature regimes prior to the morning feeding when the gut was empty. Place the insects at 4^{o}C for 15 minutes prior to use. Surface of the predators were sterilized with 0.1% Mercuric Chloride for 2 minutes and washed with sterile distilled water thrice. Under aseptic condition each insect was carefully dissected by using sterile pins, fine forceps and razors in a dissection tray filled with sterile

phosphate buffered saline (PBS) (pH 6.9). Guts were isolated individually, washed several times with fresh phosphate buffered saline to minimise the possible microbial contamination and used for the study. Wet weight of the gut was recorded for individually categorized with six temperatures for *R. marginatus* and *R. fuscipes*.

V.2.2. Enumeration of THMP of predators gut content

The isolated gut was homogenized with sterile insect ringer solution (IRB) in mortar and pestle. The homogenate was filtered through Whatmann filter paper No.1 and the pH was measured using pH meter. The filtrate was serially diluted in sterile saline and 0.1 ml of aliquot was plated on nutrient agar (NA) and Trypticase soy agar (TSA). The seeded nutrient agar plate (NA) was inoculated at 37^{o}C for 24-48 hours whereas the Serially Dilution Agar (SDA) plates were incubated in 28^{o}C for 48-72 hrs. Microbial colonies appeared after the incubation period was enumerated and the numbers of colony forming units were expressed as a wet weight of the gut

V.2.3. Identification of Microflora

Different morphological microbial colonies were selected, sub-cultured and stored at 4^{o}C on respective agar slants. Bacterial strains were identified using the criteria suggested by Cappucino and Sherman, 1999; Buchanon and Gibbons (1979) based on morphological, cultural and biochemical characterisations.

V.2.4. Hydrolytic extra cellular enzyme

The extra cellular enzymes like amylase, protease, cellulase and gelatinase activities were tested by using the nutrient media containing 0.2% (W/V) carboxymethyl cellulose (cellulase), starch (amylase) and skimmed with powder (protease) as substrates (Plate 3). Pure culture of each bacterial isolate was streaked on respective media and utilization of the substrates was determined by observing the clear zone around the colonies (Buchanon and Gibbons, 1979). All the screening experiments were replicated at thrice.

V.2.5. Statistical Analysis

Correlation analyses were made between the temperature and gut bacterial population of *R. marginatus* and *R. fuscipes* separately.

V.3. Results

V.3.1 Weight and pH of the reduviid guts

Generally, predator's intestinal pH profile was alkaline nature (Lemeke, 2003). But the present study reveals that gut content of *R. marginatus* (pH 7.6) and *R. fuscipes* (pH 7.0) were found to be either slightly alkaline or neutral. Our results revealed that the gut of these reduviids predators was neutral to slightly alkaline.

In *R. fuscipes,* gut weight was gradually diminished up to 30^{o}C then increased to 35^{o}C whereas in *R. fuscipes* gut weight was gradually decreased upto 25^{o}C then decreased from 30 to 35^{o}C. Alimentary canal weight of *R. marginatus* was higher when compared to *R. fuscipes* (figure 11). Total heteroptrophic

bacterial populations (THBP) of the whole gut homogenate of both predators are presented in figure 12. The THMP of both reduviid predators were gradually increased from 10-30^{o}C and then declined at 35^{o}C. Between the two reduviids, maximum THMP was observed in *R. fuscipes* than *R. marginatus* (Figure 12). THMP was higher the control category.

V.3.2. Bacterial Composition

Thirteen and eleven isolates of bacteria were grouped based on morphological charecters, and biochemical tests for both *R. fuscipes* and *R. marginatus.* The bacterial species isolated from the gut of *R. marginatus* includes, *Staphylococcus aureus, Bacillus cereus, B. magaterium, Enterobacter aerogenes, Micrococcus luteus, Corynbacterium kutcherii, C. xerosis, Bacillus subtilis, Escheritia coli, Pseudomonas aeroginosa and Micrococcus variance* (see table 17). Among them *Staphylococcus aureus* was found to be the most dominant specie both at 10^{o}C and 15^{O}C and *Micrococcus variance* was the dominant bacteria between 20 to 30^{o}C. *Bacillus subtilis* dominantly present at 15^{o}C and 35^{o}C for *R. marginatus* and *R. fuscipes. Micrococcus luteus, C. xerosis,* were represented in low percent at 25 and 20^{o}C respectively. Among the observed bacterial species, *Escheritia coli* was observed only at 30^{o}C. If average of all the temperatures were considered, *M. variance* constituted the dominant bacteria (40.56%) followed by *S. aureus* (31.78%) and *B. cereus* (24.10%). The most predominant bacteria observed in *R. fuscipes* (Table 18) was *M. variance* (47.22%) followed by *S. aureus* (30.50%) and *B. subtilis* (30.10%). All these bacterial populations were positively correlated (0.67, 0.50, 0.84 and 0.42 for *B. cerosus, M. variance,*

S. aureous and *C. xerosis* respectively) with different temperatures except *B. subtilis* (r^2 = - 0.35) and *Lactobacillus delbrucki* (r^2 = - 0.02) shows negative correlation to temperatures. It was more predominant (29%) at 10^oC. In general *S. aureus* and *B. cereus* were more or less similar population in all the temperatures in both reduviids.

V3.4. Hydrolytic extracellular enzymatic activity

Hydrolytic activity was observed in bacterial isolates of the whole gut in both predators. Of the four hydrolytic enzymes, cellulase activity was almost lower in these predators than the amylase and protease. Amylase activity was apparently higher at higher temperature for *R. marginatus* (65.91) and *R. fuscipes* (67.6). Another hydrolytic enzymes protease showed peak activity at 25^oC (55.1 and 47.8 for *R. marginatus* in *R. fuscipes* respectively). (Plate 3).

V.4. Discussion

The alimentary canal of insects provides a suitable substratum for the development of microorganisms because of concentrated nutrients and extended surface of the intestinal lumen. On the other hand, these associated microbes may play an important role in the digestion, nutrition and defense system of the host animals. It is possible that fastidious insoluble substrates including cellulose, alginate and chitin continue to be decomposed by the attached microbes in the fecal pellets discharged from the host animals, in addition to the alimentary canal (Pankaj *et al.*,2003).

Little is known about bacteria associated with the Reduviidae, the large group of mostly zoophagous insects comprising the haematophagous and predatory insects. In the present study we identified many novel bacterial species, which belongs to sub divisions of the Proteobacteria. Plata stingbug, *Megalopta punctatissima* also has this type of bacteria in its gut (Fukatsu and Hosokawa, 2002). The bacterial genera found in *R. marginatus* and *R. fuscipes* were *Streptococcus* spp., and *Staphylococcus* spp., and *Micrococcus* spp. In insects, there are the typical bacterial colonies found in the intestine of the all polyphagous and phytophagous insects (Brooks, 1963; Tanada and Kaya 1993). The present report was also indicating the diversity of bacteria present in the digestive system (fore, mid, hindgut) of *R. marginatus* and *R. fuscipes* (Sahayaraj and Mary Joseph 2003). Thermostable enzymes can be obtained from both mesophilic and thermophilic organisms; thermophiles represent an obvious source of thermostable enzymes. Thermostable enzymes, which have been isolated mainly from thermophilic organisms, have found a number of commercial applications because of their overall inherent stability (Santo *et al*., 1998). It was also reported that abiotic factors alter the gut bacteria populations (Tsuchida *et al.,* 2002). It may be hypothesised that the bacterial flora degraded the some acid metabolites which might have induce the pH Gut of the lepidopteran moth, *Lymantria dispar* Linn. showed lightly alkaline nature (Broderick *et al*., 2004).

Chen and Purcell (1997) suggested that environmental condition mainly affect the growth of the microorganism in the digestive system of adult reduviid predators. The gut microflora represent all the aspects of microbial relationship from pathogenic to obligate mutualism (Dillon and Dillon, 2004). Numerous

investigations have been available about the microbial flora of insect gut. Some studies have been presented the incidence of entomopathogens (Lysenko, 1985). The contradiction of gut microbiota to nutrition and disease suppression was reported extensively (Hagen, 1966; Dillon and Charnely, 1986, 1988). More over the study of the microflora associated with the insect predators may leads to the isolation of possible pathogens which may help to design the biological control agents (says Pankaj *et al.,* 2003). The present study deals with the gut microflora associated with six different temperatures reveals that indefinite growth of microbial organisms mainly depends upon the abiotic factors, such as favored temperatures at 25 and 30^{o}C.

We utilised the dilution plate method to recover the microorganism. This is the conventional technique used to isolate microorganisms in most of the microbiology related studies (Santo *et al.,* 1998). Eventhough microorganisms are capable of grow in the SDA media, another major limitation factor was the dilution. Plate method is that rare occurring or poor growing isolates will most likely go undeducted.

Small bacteria population comprises Shigella spp. like *L. casei*, and *L. delbrucki*i were recovered from the alimentary canal of these to reduviid predators. Others bacteria species from the family of *Streptococcus*, *Bacillus* and *Micrococcus* were also identified from these reduviids. From these genus common species like *Bacillus cercus, Bacillus subtilus, Micrococcus variance, Micrococcus leutus, Enterobater aerogenes* were isolated from reduviids. Many members of Enterobacteriace, Micrococococcea, Bacillacea are common in freshwater, soil,

sewage, plants, vegetables and animals including insect guts (Koch, 1967; Mckillip *et al*., 1997).

From the results, it was hypothesised that the bacterium is a mutualistic gut symbiont of *R. marginatus* which is vertically transmitted through the egg capsule or food and is essential for normal development and growth of the host insect. Previously the morphology of symbiotic bacteria of plant sucking insect species has been described (Buchner, 1965, Tustomu *et al.,* 2006). Therefore, this study is the first report of phylogenetic position of a gut symbiont in accord with various higher and lower temperatures from zoophagous reduviid bugs.

E. aerogenes was the dominantly present in the gut of *Chrysoperla rufilabris* (Burmeistre) (Mecoptera) an important biological control agent throughout the world (Harklein, 2003). In *R. marginatus*, *E aerognes* was reported at 10^{o}C. It was a common bacterium in plants, vegetables and animals including insect guts (Dash *et al.,* 1984). Temperatures alter the clima or mature bacterial community within the gut. Both lower (10 and 15^{o}C) and higher temperatures (20, 25, 30 and RT) were in favour for the colonization of *S. aureus* and *M. variance* in both reduviids. These may be due to inter specific competition among autochthonous gut bacteria of the reduviids.

V.5. Conclusion

THMB of both the species increased upto 30^{o}C for *R. marginatus* (12.37x10^{4}) and *R. fuscipes* (9.4 x 10^{4} CFU/gm). From this result it was very clear that predators maintained at room temperature had maximum gut weight with higher bacterial density. Between the two predators *R. fuscipes* has minimum bacterial density than *R. marginatus.* In lower temperatures (10,15 and 20^{o}C) microbial colony forming tendency was low in both reduviids. Bacterial strains like *Bacillus cereus, Staphlococcus aureus, Micrococcus variance* were increased when temperature increased. Though both predators have similar kind of bacterial populations such as *K. preumonsae, Lactobacillus delbruckii* and *Lactobacillus casei* were considered as autochthons bacteria of these reduviids and their populations on present only in *R. fuscipes* implies the species specificity of bacterial populations. *Staphlococcus aureus* was present dominantly between 10-20^{o}C whereas *Micrococcus variance* was higher < 30^{o}C. More than 12 bacterial isolates were identified in *R. marginatus* and *R. fuscipes* when fed with *S. litura.* Isolated bacterial strains were able to produce cellulase and amylase (C+A) followed by xylenase. Maximum hydrolytic activity was observed in cellulase and amylase producing isolates belonging to *M. variance* and *S. aureus*.

Chapter VI. MACROMOLECULES

VI. 1. Introduction

The environmental temperature on insects caused dramatic changes in behaviour, physiological activities and biochemical changes (Agrell and Landquist, 1973) particularly haemolymph lipoprotein in *Triatoma infestens* (Maria *et al*., 1991; Rolf *et al*., 1999). Jeffrey and Jesusa (2006) assessed the biochemical fitness of a predator, *Podisus maculiventris* in relation to food quality and effect of five preys. The information about the influence of temperature on various biochemical entities were available in the literature (Himano, 1979, Maa, 1987). Proteins constitute the basic entities in the living being and undergo both qualitative and quantitative changes during development (Engelman, 1979). The fat body is the principal store house of lipid in insects. Most of the lipid is present as a triglycerol which commonly constitutes more than 70% of the dry weight of the insect fat body (Chapman, 1998). Lipids are synthesised from the fat body and secreted into haemolymph through physical activities (Brooks, 1969; Beenakkers *et al*., 1985). Its importance along with protein was also available for heteropteran insects (Kunkel and Nordin, 1985; Helosia *et al*., 1997). Further more Beenakkers *et al*., 1985 viewed that the whole body content is an important carbohydrate reserve in many insects (Beenakkers *et al*., 1985).

Earlier molecular profiling provides a rapid means of quantifying prey diversity within predators but when there are specific prey DNA targets with group specific primers is the principal method of choice (Symondson, 2002). This is fine

for sample laboratory studies, but when there are multiple potential target prey species (Sheppard *et al*., 2004) and fragments (Hoogendoorn and Heimpel, 2001) the time required to assay each predator potential target becomes limiting.

In field studies, the mean number of prey items in a generalist predator gut may be as a few separate PCR assays evaluated (Harper *et al*., 2005). This technique is effectively peculiars for many useful field-based ecological studies. Rapid PCR – based screening systems for the study of the prey diversity of generalist predators have been developed to expend the potential of molecular detection in to various areas of food web research (Dodd, 2004). From the published results, it was very clear that except the haematophagous reduviids such as *Trypanosoma rangeli* and *Trypanosoma cruzi* (Moser *et al*., 1989; Breniere *et al*., 1995; Russomando *et al*., 1996; Shiankal *et al*., 1996; Vallejo *et al*., 1999), till date, no information was available for the polyphagous reduviid predators.

The Polymerase Chain Reaction (PCR) technique was developed by Ehrlich in 1989. It is one of the simplest, fastest and least expensive molecular approaches is to use RAPD – PCR (Randomly Amplified polymorphic DNA) (Shappiro *et al.,* 1988) is used to amplify a region of DNA that lies between two regions of known sequence (Teresa *et al*., 2002). It has used in many fields including to know the genetic variability in insects (review of Sheppard and Hardwood and Obrycki, 2005b).

Therefore, it was envisaged to analyse quantitatively and electrophoretically in relation to temperatures modification also on imperative one. This chapters deals with changes of whole body and egg macromolecules

(carbohydrate, protein and lipids) of two reduviid predator(s) by spectrophotometer methods, whole gut protein by electrophoresis method; gut DNA polymorphism by RAPD – PCR analysis by AGE in relation to six different constant temperatures on *R. marginatus* and *R. fuscipes*.

VI.2. Materials and Methods

VI.2.1. Total carbohydrate, protein and lipid

Eggs were incubated for 12 to 15 days (10,15, 20^{o}C), 6 to 7 days (25 to 30^{o}C) and 4 days (35^{o}C) have been used for analysing the protein (Lowry *et al.*, 1951), carbohydrate and lipids (Bragdon, 1951). 30 adult reduviids of both male and female were kept in the BOD incubator and maintained at 10, 15, 20, 25, 30 and 35^{o}C separately untill their death. After the (one month) stipulated period 10 insects were randomly selected and their whole body total carbohydrate, protein and lipid content were estimated using the above-mentioned methods.

VI.2.2. Procedure for Electrophoresis

SDS polyacrylamide slab gel electrophoresis was carriedout using Leamli (1989) method with minor modifications. A sandwich was made with two glass plates separated by spacer strips. The spacer strips are coated with vaseline for adhering mechanisms. The glass plate was kept vertically by placing it on to a stand, which can hold the plates vertically. Few ml of distilled water was poured between the plates to check leakage if any. The resolving gel of 12% (P^{H}7.6) was poured in to the space between the glass pates after removing distilled water. The level should be about 2cm below from the notch. It was kept for polymerization for

about 30mts. Then made a layer of distilled water on the surface of the resolving gel, to avoid the contact between the gel surface, air and also to make an even surface.

When polymerisation was completed poured off the distilled water and stacking gel was poured (7.5% P^H7.6) over the resolving gel and the Teflon comb with fingers (each finger with 7cm wide) was inserted to into the gels, and allowed to polymerize for 30 minutes. After polymerisation, the glass plates were clipped out from the stand and also, removed the bottom Teflon spacers. Both of the slab gel was made clean with filter paper and attached the plate to the electrophoresis apparatus. The electrode buffer (TBE) was poured to the lower and upper chamber. Then the Teflon comb was carefully removed from the gel, supernatant of the previously prepared sample was added in each well in about a volume of 15µl with the help of microtitre syringe. Marker protein of 14-100 KDa (Genei, Bangalore, India) was loaded in one well as a reference. Initially a current of 60V was supplied with the sample entered in to the separating gel and electrophoresis was continued at 120V till the marker dye reached the bottom of the separating gel (resolved gel). At the end of electrophoresis run glass plates were gently moved apart with a spatula, by running a stream if electrode, the gel in to a solvent resistant plastic trough for staining (Coomassive brilliant blue- CBB) for over night and destaining (24 hrs) until clear band can be seen.

VI.2.3. Protein profile Studies

Under six various temperatures subjected *R. marginatus* and *R. fuscipes* whole gut was used for this present study.

VI.2.3.1. Reagents preparation

VI.2.3.2. Stock solutions

i. Acrylamide 30%-Bisacrylamide (29.2 : 0.8) prepared by mixing acrylamide (29.2 gm), and bisacrylamide (0.8 gm) in 100 ml distilled water. The solution is filtered through Whatman No.1 filter paper and stored at 40^{o}C in a dark bottle.

ii. Separating gel buffer (resolving gel) (1.5 Tris HCl. - pH 8.8)- 18.17 g Tris was dissolved in approximately 40 ml of distilled water and adjust the pH to 8.8 with 1N HCl using pH meter. Make the final volume up to 100 ml.

iii. Stacking gel buffer (0.5M Tris HCl pH 6.8) 0.057 g of Tris was dissolved in approximately 40 ml of distilled water and pH adjusted the pH to 6.8 with 1N HCl and made the final volume up to 100 ml. The solutions i, ii, and iii were filtered through Whatman No.1 filter paper and stored at 4^{o}C in a dark bottle.

iv. 10% SDS (Sodium Dodecyl Sulphate) - 1 gram of SDS was dissolved in 10 ml of doubled distilled water. The solution was clear and colorless and kept at room temperature.

v. Ammonium per sulphate (APS) 10% –100 mg of APS was dissolved in 1 ml of distilled water APS. The solution is unstable and

decomposes readily at room temperature and hence it should be made fresh just before use.

vi. TEMED (N, N, N, N, - Tetra methyl ethylene diamine) - This reagent was acting as a catalyst for gel formation.

vii. Electrode buffer (reservoirs Buffer) –3.028 gm of Tris, 14.45 gm Glycine, 0.5 gm SDS were mixed with 500 ml of distilled water. The solution was stored at 4^{o}C in a dark bottle.

viii. Coomassie Brilliant blue stain – R – 250 - 50 ml methanol, 7 ml acetic acid, 200 mg coomassic blue were mixed with 43 ml distilled water. The solution is blue in color and kept at room temperature.

ix. Destaining solution - 30 ml ethanol, 67 ml of distilled water were mixed with 7 ml of a acetic acid. The solution is colorless and kept at room temperature. This solution once used can be reused. For this, pupae after destaining add a teaspoon of activated charcoal to this solution and allow settle the impurities properly. Then the blue color disappears and the solution is filtered through Whatman No.1 filter paper and this can be used for destaining again.

ix. Sample buffer (3ml) – prepared by mixing 3 ml each of 0.5 M Tris – HCl (6.8) and 10 % SDS 0.3 ml, β - mercaptoethanol 2.4 ml, 3 ml, Glycerol 3 ml, Distilled water 3 ml, Bromophenol blue 1 pinch. The solution is blue in color and is stored at 4^{o}C.

xi. Resolving gel (125- 15ml) – Prepared by mixing 4.9 ml distilled water, 6.0 ml acrylmaide, 3.8 ml Tris (8.8), 0.15 ml 10 % SDS, 0.15 ml 10 % A PS, 0.006 ml TEMED and allows 20-30 minutes for polymerisation.

xii. Stacking gel (125 – 4 ml) – Prepared by mixing 2.70 distilled water, 0.067 ml acrylmaide, 0.50 ml Tris (6.8), 0.04 ml 10 % SDS, 0.04 ml 10 % APS and 0.004 ml TEMED.

xiii. 7 % Acetic acid – mix 7 ml acetic acid and 93 ml distilled water and this solution is used for the preservation of gel.

Insect Ringer solution (IRS) – It was prepared by mixing 7.5 gm sodium chloride, 0.035 gm Potassium chloride, and 0.22 gm Calcium chloride with 1000 distilled water. This solution was kept at room temperature.

VI.2.3.3. Protein sample preparation for Electrophoresis

6 to 10 adult of *R. marginatus* and *R. fuscipes* were selected separately from the stock insects which maintained in different temperature including the room temperature categories. Foregut was dissected out from the predators and homogenised with homogeniser. Eppendrof tubes containing 75µl of gut sample was boiled at 50-60^{o}C for 3 mts and allowed to cool at room temperature. The sample was then centrifuged at 10,000 rpm supernatant was collected and used as the protein sample for electrophoresis.

VI.3. DNA Extraction and amplification

Six to ten reduviid adults predators were reared at different temperature regimes for more than a month were randomly selected and homogenized with 0.5 ml extraction buffer (8% DTAB, 1.5M Nacl, 100mM Tris, 50M EDTA 10% SDS and proteinase K) and grind further. The extract was incubated for 2-3 hrs at 50^0C–60^0C to allow the separation of DNA also for the denaturation of proteins. The mixture was centrifuged for 5 minutes at 10,000 rpm. The supernatent was cleaned away from the protein and lipids by phase extraction with an equal volume of phenol, chloroform and iso amyl alcohol (25:24:1). DNA was precipitated by adding one-tenth volume of 3M NaCl + two volume ice-cold 95% absolute ethanol and incubated for one hr at -20^{o}C. The precipitated DNA was centrifuged, then washed with 70% ethanol, DNA was vacuum dried and resuspended in 100µl TE buffer, pH 8.0). The concentration and purity of extracted DNA was determined spectrophotomectrically (UV- instrument) at 260 nm and 280 nm. Samples showing the one OD (optical density) equivalent to 50 µg and purity (determined by the ratio of 260 nm and 280 nm) 1.5 to 1.8 alone were taken for further analysis. Template DNA extracts were stored at -20°C and thawed at room temperature for further amplification.

VI. 3.1. PCR Amplification

The extracted DNA from the experimental predators were subjected to PCR analysis using 6 universal primers among six, further proceed for amplification 3 primers were selected such as KTG-3-(5′-GTAGACCCGT-3′), KTG-5 (5′-AACGCGCAAC-3′) and OPE 8-(5′-AACGGCGACA-3′) (GENEI

scientific supplies, Bangalore). PCR reactions were performed in 25 µl of reaction mixtures contained 1 mM dNTP mix (5.0 µl), 1.0 µl template DNA (50 ng / µl), 10 mM RAPD primer (2.5 µl), 10X Reaction buffer, 25 mM Mg Cl_2 (1.5 µl) 2.5 units of Taq polymerase enzyme (5U / µl) (Bangalore Genei, India) and sterile de-ionsed water. Above-mentioned 25 µl of reaction mixtures was placed in PCR tubes in two layers. The bottom layer consists of all reagents except *Thermus aquaticus* (Taq), sterile distilled water and sample DNA. The upper layer consits of Amplification was performed with thermocycler (Master cycler ep's eppendrof, India) for 40 cycles. Thermal cycles were programmed for initial denaturation at 94^oC for 2 minutes. Each cycle consisted of 40 seconds annealing at 94^oC and also with 1 minute annealing at 48^oC, followed by 72^oC for 5 minutes as final extension. Amplified, samples were stored at 4^oC prior to electrophoresis. PCR amplified products were separated on 1.4% agarose gels submerged in 1X TBE, and the banding profiles was visualised with ethidium bromide. Gels were documented using Biotech documentation and analysed with Gel Del TM software (Bangalore) (Carezza Booto *et al.,* (2005). Genetic similarity and Dissimilarity dendrogram was made from the similarity data using UPGMA method of the programme Digital Gel Documentation (Biotech, Tamil Nadu, India)

V.3.2. Statistical analyses

Using three selected primers with randomly selected six temperatures treated both predators were comparative analysis was made. RAPD patterns and gut protein polypeptide profiles were visually analysed and scored form photographs. For the analyses and comparison of the patterns a set of distinct, well

separated bands were selected. The genotypes were dietermined by record the presence (i) or absence (o) of these bands and neglecting other (weak and unsolved groups) bands. Genetic identity (GS) and genetic distance (DS) values between the total six temperatures were calculated using the data generated from the RAPD profiles using digital gel documentation (Biotech, Tamil Nadu, India). Genetic distance values were utilized to contracted to dendrogram through clustering analyses (UPGMA) to determined the relationship among the six various temperatures on the predator of *R. marginatus* and *R. fuscipes*

VI.4. Results

VI.4.1. Whole animal macromolecules

VI.4.1.1. Carbohydrate

Total carbohydrates, protein and lipid content of *R. marginatus* in relation to different temperature regimes (10 to 35^{o}C) is presented in figure 13. From the figure it was very clear that, total carbohydrate content was lower at 10^{o}C (22.2 μg/mg). It gradually increased when the temperature was increased (23.5, 25.11, 27.8 and 28.8, μg/mg for 15, 20, 25 and 30^{o}C respectively) and attains its peak at 35^{o}C (30.14μg/mg). Statistical analysis between control (25.3μg/mg) with different temperature showed that 25, 30 and 35 were significant at 5% level. Similar observation was also recorded in *R. fuscipes* too. Between the two reduviids *R. fuscipes* had maximum carbohydrate content than *R. marginatus*.

VI.4.1.2. Lipid and Protein

In contrast to the carbohydrate, lipid content was gradually decreased from the lower temperature to the higher temperature (Figure 11). As observed for carbohydrate, the lipid content was low and high in *R.marginatus* respectively. In *R. marginatus*, the protein content the control category (291.20 μg/mg) and 20^{o}C (290.71 μg/mg) and 25^{o}C (293. 54μg/mg) were more or less similar. However, in *R. fuscipes*, protein level in control (272.0 μg/mg) and 25^{o}C (273.28 μg/mg) categories were similar. Statistical analyses were made between control and 10 to 30^{o}C reveals that all the comparisons were significant at 5 % level.

VI.4.1.3. Protein

Protein content was gradually increased from 10 to 25^{o}C and 10 to 30^{o}C for *R. marginatus* and *R. fuscipes* respectively.

VI.4.2. Temperature and gut protein profile of *R. marginatus* and *R. fuscipes*

R. marginatus adults gut protein analyses by SDS PAGE. Showed that both 35, 30^{o}C produce 5 polypeptides where as 25, 20^{o}C and 15^{o}C produced seven bands with molecular weight between 6.5 to 205 kDa. Two molecular weight polypeptide such as 56.0 and 205 kDa were absent at 10^{o}C in *R. marginatus* (Plate 4a). From the dendrogram analyses explained that gut protein profile, showed higher genetic identity (GS) at 20^{o}C (0.83), than the lower temperatures such as 10, 15^{o}C (GS = 0.77). (Figure 15(a)). As in the case of dissimilarity (Genetic distance-GD), minimum value was recorded (0.16) temperatures of 35, 15 and

20°C. It was further reduced when the reduviid was subjected between 25 and 30°C (0.11) (Figure 15b).

Plate 4(b) depicted gut protein profile of *R. fuscipes.* Four uniform polypeptides appeared at 35, 30, 25 and 20°C (between the molecular weight range 2.0 to 205 kDa) except at 10°C. However, at 35 and 20°C once polypeptide (<205 kDa) was peculiar, in gut protein because, absence of this higher Mw. The dengrogram analyses showed the highest genetic similarity between 25 and 30°C (0.81). Lowest genetic similarity (0.22) was noted at 10°C and 15°C. Although dissimilarity (DS) was did not possessed striked main variations, even though they ranged between 0.23 to 0.11. A dendrogram was predicated to find out the genetic relationship in *R. marginatus* and *R. fuscipes* which subjected to various temperatures. From the results it was very clear that predators reared at 30° C had closer relationship with 25°C and also another category of 10 and 15°C in *R. marginatus,* whereas in *R. fuscipes,* relationship was recorded between the temperatures 10 and 15°C.

VI.4.2.1. Eggs macromolecular profile

In another study biochemical composition of eggs in relation to temperature was evaluated. Regarding the total carbohydrate, maximum content was recorded at 35°C for the two reduviids. Furthermore, egg protein contents were gradually increased up to 30°C for both the reduviids. In contrast, lipid content was gradually decreased up to 35°C in *R. marginatus* and *R. fuscipes* (Figure 14).

VI.4.3. DNA Amplification of *R. marginatus* and *R. fuscipes*

PCR amplified products having 400 and 600 bp were common irrespective of the primers in *R. marginatus*. Such a similarity was not observed when *R. fuscipes* whole body DNA was amplified with OPE-8, KTG-3 and KTG-4 Primers. Interestingly OPE-8, KTG-3 and KTG-5 produced a unique bp product of 1200-150 and 50 bp in *R. marginatus*. Similarly these 3 primers produced 950, 200 and 300 and 900 bp in *R. fuscipes*. Present study reveals that RAPD markers were efficient for the assessment of genetic similarity and dissimilarity coefficient using Digital Gel Documentation between the six temperatures within the same species described in the dendrogram. Apparently the resulting data present in Tables, 19, 20 were further processed for cluster analysis using the unweighed paired group of average method (UPGMA). Totally seven primers were tested, four primers (KTG- 1, 2 and 4 and OPE-8) yielded no clear or any scorable bands, but remaining 3 primers primers (KTG –2, KTG – 5 and OPE – 8) were amplified, produced scorable with polymorphic bands. Primers KTG –3 and KTG – 5 amplified maximum numbers of polymorphic bands ranged about 31 to 34, in *R. marginatus.* Primers such as OPE-8 and KTG –3 produced 26 and 32 bands in *R. fuscipes*.

VI.4.4. Genetic similarities (GS) in *Rhynocoris marginatus*

4.4.1. Primer – KTG – 3

Figure 17a shown KTG –3 primer predicted dendrogram, it consists of two clusters. Cluster – 1, and this deserved higher GS value was higher (0.84) than the remaining temperatures. As in the case of another temperatures held in cluster – II,

this also again stands for only one temperature at 20^{o}C, and it consisted estimated GS value was 0.80. Where as, cluster II again divided into II-b1 and C-II b2. These subclusters belong to the temperatures of 30 and 35^{o}C also possessed similar GS value and15 including 25 and 10^{o}C respectively. Of these four temperatures of 15^{o}C (C-II b2) consisted estimated GS value was higher (0.70) this was 10% increased away from C-II b1 consisted 30 and 35^{o}C. Finally C-II b2 represented temperature of lower (10^{o}C) and optimum (25^{o}C) both the temperatures shared equal GS value of 0.40. From this result clearly showed 40.4% deviation were observed between RT and lower temperatures 10^{o}C.

4.4.2. Primer KTG – 5

When estimating the KTG – 5 primers (Figure), dendrogram revealed mainly two sub clusters. From this cluster – 1 had been the temperature at 30^{o}C expressed GS value was (0.76). Then the cluster –II again divide into two sub-clusters, such as C-II-a and b. Here, cluster – II a represented at 35 and 15^{0}C, both the temperatures shared another higher as well as similar (GS) values of 0.57, followed by cluster II – b consisted remaining temperatures and its observed GS values were RT^{0}C (0.6b), 25^{0}C (0.65), and following lower temperatures 20 and 10^{o}C noted GS value of 0.29. Since the overall results clearly noted highly diverged 0.53% at 35^{0}C (0.23). Since the overall results clearly noted highly diverged 0.47% at 10^{0}C from the initial genetic similarity index of 0.76 at 30^{o}C (Table 19).

4.4.3. Primer OPE – 8

Based on *R. marginatus* OPE-8 primer could be provided dendrogram. Consisted of two cluster, They were cluster I and cluster II. C-I mainly stands from temperatures (RT) expressed highest GS value of 0.84. Since cluster –II broadly divided into two subclusters namely Cluster II a and Cluster II b. At 35^{o}C category include C-II b. Interestingly both temperatures shared GS value of 0.75. Similarly remaining adjacent temperatures such as 20 and 25^{o}C represented C-II a and 10 and 15^{o}C stands for C-II b. Since these four temperatures (10-25^{o}C) had been possessed the similar value of 0.50. Here this primer predicted overall similarity that was 0.34% deviation observed form RT initial GS index of 0.84 noted 10-25^{O}C. This result concluded closely relationship seen between similar at 10-15, 20-25 and 30-35^{o}C.

VI.4.5. Genetic similarities (GS) in *R. fuscipes*

VI. 4.5.1. Primer KTG-3

KTG-3 primer had drawn a dendrogram predicted results clearly visible in figure 18a. From this figure expressed smaller accessions instead of clusters arranged with each temperature as a decreased manner in accord with GS estimated values. The higher genetic identity or GS observed at higher temperature at RT (0.95) followed by temperature consisted GS value was 30^{o}C (0.94), 25^{o}C (0.93), 20^{o}C (0.90) and 15^{O}C (0.85). Finally medium GS value (0.67) was denoted at lower temperature (10^{o}C) as well as higher temperature (35^{O}C). Each temperature reported all the Genetic Similarities were similar as soon as they had been little deviation range about 0.01% between RT-25^{o}C and 0.05% for 20-15^{o}C and 0.20% shows at 15-35 respectively. The overall results indicated 0.28% away from initial GS (0.95) to RT-10^{o}C.

VI. 4.5.2. KTG-5

Apart from the primer KTG-5 revealed dendrogram see figure 18c. In this primer does not divide into clusters but they were arranged the separate accessions and it stands for lower and higher temperatures (RT-10^{o}C) instead of cluster. As in the case of estimated higher GS value also at the RT (0.88) then the lower temperatures (35^{o}C) secured adjacent value of 0.86. Similarly following temperatures had been little variation noted in GS value such as 30 ^{o}C (0.84) and 25 ^{o}C (0.77), 20 ^{o}C (0.66). Finally at 15 and 10 ^{o}C lower temperatures noted lowest GS value noted 0.34. The overall results shows closely related GS range 0.02% to more deviation 0.54% observed away from initial GS index value at RT (0.88) seen among the each six temperatures.

VI. 4.5.2. OPE-8

Based on OPE-8 primer showed cluster analysis (figure 18b) mainly divided into two clusters that was namely cluster I and II. Cluster I consisted only one temperature of RT and it was also secured higher GS value of 0.92. Another cluster II again broadly divided into two subclusters (cluster IIa and cluster II b). At 10 and 20 ^{O}C had been possessed another higher as well as similar estimated GS value of 0.87. These two temperatures represented Cluster II a stands for remaining temperatures of 35, 15^{o}C and both temperatures observed GS value Cluster II a and C-II b was similar 0.75. (Table 20). At 25 ^{o}C and 10 belongs to cluster II b both temperatures also had been shared similar estimated GS value 0.75 as like that C-II a. As a resulted dendrogram showed a did not found more deviations (0.57%) made between RT-10^{o}C, at the same time a meager variation 0.17% revealed among these six temperatures.

VI.5.1. Discussion

VI.5.2. Macromolecules

Macromolecules like carbohydrate, protein, lipid content of *R. marginatus* and *R. fuscipes* had been dearth in the life time. Previously George and Ambrose (1999b, George *et al.*, 2002) demonstrated that insecticide affect carbohydrate, protein, lipid content of *R. marginatus*. Among the three macromolecules initially the whole body carbohydrate content could be attributed to its higher utilization as well as energy releasing site were warranted by altered metabolism due to agreement observation discussed in other insects belongs to Coleoptera (Price 1965; Pigman and Horton, 1970). Such high energy demands for various endothemic biochemical reactions can be readily react on carbohydrate reserves because they are principal and immediate chief source of energy precursors (Wyatt, 1976). More over prolonged treatment of cooling as well as higher temperature affected functional either quantitatively nor qualitatively at the range of metabolical changes. It leads to reduce the synthesis of protein by deranging the protein synthetic machinery.

Appearance of some protein substances mainly affected by temperature variation says Bradford (1976). According to Ryan and Dick (2001), when ever temperature reach to peak (or) higher (or) exceeded at 35^{o}C, adult insects were enable to ultimately adopted and also this changes caused mainly depends upon the treated temperature. This kind of opinion was also agreed by Shappiro *et al.* (1988). Temperature changes also affect the Lepidopteran pests, its may be related to heat shock proteins induced by sub-lethal temperature an other environmental

stresses (Dodd, 2004; Zhang *et al.,* 2007), reported that major during heat shock they should be disintegrate induced by conditioning at 33 - 41^{o}C after for 2 to 3 hr at 20^{o}C. Further more of the energy as well as break down of food particles of insects and metabolic process, directly or indirectly controlled by biotic factors suggested by Salt, 1970.

VI.5.3. Gut Protein Pattern

Gut protein pattern shows significant reduction in the number of polypeptides when the redvuiid was subjected to difficult temperatures. Molecular weights of gut protein of two predators were range between 205 to 14.3 kDa and below 205 to 14.3 kDa in *R. marginatus* and *R. fuscipes* respectively. The electrophoretic variation of the protein bands in the whole alimentary canal of adult these predators showed Plates (6a and b). Such qualitative profiles of protein was observed during adult transformation with temperature confirms earlier findings (Tefler *et al.,* 1983; Ryan and Dick, 2001). As in the case of whole gut of *R. marginatus* showed six polypeptides at room temperature. Intensity of polypeptides, position, size and shape for all 6 temperature determined slightly qualitative changes between 10-15, 30-35 and 20-25^{o}C. In *R. fuscipes* higher and lower temperature had been peculiar high molecular weight polypeptides. This agreed with earlier findings in Coleopteren predators did not based upon only temperature but endepeptidases in alimentary canal (Addedire and Balogun, 1995).

VI 5.4. RAPD – PCR

The inter population profile of the six temperature combination showed a remarkable banding patterns of powerful band, high intensities of genetic variation or suggesting heterogenous as well as homogenous amplified DNA between 10 to 15, 20 - 25, 25 - 30 and finally 35^{o}C envisaged faint banding pattern which ranged then 2000 to 200bp MW and 1000 to 300bp MW in *R. marginatus* and *R. fuscipes* respectively. In *R. fuscipes,* KTG - 5, KTG - 3 primer produced amplified products were homogenous with respect to all temperatures. This results demonstrate precisely KTG and KTG -3 primer in *R. marginatus* adults revealed that weak and powerful band were observed between the 10^{o}C to 35^{o}C and in OPE – 8, we recorded low molecular bp bands with uniform pattern.

This result clearly showed higher and lower temperatures caused changes IN the DNA pattern and level both in terms of qualitatively and quantitatively. The success of PCR depends on the quality of the DNA, must be free from any contaminants and from protein, nuclease that interfere the amplification process. Greenstone *et al.,* 2005, King *et al*., 2008.

PCR based protocol shows great power for quick and simple characterisation of genetic variation within and among the population (Whittman, 2005). PCR techniques has been successfully used in studies on DNA of reduviids, *T. cruzii* (Breniere *et al.*, 1992; Carezza Botto *et al.,* 2005) and preliminary studies on the selection and activity cycles on heteroptera reduviidae (Canals *et al.,* 1997; Moser *et al.,* 1989; Russomodo *et al.,* 1992). Qualification and Quantification of DNA under such circumstance was becomes necessary to amplify the DNA

polymerase chain reaction and then quantify the PCR product. The logic being that the amount of product would be measured and the amount as well as purity of the extracted initial DNA. Due to extreme sensitivity of the PCR reaction, however even very small variation in the reaction efficiency would result in significant differences in the amount of final product formed. RAPD analysis allowed grouping the insects in accordance to the place of capture in contrast yo the molecular analysis. It is possible that this grouping could be due to the phenotypic plasticity, the expression of different phenotypes in single genotype when subjected to different environments (Whitman, 2005)

These result indicated that high quality DNA can be isolated from both of the predators reared under range of temperatures. In addition, successful PCR is possible when the amount of DNA specifically with temperature produced amplification product was widely varied. This demonstrates that quantity of DNA was a initial process for amplification of isolated DNA molecules. The study also had been addressed the suitability of reagents used to stored the experimental adult predators for subsequent DNA isolate in Deep freeze (-20^{o}C). Storage conditions are apparently not critical for experimental samples stored less than 6 months.

Temperature mainly determined biologically and biochemical functional activities including macromolecules of DNA and RNA content (Dodd, 2004; Carreza Booto *et al.,* 2005). Result also indicate that when we analysed the trees, which generated from both predators with 3 various primers, KTG- 3, KTG-5 and OPE-8 denoted that the tree constructed using the KTG-5, KTG-5 were shown variable than OPE-8 and in *R. marginatus.* In another way in *R. fuscipes* predator,

KTG - 5 and OPE 8 showed similar variation but they did not observed more variation between 10^{o}C to 35^{o}C.

From this study we understood that when the temperature reached at 35^{o}C or more or less than 10^{o}C amplified DNA of the predators were less intense compared to optimum normal temperatures banding profile. Notably this similar trend was also observed by Garnoel and Barett, 1993. Some individual of the heat treated group of insects exhibited a faint band, indicating temperature fluctuation its also caused or accompany with presence of small amount of the bacterium present on the alimentary canal of an insects previously reported by Tsuchida *et al.*, (2006).

VI. 6. Conclusion

We concluded that PCR is an excellent tool that can be applied to identity genetic polymorphism as well as change the genetic constituents depends upon the temperature variation within and among the same individual of this predators. PCR employed here is a method, which has the large applicability of RAPD but also can generate differences the banding pattern that are more information for population analysis. More ever, this result indicates prolonged temperature (highest above 35^{o}C) stores could be reduce or denature the protein molecules. In RAPD banding profiles of difference temperature (10-35^{o}C) differ from each other in terms of both the numbers as well as size of the amplified fragments with size and MW ranged between 2500 and 100dp and 1500 bp to 4300 bp in *R. marginatus* and *R. fuscipes* respectively.

Chapter VII. INDIRECT ELISA

VII.1. Introduction

Use of ELISA in nutritional studies was suggested by Hagler (1998). This enables the rapid screening of predators to obtained accurate data on gut content (reviewed by Van Weeman and Schuurs, 1971a, b; Sundarland, 1988; Greenstone, 1996). The most important factors which considered in the development of antibody-based assay was the level of sensitivity and specificity achieved (Sheppard and Harwood, 2005). Laboratory optimisation is necessary to quantify the rates of antigen decay, the effects of temperature on decay rates, the consequence of alternative prey consumption on detection periods and difference is detection limits between the predators. All these factors can influence the detection of prey material (Sunderland, 1996).

Furthermore, the mass collection of arthropods forgut content analysis can yield false-positive data due to surface level contamination with target prey or increased interaction between the predators and prey due to inappropriate sampling protocols (Harwood and Obryckii, 2005a and 2005b). The simplicity of screening protocols associated with antigen-antibody based assays has been allowed large-scale field analyses of predators prey interactions (eg. Harwood *et al*., 2004; Hagler and Naranjo, 2005). This technique of using pest specific antibodies was pioneered in the early 1998 (e.g. Hagler *at al.*, 1992; Hagler and Naranjo, 1994 a, b). In the largest gut-content study reported (Hagler and Naranjo, 2005) assayed predators by indirect ELISA. Most investigators employing gut content

immunoassay have used whole body homogenates for their assays (Fitcher and Stephen, 1984; Hagler *et al.,* 1992; Hagler and Naranjo, 1997). Microscopic gut content analyses are easy and affordable, but they are ineffective for most predators because the prey was liquefied (or) chewed into tiny unrecognizable pieces (Miles, 1972).

Visual identification of gut content revealed the feeding preferences of coleopteran predators (Forbes, 1983) of coccinellid feeding on pollen or aphids. Further more the additional investigation had been developed an indirect enzyme – linked immunosorbent assay (ELISA) which employs a species and antibody specific for examining predators of the *Pectinophora gossypiella* (Saunders) on the pinkbooworm eggs (Hagler *et al.,* 1994; Hagler and Naranjo, 1997). Valuable information can be gathered and gut dissection has enable the identification of prey remains from museum specimen. Though reduviids are good biological control agents (Schaefer, 1988; Ambrose, 1999; Sahayaraj, 2007a), till now no information was available about the usage of gut content analyses using ELISA. Hence, this study was proposed to examine the effect of total protein content on the efficacy of *R. marginatus* and *R. fuscipes* maintained with six temperatures and fed with three chosen pests using indirect ELISA.

VII.2. Materials and Methods

20 to 25 adults of *R marginatus* and *R. fuscipes* (> 15 days old) were maintained separately in environmental chambers at 10, 15, 20, 25, 30 and 35^{o}C on *C. cephalonica*, *S. litura* and *D. cingulatus* separately. Predators were removed from the environmental chambers after 15 days and immediately frozen at - 20^{o}C.

Each predator were homogenised in 500 µl of PBS and assayed for *S. litura, C. cephalonica* and *D. cingulatus* remains in the gut and haemolymph of the predators. Antibody was determined through immunoassays performed into round - bottom wells of polystyrene microtittre plates coated with 500 ng of *S. litura, C. cephalonica* and *D. cingulatus* purified protein separately. After antigen sensitisation, the free reactive sites of the wells were blocked with 1 % BSA.

VII.2.1. Haemolymph collection

Haemolymph was collected from 6 to 10 adults of *R. marginatus* and 10 *R. fuscipes* by puncturing the Antennae region with a 0.33 mm diameter needle attached to a 1 ml syringe and withdrawing haemolymph from the antennae taking care to avoid contamination of haemolymph with body fluid. Saturated phenylthiourea (2 µl) was added to the pooled haemolymph sample to prevent coagulation. Samples were centrifuged at 12000 g for 3 hours and the supernatant used for ELISA.

VII.2.2. Preparation of Pest Antigen

In a small beaker, take 25 ml of distilled water and placed a dialysis membrane for 10 to 20 minutes and then replaced in to boiled water for 10 minutes inorder to remove impurities, unwanted proteins and any inactive enzymes. Make sure the dialysis membrane did not touch the wall of the beaker. Check the leakage of the membrane using squeeze bottle. Desired length was selected and both inner and outer sides were rinsed with distilled water. One end of the membrane was tied securely with the thread. Whole body of three pests (*S. litura, C. cephalonica* and

D. cingulatus) were homogenised with 500 µl to 1 ml of cold PBS solution then passed the crude content through the dialysis membrane (29 mm) and placed inside a beaker containing 500 ml of PBS. Open end of the membrane was closed securely by cotton thread. The membrane was then placed in 500 ml of PBS buffer solution and dialysed for over night at 4^{o}C. During this process PBS buffer was changed 3 to 4 hours in order to remove the impurities. Finally the purified protein adhered on the inner surface of the membrane was used as pest antigen.

VII.2.3. Indirect Gut content ELISA

Samples were prepared for ELISA by homogenising individual predators using 500µl phosphate buffered saline in 96-well assay plate were coated separately with a 100µl of aliquot for each antigen sample and incubated at 4^{o}C overnight. The unbound antigen was discarded from the assay plate and 300 µl of 1.0% (10.0 mg/ µl) BSA in distilled H_2O was added. Allow it for 30 minutes at room temperature to block any unoccupied protein binding sites in the wells. Wells were rinsed three times with PBS – Tween 20 (0.05%) and twice with PBS. Fifty micro litters of the pest antigen was then added separately to each well (Hagler *et al.*, 1994). Then the plates were incubated for 1hr for at room temperature then rinsed by the above said manner. Aliquots (50 µl) of anti-rabbit's lgG conjugated to alkaline phosphates diluted 1:500 in 1.05% BSA was added to each well of the plates and incubated for 1 hour. Plate contents were discarded and again plates were rinsed three times as described above. A 50 µl aliquot of substrate solution was added to each well using the regents supplied in are alkaline phosphate substrate kit (Nune, Mexisorp, UK) following the addition of 50µl of 2N H_2SO_4

Then absorbance of each well was measured using a SLR 36 ELISA strip reader (Glaxo, Mumbai) at 450nm. Each pest's antigen was considered as positive sample separately.

VII.2.4. Effect of predator total protein content in Indirect ELISA sensitivity

Predators devoid of the pest's antigen were frozen at-40^{o}C for 3 days. Separately stock solutions were prepared by homogenising 10 to 15 (*D. cingulatus*), 9 to 17 (*C. cephalonica*) and 3 to 10 (*S. litura*) with to 5 to 10 ml of PBS. 50 µl aliquot of this stock solution was equivalent to a single pest's antigen. 50 µl aliquot of stock pest antigen solution was added to each predator sample for total volume of 500 µl. Six to ten predators were homogenised in 500 µl PBS and treated as a negative controls. The total protein content of each individual was determined method (Bragdon, 1976). Then the samples were assayed for indirect ELISA as described in session VII.2.2. The mean ELISA absorbance value was recorded for each temperature treatment separately using ELISA reader (Glaxo, India).

VII.3. Result

Protein content of *Rhynocoris marginatus* in relation to three pests such as *S. litura, D. cingulatus* and *C. cephalonica* were significantly different. For instance the overall mean protein concentration was 400, 330, 400, 475 and 425 µg/insect for *R. marginatus, R. fuscipes, S. litura, D. cingulatus* and *C. cephalonica* respectively. We have not maintained any negative control. The

standardized ELISA consisted of homogenate each predator, regardless of its total protein content, in 500 µl of PBS with *S. litura, D. cingulatus* and *C. cephalonica* as pest antigen. Pest antigen was detected in every *R. marginatus* sample that was spiked with a single pest yielding a mean ELISA absorbance value (Figures 21 and 22). The *R. marginatus* samples values were increased with pest antigen was immuno reactive. We are used a standardized indirect gut and haemolymph content ELISA for all temperature reared predators, because we would like to find out the qualitative feeding behavior of these predator in relation to various pests.

We standardized the first step by coating the pest antigen predator/ 500 µl of PBS in the indirect ELISA plates. However, it reveals from the result that irrespective of the prey consumption, 500-µl dilution yielded maximum protein content both in *R. marginatus* and *R. fuscipes*. To minimise the high frequency of the ELISA false-negative reactions, first we added equivalent amount of pest antigen to *R. marginatus* and *R. fuscipes* samples that were homogenised in with 500 µl (500 µg/well) to 1500 µl PBS. Then we analysed each sample by an indirect ELISA. From the observations we understood that a single well which contain 100 µl homogenate is required for this study.

VII.3.1. Effect of predator protein content on ELISA sensitivity

Mean protein content of *R. marginatus* fed with three pests is presented in figure 21. Results revealed that protein content was gradually increased up to 20^{o}C, and then declined to 25 and 30^{o}C when *R. marginauts* was fed with *C. cephalonica* and *S. litura*. However, protein content was gradually increased up to 30^{o}C in *R. marginatus* and *R. fuscipes* fed with *D. cingulatus*. Similar trend was also

observed in *R. fuscipes* fed with *S. litura, D. cingulatus* and *C. cephalonica.* The indirect ELISA was unreliable of these detecting immune response for two heteropteran predator (Figures 21and 22).

VII.3.2. ELISA response on gut of both predators.

R. marginatus was maintained at lower temperature threshold ($\leq$ 25^{o}C) after *S. litura* feeding *R. marginauts* showed more immune response than the individual reared at 35^{o}C (Fig. 20). There was a irregular manner of positive response recorded at all temperatures on the tested pests. But declined level was visibly found between 25 to 35^{o}C (Fig. 23). In *D. cingulatus* fed individuals more immune response was recorded between 20 to 30^{o}C, and then it was declined at 35^{o}C. However, in *R. marginatus* fed with *C. cingulatus* adults revealed that immune response was increased linearly from 10 to 30^{o}C and then the response declined at 35^{o}C. As in the case of *R. fuscipes* fed with *S. litura,* gut immune response did not have a more variation between 10 to 20^{o}C, eventhough suddenly increased up to 20 to 30^{o}C, again slowly declined towards at 35^{o}C, this kind of similar immune response observed when fed with other two pests (Fig.24). Among the three pests, *S. litura* provide more gut immune response to these two reduviid predators than *D. cingulatus* and *C. cephalonica.*

VII.3.3. ELISA response on Haemolymph

In another study, immune response was recorded using predators haemolymph. It was shown that the antigenic protein replied immunoreactive absorbant value was higher in *R. marginatus* (0.72 $\pm$ 0.02) on *S. litura* followed by

C. cephalonica (0.63 ± 0.03) and *D. cingulatus* (0.62 ± 0.03) at 30°C. Similarly in *R. fuscipes*, higher response was also noted at 30°C for all the three pests (0.75 ± 0.01, 0 .68 ± 0.02, 0.54 ± 0.02 for *S. litura, C. cephalonica* and *D. cingulatus* respectively). In all other temperatures revealed the immune response was more or less equal for all the pests of these two predators. Among the three pests, maximum response was observed on *S. litura* followed by *C. cephalonica* and *D. cingulatus* (Table 21a and 21b).

VII.4. Discussion

The results of this study suggested that selective prey consumption of reduviid predators with optimum temperature reflect that these reduviids preferred *S. litura*. Result also confirmed that haemolymph possessed more immune response which immune reactive property (or) tendency normally decay (or) disrupt at higher (< 35°C) and lower temperature (> 15°C). Results also revealed that immune response of both predators fluctuated according to the temperature regimes quoted by Hagler and Naranjo (1997). In other findings of indirect ELISA revealed that the predator gut immune response was decreased and increased based upon meal size, suitable prey consumed, temperature regimes and prey detective interval (Sunderland *et al.*, 1987; Sopp and Sunderland, 1989). Most of the studies attributed interspecies differences in the prey detection to variable metabolic rate as a function of time and temperature (Engval and Perlman, 1972 a, b; Fitcher and Stephen, 1981; Sopp *et al.*, 1992; Greenstone and Hunt, 1993; Hagler,1998). From this results it was clear that ELISA study can also be considered to known the temperature depend immune reaction of both *R. marginatus* and *R. fuscipes.* It was

also noticed that there was a rapid decline with preys like *S. litura* and *D. cingulatus.* In addition, Naranjo and Hagler (1997) recorded considerable species gut content immune response variation present on predators gut by immunoassays performance. Previous studies of Sahayaraj, (2000); Sahayaraj *et al.* (2004) showed that when *R. marginatus* was provided with *H. armigona* and *S. litura,* reduviid preferred *S. litura.* Prey preference studied also revealed that *R. marginatus* preferred mostly *S. litura* followed by *D. cingulatus* (Sahayaraj, 1994).

From the present experiment it was noted that both reduviid predators possessed haemolymph and gut content by ELISA detected immune response was adversely affected depends upon the lower as well as higher temperatures. These results are also in conformation with the report of Sopp and Sunderland (1989). This indirect ELISA study clearly indicated that between the two predators, reduviid *R. fuscipes* (145 mg) (0.072) gut content revealed more immune response than the larger predator of *R. marginatus* (310 mg) (0.068). A similar result was also recorded by Hagler and Naranjo (1996). Stuart *et al.,* (1990) reported that optimal concentrations of reagent were determined through sequential check board of primary antibodies followed by primary antibody versus standard antigen dilutions, its leads to standard curves generated in an indirect ELISA. The results revealed that the marked differences of pest specific with higher immune response explained mainly depends upon the given temperature regimes. For instance the response was in favour at 25^{o}C for *S. litura,* 30^{o}C for *C. cephalonica* and at 35^{o}C for *D. cingulatus* which concordant observation was made by Ma *et al.*, 1984. Because, the prey consumed by less protein contain small predators have a greater

chance for attached or adhered to the ELISA microplate matrix than the prey consumed by large protein - rich predators. The total protein concentration present in the ELISA samples should not exceed 125 µg / samples to minimize the probability of ELISA false negative reaction. This relationship suggests that there is a rapid initial decay of ELISA sensitivity occurs as protein content increases (Sundarland *et al.,* 1978). In effect, the extraneous, non-target proteins associated with large predators "block" the targeted prey proteins from binding on to the ELISA matrix (Pickel, 1981). The net result is a higher frequency of false-negative reactions with large predators (Ma *et al.,* 1984).

In summary, factors such as variable predator digestive rates (Symondson and Liddel, 1993), prey sizes (Sopp and Sunderland, 1989; Symondson and Liddell, 1996), temperature (Hagler and Narganjo 1999a, b), predator metabolic status and developmental stage of the prey (Hagler *et al.,* 1992) can all effect the quantitative out come of immunoassays (Sunderland 1996). However very few investigations have considered the total protein content present in the samples as an important variable which affecting the qualitative and quantitative outcome of indirect immunoassays.

VII.5. Conclusion

R. marginatus and *R. fuscipes* subjected to various constant temperature and fed with *C. cephalonica*, *S. litura* and *D. cingulatus.* Of these 3 pests, *S. litura* fed individuals were more immuno reactive at both 25 (0.61) and 30^{o}C (0.63) in *R. marginatus* and *R. fuscipes* (0.69 and 0.71 at for 25 and 30^{o}C) than *C. cephalonica* and *D. cingulatus*. The results showed that remaining temperature

had an immune response positive but lower than that of 25^{o}C, 35^{o}C. It was also concluded that *R. fuscipes* haemolymph was more immunoresponse than *R. marginatus.*

Table 21. Various temperatures (^{o}C) ELISA versus qualitative haemolymph analysis *R. marginatus* (a) and *R. fuscipes* (b) with *Corcyra cephalonica* (Cc) *Dysdercus cingulatus,* (Dc) and *Spodoptera litura* (Sl).

(a)

Pests	10	15	20	25	30	35	RT
C. cephalonica	0.43	0.45	0.50	0.66	0.63	0.43	0.61
D. cingulatus,	0.41	0.44	0.50	0.63	0.62	0.42	0.62
S. litura	0.46	0.52	0.52	0.69	0.72	0.49	0.72

(b)

Pests	10	15	20	25	30	35	RT
C. cephalonica	0.42	0.42	0.44	0.68	0.68	0.56	0.68
D. cingulatus,	0.40	0.41	0.42	0.57	0.54	0.48	0.55
S. litura	0.44	0.55	0.59	0.73	0.75	0.55	0.75

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SUMMARY

1. Laboratory study was undertaken to investigate the biology, bio-efficacy, bacterial population, macromolecules and antibody profiles of two reduviid predator under constant and fluctuating temperature regimes. From this study we understood that nymphal development, survival and reproductive performances were optimum between the temperatures of > 24.31 and < 32.31 for *Rhynocoris marginatus* and >25.41 and <37.28 for *Rhynocoris fuscipes*.

2. Predatory rate, approaching time, consumption time, weight gain were maximum in 25 and 30^{o}C than other temperatures in both reduviid predators. Meanwhile predatory rate was also higher at higher temperature and also according with the age of the nymphal instars of both predators. However, in *R. fuscipes* adult, the predatory rate was lower when compared with fifth instars nymphs.

3. Predators gut enzyme studies revealed that amylase, protease, invertase and esterase activities were higher at 35^{o}C. Moreover, enzyme activities were temperature dependent one in both predators.

4. 11 and 13 bacterial species were recorded from *R. marginatus* and *R. fuscipes* gut respectively. Total heterotrophic bacterial population (THBP) was higher in *R. fuscipes*. Both lower and higher temperatures alter the

bacterial species and their population level. Although a *Lactobacillus delbruckii* and *Lactobacillus casei* were present only in *R. fuscipes*.

5. In both reduviids macromolecular (crude protein, carbohydrate and lipids) profiles of eggs and whole body of these reduviids varied according to lower as well as higher temperatures. SDS-PAGE analyses for gut protein of *R. marginatus* showed unique polypeptide at 10^{o}C (116.0 kDa), 20^{o}C (405 kDa) and 35^{o}C (590.0 kDa). Whereas *R. fuscipes* produced a specific polypeptide at 35^{o}C (66.0 kDa). Similarly, PCR amplified DNA products produced better amplification with KTG-3 and KTG – 5 in *R. marginatus* and OPE – 8 and KTG-5 in *R. fuscipes*.

6. ELISA studies reveal that between these two predators *R. fuscipes* was more reactive than *R. marginatus*. However, the reduviids were highly immunogenic both at 25 and 30^{0}C when they were fed with *S. litura* followed by *C. cephalonica* and *D. cingulatus.* Between gut and haemolymph, haemolymph possessed were immune response than gut of both predator.

Table 13. Bio-efficacy of *R. marginatus* life stages on *D. cingulatus* at different constant temperatures (^{O}C)**

Temp. (^{o}C)	Pest stage	Initial weight	Approaching time	Handling time	Weight gain
Third instar					
10	II	46.11 ± 1.66*	6.22 ± 2.38*	0.44±0.17*	3.23±0.78*
15	II	44.85 ± 1.12*	5.85 ± 3.26*	0.51±0.51*	4.2±0.78
20	II	44.0 ± 2.00*	4.60 ±1.09*	0.56±0.23*	5.85±0.45*
25	II	35.8 ± 1.03*	2.04 ± 0.24*	2.27±0.27*	12.3±1.46*
30	II	33.13 ± 2.0*	.035 ± 0.41*	2.41±0.46*	15.56±1.11*
35	II	27.25 ± 2.14	0.14 ± 0.03*	1.22±0.17*	7.41±0.55*
RT	II	34.03 ± 3.42	0.48 ± 0.10	2.17±0.62	11.19±0.29
Fourth instar					
10	IV	76.1 ± 5.83*	5.61 ± 3.28*	1.28 ± 0.37	3.06 ± 0.18*
15	IV	73.9 ± 3.78*	5.23 ± 0.52*	1.40 ± 0.11*	5.51 ± 0.46*
20	IV	79.7 ± 1.49*	4.57 ± 0.44	1.52 ± 0.29	5.64 ±1.53*
25	IV	67.7 ± 1.78*	1.15 ± 0.25*	1.57 ± 0.52*	17.71 ± 3.66
30	I V	65.0 ± 1.41*	0.37 ± 0.12*	2.30 ± 0.16	15.28 ± 3.69
35	IV	47.22± 3.08*	0.10 ± 0.06	1.20 ± 0.39*	9.59 ±1.13*
RT	IV	63.11 ± 4.62*	0.28 ± 0.04	2.09 ± 0.05*	14.90±2.06*
Fifth instar					
10	V	96.26±13.49*	6.11±1.00	0.47±0.07	.94±0.21*
15	V	93.16±12.77*	5.07±0.48*	.56±0.23*	4.04±0.86
20	V	91.70±14.02*	4.19±0.31*	1.10±0.16	5.90±0.35*
25	V	83.0±4.06*	2.20±0.5*	2.00±0.50	13.41±2.08*
30	V	82.4±6.10*	0.43±0.26	2.41±0.56	16.05±0.87*
35	V	73.85±6.13*	1.08±0.41	1.08±0.03*	11.29±2.60
RT	V	83.5±7.15*	0.39±0.05	2.25±0.13	15.3±2.61
Adult					
10	V	163.33±21.10*	7.28±1.033	0.30±0.25	1.22±0.08*
15	V	158.57±16.13*	7.16±0.24	0.44±0.17	3.56±1.60*
20	V	152.9±6.64*	5.21±0.62*	1.17±0.52	12.75±0.78*
25	V	150.7±1.70	2.07±0.42*	2.55±0.36	19.41±2.08*
30	V	147.2±3.27*	0.52±0.55	3.10±0.12	19.93±0.45*
35	V	123.61±20.04*	0.11±0.03	2.45±0.98*	7.64±0.21*
RT	V	147.0±2.19*	0.33±0.02*	2.43±0.24*	21.6±2.61

*Significance at 5% level

Table 14. Bio-efficacy of *R. marginatus* life stages on *S. litura* at different constant temperatures (^{O}C)**

Temp. (^{o}C)	Pest stage	Initial weight	Approching time	Handling time	Average weight gain
Third instar					
10	II	38.70±2.60*	4.05±1.03	0.54±0.23*	4.69±1.51*
15	II	37.11±3.06*	2.29±0.18	1.08±0.50*	9.30±1.22*
20	II	35.26±7.58*	2.52±0.22	1.34±0.56*	9.74±2.02*
25	II	27.09±2.84*	1.19±0.74	2.53±0.46	22.01±4.11*
30	II	27.25±1.09*	0.27±0.14*	2.86±1.49	22.31±2.59*
35	II	22.18±2.25*	0.12±0.04*	2.15±0.09*	16.92±2.96*
RT	II	29.27±2.66	0.40±0.17*	2.36±0.55*	25.75±1.02*
Fourth instar					
10	III	64.70±2.72*	4.28±2.61	1.23±2.70*	5.40±0.13*
15	III	62.06±0.76*	3.15±0.69	1.81±0.51*	11.72±2.06*
20	III	59.33±1.64*	2.10±0.37	2.29±0.71*	21.05±2.16*
25	III	52.64±3.13*	1.25±0.51	2.55±2.07*	28.92±2.09
30	III	49.28±7.81*	0.43±0.08*	2.25±0.48*	29.22±2.55
35	III	44.71±3.68*	0.08±0.04*	2.44±0.55*	19.08±2.16*
RT	III	54.11±1.15*	0.46±0.82	3.14±0.42*	26.16±2.10*
Fifith instar					
10	IV	96.42±2.36*	7.28±2.14	0.21±0.07	04.5±2.10*
15	IV	96.25±3.93*	6.46±0.16	0.40±0.06	7.13±0.76*
20	IV	90.11±5.16*	5.85±1.14	1.12±0.30	19.97±4.46*
25	IV	84.62±6.09*	2.46±0.95	2.53±0.62	34.38±5.29*
30	IV	79.07±10.22*	0.43±0.21*	3.20±1.08	36.19±5.08*
35	IV	62.56±7.95*	0.08±0.06*	2.17±0.54	36.55±11.27*
RT	IV	82.01±7.70*	0.40±0.24	2.56±0.55	26.91±8.16
Adult					
10	V	238.20±4.16*	5.56±0.10	1.22±0.14*	6.70±2.46*
15	V	229.05±2.49*	5.41±0.16	1.31±0.18*	8.16±0.11*
20	V	164.01±2.21*	4.18±1.20	2.44±0.93*	20.32±0.17*
25	V	159.22±2.58*	1.07±0.05*	3.43±1.87	34.4±2.97
30	V	136.36±2.31	0.50±0.44*	3.51±0.55*	37.7±2.63*
35	V	130.60±2.37*	0.21±0.02*	2.27±0.03*	18.32±6.12*
T	V	134.09±8.21	0.46±0.07	2.28±1.01	36.00±3.60

*Significance at 5% level

Table 15. Bioefficacy of *R. fuscipes* life stages on *S.litura* at different constant temperatures (^{O}C**)**

Temp. (^{o}C)	Pest stage	Initial weight	Approaching time	Handling time	weight gain
Third instar					
10	II	13.23±2.1*	5.45±1.03*	0.53±0.28	4.48±0.82*
15	II	18.02±1.01*	5.40±1.68*	1.12±0.19	5.54±1.15*
20	II	15.61±2.34*	4.22±1.77*	1.40±0.15	10.5±0.86*
25	II	15.58±2.09*	1.06±0.43	2.14±0.8	10.49±2.07*
30	II	12.60±2.14*	0.53±0.10*	2.28±0.49	15.83±3.06*
35	II	8.06±1.22*	0.24±0.08*	1.27±0.30	8.37±1.57*
RT	II	12.36±0.26*	0.40±0.26*	2.10±0.29	15.2±3.11*
Fourth instar					
10	III	38.51±6.01*	5.03±1.18	0.50±0.12*	2.31±0.70*
15	III	38.11±3.7*	5.12±2.44	1.20±0.55	4.01±0.23*
20	III	35.48±2.73*	3.66±1.26*	1.55±0.32*	7.99±0.83*
25	III	33.25±2.90*	1.07±0.33*	2.11±1.68*	13.56±0.48*
30	III	31.28±1.56*	1.06±0.24*	2.36±0.40*	13.25±1.30*
35	III	25.20±2.21*	1.26±0.21*	1.50±0.90*	11.26±0.76
RT	III	30.16±5.20*	0.43±0.22*	2.27±0.51*	11.70±0.23*
Fifth instar					
10	IV	43.11±2.95*	6.47±0.71	0.52±0.39	4.60±1.06*
15	IV	43.00±1.98*	5.36±0.24	1.22±0.52*	6.82±1.34*
20	IV	41.44±1.96*	4.02±1.34	1.50±0.16*	8.17±2.78*
25	IV	38.00±2.01*	1.34±0.26	1.55±0.22*	12.28±2.51*
30	IV	37.51±0.62*	0.42±0.31	2.07±0.18*	11.66±3.27*
35	IV	30.28±2.44*	0.14±0.09	1.26±0.18*	9.36±2.05*
RT	IV	36.40±2.63*	0.30±0.80	2.49±0.40*	12.25±5.28*
Adult					
10	IV	65.30±2.1*	6.13±2.72*	0.45±0.06*	2.80±0.34*
15	IV	63.01±2.89*	5.56±1.74*	0.51±0.39*	5.33±0.35*
20	IV	60.70±1.33*	4.36±1.92	1.44±0.03*	15.71±0.45
25	IV	52.40±2.21*	2.10±0.39	2.36±0.55	15.43±4.01
30	IV	52.00±2.19*	1.38±0.14	2.36±0.78	13.51±2.07*
35	IV	44.15±2.83*	0.18±0.03	2.56±0.77	8.90±3.18*
RT	IV	50.18±3.56*	1.20±0.07	1.39±0.51	16.10±2.11*

*Significance at 5% level

Table 16. Bioefficacy of *R. fuscipes* life stages **on *D. cingulatus*** at different constant temperatures (^{O}C**)**

Temp. (^{o}C)	Pest stage	Initial weight	Approaching time	Handling time	weight gain
Third instar					
10	II	18.66±2.54*	5.43±1.14	0.31±0.14	2.43±0.40*
15	II	17.39±3.81*	5.20±2.28	1.56±0.23*	6.31±1.03*
20	II	16.02±1.38*	4.30±0.05*	2.04±0.11*	6.48±2.05*
25	II	13.74±.040*	1.14±0.50*	2.33±0.62*	10.43±2.47*
30	II	12.26±2.16*	1.28±0.06*	2.11±0.34*	10.86±2.08*
35	II	7.24±1.85*	0.23±0.07*	1.00±0.28*	8.02±1.05
RT	II	13.02±2.21*	2.38±0.10	1.46±0.27	10.07±2.09*
Fourth instar					
10	III	34.10±0.20	6.34±1.14	0.28±3.29*	1.26±2.25*
15	III	33.20±5.21	5.57±0.29	0.41±1.09*	2.87±2.09*
20	III	30.60±2.57	2.58±0.27	0.20±1.63*	6.82±2.40*
25	III	27.56±0.55	2.10±0.27*	1.63±0.19*	8.91±2.23*
30	III	26.11±0.60*	1.25±0.25*	1.86±0.18*	13.29±2.11*
35	III	22.01±0.72*	0.12±0.14*	0.52±0.07*	6.75±2.43*
RT	III	27.02±0.34*	0.36±0.31*	2.32±0.01*	10.96±2.70*
Fifth instar					
10	IV	65.28 ± 5.79*	6.86 ± 0.21	0.54 ± 0.30*	3.02 ± 0.17*
15	IV	63.10 ± 4.03*	6.17 ± 0.20	1.13 ± 0.09*	5.3 ± 0.78*
20	IV	59.70 ± 0.84*	5.30 ± 0.29	1.37 ± 0.39*	4.85 ± 0.20*
25	IV	52.29 ± 6.28*	3.58 ± 0.53	2.08 ± 0.32*	6.57 ±1.32*
30	IV	46.13 ± 2.66*	0.43 ± 0.27*	2.06 ± 0.14*	12.48 ± 2.44
35	IV	50.8 ± 2.84*	0.21 ± 0.21*	0.54 ± 0.11*	8.02 ±1.06*
RT	IV	50.8 ± 2.60*	0.32 ± 0.08*	2.40 ± 0.02*	10.48 ± 1.40*
Adult					
10	IV	52.10 ± 0.84*	5.20 ±1.21	1.23 ± 0.39	4.06 ±1.06*
15	IV	51.08 ± 0.68*	5.17 ± 0.40	1.35 ± 0.25	4.38 ± 0.16*
20	IV	49.85 ± 2.45*	4.38 ± 2.26	1.49 ± 0.28	8.56 ± 0.03*
25	IV	45.25 ± 0.66	2.50 ± 2.03	2.42 ± 0.18	17.13 ±1.44*
30	IV	43.88 ± 5.87*	1.43 ± 0.29	2.55 ± 0.13	17.55 ± 0.81*
35	IV	32.00 ± 2.75*	0.44 ± 0.15	0.64 ± 0.03	7.46 ± 2.18
RT	IV	46.88 ± 0.81*	0.55 ± 0.63	2.38 ± 0.40	14.34 ± 2.05*

*Significance at 5% level

Table 19. Data of dendrogram analysis with 3 primers (KTG – 3, KTG – 5 and OPE – 8 shows genetic similarity (GS) on *R. marginatus* at various temperatures (^{o}C)

Temperatures	KTG – 3	KTG – 5	OPE – 8
	GS	GS	GS
10	0.40	0.29	0.50
15	0.70	0.57	0.50
20	0.80	0.29	0.50
25	0.40	0.65	0.50
30	0.60	0.76	0.75
35	0.60	0.57	0.75
RT	0.84	0.66	0.84

Table 20 . Data of dendrogram analysis with 3 primers (KTG – 3, KTG – 5 and OPE – 8) shows genetic similarity (GS) on *R. fuscipes* at various temperatures (^{o}C)

Temperatures	KTG – 3	KTG – 5	OPE – 8
	GS	GS	GS
10	0.67	0.40	0.75
15	0.85	0.70	0.75
20	0.90	0.80	0.87
25	0.93	0.40	0.75
30	0.93	0.60	0.87
35	0.94	0.60	0.75
RT	0.95	0.89	0.92

Table 18. ***R. fuscipes*** **adults gut bacterial population (in %) in related to temperatures (°C)**

Bacteria	Temperature (°C)							
	10	**15**	**20**	**25**	**30**	**35**	**RT**	**Mean**
Bacillus subtilis	-	44.15	23.17	-	-	23.07	-	30.1
B. cereus	41.20	10.21	17.30	16.05	-	-	-	21.2
B. megaterium	-	-	19.26	27.14	-	-	-	23.20
Corynbacterium kutcheri	-	-	-	4.39		-	-	4.39
C. xerosis	-	-	10.33	8.16	9.10	5.13	9.10	8.8
K. pneumoin	-	-	3.21	-	-	-	-	3.21
Lactobacillus dellbruckii	29.0	-	-	-	0.91	-	0.91	10.8
L. casei	-	11.60	-	-	-	-	-	11.60
Pseudomonas aeroginosa	-	-	-	-	0.71	2.0	0.71	1.14
Micrococcus variance	-	-	40.13	48.98	53.97	40.09	53.97	47.42
M. luteus	-	-	-	2.39	-	-	-	2.39
Enterobacter aerogenes	18.11	-	23.04	28.13	31.07	-	-	25.08
Stephylococus aureus	55.1	44.15	15.99	15.08	22.7	36.92	22.7	30.37

Table 17. *R. marginatus* adults out bacterial population in related to temperatures (^{o}C)

BACTERIA	Temperature (^{o}C)							
	10	**15**	**20**	**25**	**30**	**35**	**Room t^{o}**	**Mean**
Bacillus cereus	37.26	31.2	15.39	12.21	-	-	-	96.06
B. megaterium	-	-	10.2	-	-	-	-	10.2
B. subtilis	-	21.0	10.01	10.0	14.83	25.07	14.83	15.95
Corynbacterium kutcherii	-	-	-	9.45	-	-	-	9.45
C. xerosis	-	-	17.21	-	2.18	2.47	2.18	6.01
Micrococcus variance	-	-	25.59	32.28	52.70	40.00	52.70	40.68
Micrococcus luteus	-	-	-	3.68	-	-	-	3.68
Enterobacter qrogenes	13.72	-	11.25-	15.36-	28.36-	-	-	17.2
Escherichia coli	-	-	-	-	1.63	-	-	1.63
P. aeroginosa	-	-	-	-	0.55	6.34	0.55	2.8
Staphylococcus aureus	49.0	47.3	23.18	20.32	28.09	26.36	28.09	31.75

Table 1. Nymphal developmental period (in days) of *R. marginatus* at various temperatures (^{o}C)

Tempera -tures	I	II	III	IV	V	I-Adult
10	20.4 ± 0.8	22.6 ± 1.1	0	0	0	43.0 (I-II)
15	19.1 ± 2.2	20.5 ± 3.2	17.5 ± 2.9	0	0	57.1 (I-III)
20	19.0 ± 2.2	15.4 ± 0.5	18.2 ± 0.8	16.2 ± 2.6	18.40 ± 2.6	87.2
25	8.8 ± 0.8	8.2 ± 1.05	7.2 ±1.3	9.2 ± 0.4	16.40 ± 0.7	49.8
30	9.8 ± 0.8	8.8 ± 0.5	8.0 ± 0.7	7.3 ± 0.7	14.0 ± 0.7	48.0
35	4.8 ± 0.3	4.2 ± 1.3	0	0	0	9.4 (I-II)
RT	8.2 ± 0.7	6.8 ± 0.4	8.0 ± 0.6	7.8 ± 0.4	15.2 ± 0.8	46.0

Table 2. Nymphal developmental period (in days) of *R. fuscipes* at various temperatures (^{o}C)

Tempera-tures	I	II	III	IV	V	I-Adult
10	21.56 ± 1.7	20.8	0	0	0	42.3 (I-II)
15	19.00 ± 1.5	18.28 ± 3.4	0	0	0	54.3 (I-II)
20	16.80 ± 3.1	15.80 ± 1.9	16.20 ± 2.41	16.36 ± 2.2	16.00	82.0
25	9.20 ± 1.3	7.60 ± 1.1	7.80 ± 1.3	7.60 ± 1.1	15.53 ± 2.1	47.4
30	8.0 ± 7.7	6.80 ± .8	6.60 ± 1.1	8.80 ± 1.5	14.40 ± 1.1	44.6
35	4.20 ± .8	5.40 ± 1.7	0	0	0	9.6 (I-II)
RT	8.2 ± 0.7	6.88 ± 0.4	7.2 ± 0.7	8.0 ± 0.6	15.3 ± 0.8	45.5

Table 3. Different temperatures (^{o}C) on *R. marginatus* nymphs and eggs survival rate (in %)

Temperatures	I	II	III	VI	V	TNSR	TESR
10	64.7	52.8	18.2	0	0	18.2 (I-II)	68.2
15	87.1	62.9	38.8	0	0	38.8 (I-II)	89.3
20	92.3	87.5	47.9	36.2	34.3	34.3	96.5
25	92.5	87.91	74.6	61.4	55.8	52.3	100
30	100	89.3	72.9	67.5	61.0	58.6	100
35	72.0	47.2	29.2	20.8	0	20.8 (I-IV)	77.51
RT	97.5	92.4	87.3	81.3	77.7	88.3	100

TNSR – Total Nymphal Survival Rate, RT- Room temperature, TESR- Total Egg Survival Rate

Table 4. Different temperatures (^{o}C) on *R. fuscipes* survival rate (in %)

Temperatures	I	II	III	VI	V	TNSR	TESR
10	66.7	41.7	15.4	0	0	15.4 (1-II)	67.2
15	72.1	61.5	22.2	16.9	0	16.9 (I – IV)	76.9
20	82.1	65.2	63.9	47.8	34.4	32.0	85.2
25	93.1	92.5	74.2	62.9	51.3	49.8	98.6
30	100	84.2	78.9	67.2	54.7	50.1	100
35	61.8	47.6	16.5	0	0	16.35 (I-III)	65.70
RT	94.02	80.36	78.01	73.58	68.17	83.25	98.1

TNSR – Total Nymphal Survival Rate, RT- Room temperature, TESR- Total Egg Survival Rate

Table 5. Influence of different temperatures (^{o}C) on reproductive parameters of freshly mouled *R. marginatus* adults

Parameters	Temperatures						
	10	**15**	**20**	**25**	**30**	**35**	**RT**
Preoviposition	-	-	58.1 ± 1.9	19.6 ± 6.8	4.80 ± 4.2	4.60 ± 1.8	12.9 ± 0.9
Oviposition	-	-	27.1 ± 5.4	26.20 ± 9.4	21.53 ± 9.2	12.62 ± 5.	57.2 ± 5.0
Post- oviposition	-	-	13.7 ± 4.01	8.20 ± 3.6	8.6 ± 0.6	2.11 ± 2.3	18.1 ± 0.7
Total no. of eggs	-	-	112.0 ± 6.3	154.3 ± 8.6	175.0 ± 5.5	147.0± 5.5	119.6 ± 8.5
Maximum no of eggs / batch	-	-	66.7 ± 5.3	80.30 ± 11.2	98.0 ± 13.8	89.0± 13.7	78.5 ± 9.1
Minimum no of eggs / batch	-	-	31.7 ± 3.0	74.01 ± 18.3	77.0 ± 5.2	29.03 ± 5.2	36.7 ± 4.3
Incubation period	-	-	19.2 ± 1.8	8.1 ± 1.4	8.49 ± 1.3	5.44 ± 1.1	7.8 ± 0.5
Hatchability (in %)	-	-	9.6	97.5	94.7	74.7	95.5
Egg mortality (in %)	-	-	10.46	2.6	5.3	26.32.14	1.09
Oviposition Index	-	-	3.79	3.19	2.50	5.98	3.16

Table 6. Reproductive parameters of freshly moulted adult *R. fuscipes* at various temperatures (^{o}C)

Parameters	Temperatures ^{o}C						
	10	**15**	**20**	**25**	**30**	**35**	**RT**
Preoviposition	-	-	-	36.02 ± 7.41	21.33 ± 5.1	-	21.3 ± 4.5
Oviposition	-	-	-	22.78 ± 16.21	15.06 ± 2.79	-	20.6 ± 3.3
Post oviposition	-	-	-	11.34 ± 4.20	5.83 ± 2.26	-	4.8 ± 2.2
Total no. of eggs	-	-	-	98.36 ± 7.41	73.72 ± 3.55	-	229.7 ± 5.1
Maximum no of eggs/batch	-	-	-	61.50 ± 10.80	65.02 ± 3.09	-	221.1 ± 6.7
Minimum no of eggs/batch	-	-	-	29.07 ± 2.13	35.07 ± 1.08	-	8.03 ± 1.2
Incubation Period	-	-	22.17 ± 0.53	9.4 ± 1.47	8.20 ± 1.0	-	8.66 ± 0.7
Hatch out %	-	-	71.42	88.23	96.71	-	92.8
Egg Mortality%	-	-	28.38	11.77	3.29	-	7.2
Oviposition Index	-	-	-	2.80	2.11	-	2.83

Table 7. Reproductive parameters of *R. marginatus* maintained on various temperatures (^{o}C)

Parameters	Temperatures (^{o}C)						
	RT	**10**	**15**	**20**	**25**	**30**	**35**
Preoviposition	12.9 ± 0.9	-	-	30.28 ± 12.4	19.25 ± 2.5	19.75±6.49	-
Oviposition	57.2 ± 4.0	-	-	16.50 ± 4.7	17.00 ± 9.8	12.83 ± 3.7	-
Post oviposition	18.1 ± 0.7	-	-	23.50 ± 7.5	26.75 ± 3.2	29.5 ± 5.4	-
Oviposition index	0.50	-	-	0.51	0.65	0.44	-
Incubation period	7.58 ± 1.5	-	19.8 ± 1.2	16.03 ± 2.6	9.25 ± 12.9	8.5 ± 0.9	-
Maximum no of eggs/batch	128.3 ± 17.2	-	-	72.08 ± 5.26	114.03 ± 10.5	120.07 ± 9.6	-
Minimum no of eggs/batch	69.80 ± 14.31	-	-	18.39±11.5	32.60 ± 17.3	58.39 ± 9.09	-
Total no. of eggs	201.30	-	-	91.25 ± 1.6	146.08 ± 3.1	180.36 ± 5.11	
Hatch out %	95.5	-	-	89.40	96.09	98.23	-

RT- Room temperature

Table 8. Reproductive parameters of *R. fuscipes* maintained on various temperatures (^{o}C)

	Temperatures ^{o}C						
Parameters	**10**	**15**	**20**	**25**	**30**	**35**	**Control**
Preoviposition	-	-	-	6.80 ± 3.4	7.60 ± 1.51	-	4.83 ± 2.26
Oviposition	-	-	-	11.75 ± 4.4	13.05 ± 2.2	-	20.66 ± 3.55
Postoviposition	-	-	-	18.20 ± 5.3	6.50 ± 1.9	-	21.48 ± 4.52
Maximum no. of Eggs / batch	-	-	-	68.26 ± 17.7	75.8 ± 14.5	-	79. 71 ± 5.27
Minimum no. eggs / batch	-	-	-	29.16	43.52	-	47.16
Total no. of egg / batch	-	-	-	92.52 ± 2.84	118.11+2.73	-	120.89 ± 1.31
Oviposition index	-	-	-	0.65	0.36	-	0.90
Incubation period	-	-	20.15	9.20	8.11	4.50	7.44
Hatching percentage (in %)	-	-	-	98.20	98.81	-	99.61

Table 9 (a). Linear thermal unit models for lower threshold temperatures (T_o) (^oC) and mean thermal unit recruitment (K) for development of by nymphal instars of *R. marginauts*

Life Stages	Regression equation	r^2	To	K : DD
Egg	Y = 0.0267 t – 0.2591	0 . 0182	19.51	52.4
I instar	Y = 0.0422 t – 0.5610	0.0212	21.16	65.6
II instar	Y = 0.0394 t – 0. 5270	0.4813	16.92	73.6
III instar	Y = 0.0433 t – 0. 3114	0.2575	12.09	60.4
IV instar	Y = 0.860 t – 0. 3526	0.5250	18.05	43.51
V instar	Y = 0.0229 t – 0.4611	0.8103	18.40	41.4
I – Adult	Y = 0.0058 t – 0.0691	0.8514	24.31	284.5
Total	Y = 0.0031 t – 0.0590	0.140	24.8	336.0

Y = reciprocal of mean developmental times; r^2 = coefficient of correlation; Degree days (DD) requirement to complete instar.

Table 9 (b). Linear thermal unit models for lower threshold temperatures (T_o) (^oC) and mean thermal unit recruitment (K) for development of by nymphal instars of *R. fuscipes*

Life Stages	Regression equation	r^2	To	K : DD
Egg	Y = 0.0145 t – 0.1462	0.2820	20.53	67.55
I instar	Y = 0.0327 t – 0.3230	0.1723	19.41	64.71
II instar	Y = 0.0512 t – 0.6410	0.4531	22.48	72.60
III instar	Y = 0.0659 t – 0.6337	0.8472	15.58	63.1
IV instar	Y = 0.0996 t – 0. 3253	0.528	14.63	54.0
V instar	Y = 0.02425 t– 0.1253	0.635	12.07	52.96
I – Adult	Y = 0.0511 t – 0. 0528	0.570	25.01	289.67
Total	Y = 0.0062 t – 0.0674	0.756	23.92	362.76

Table : 10 (a): Linear thermal unit models for higher threshold temperatures (T_o) (^oC) and mean thermal unit recruitment (K) for development of by nymphal instars of *R. marginauts*

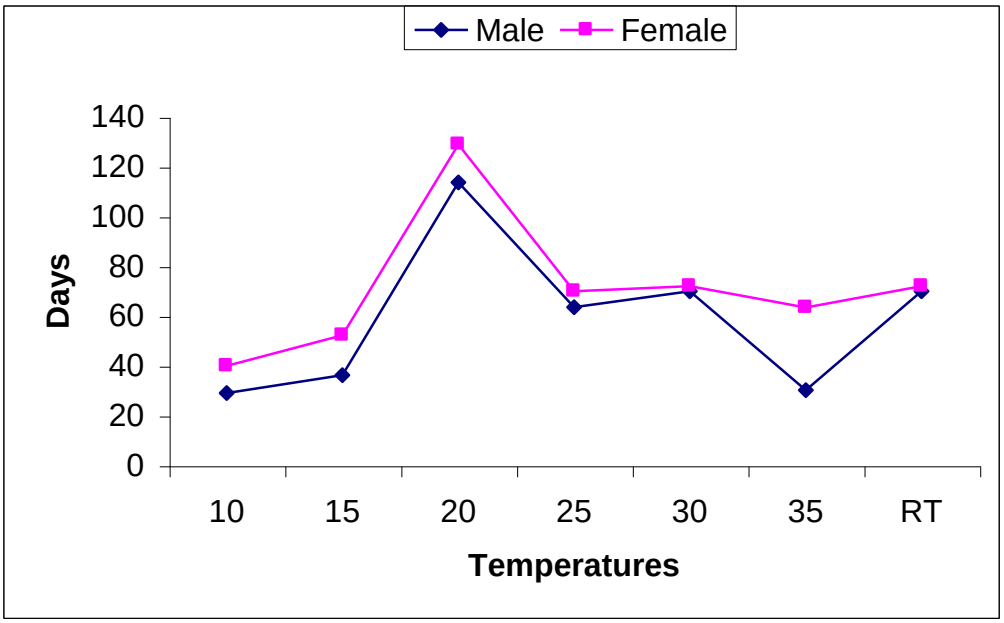
Life Stages	Regression equation	r^2	To	K : DD
Egg stage	Y = 0.0267 t– 0.2591	0.830	24.5	52.45
I instar	Y = 0.0622 t– 0. 5610	0.671	23.16	65.6
II instar	Y = 0.0394 t – 0.527	0.655	35.92	73.6
III instar	Y = 0.0433 t – 0.3114	0.497	33.09	73.6
IV instar	Y = 0.0433 t – 0. 3526	0.572	31.05	60.4
V instar	Y = 0.0229 t – 0.462	0.568	35.27	41.4
I – Adult	Y = 0.0053 t – 0.690	0.842	32.31	284.51
Total	0.0031 t – 0.0590	0.788	24.8	358.07

Table 10 (b) : Linear thermal unit models for higher threshold temperatures (T_o) (^oC) and mean thermal unit recruitment (K) for development of by nymphal instars of *R. fuscipes*

Life Stages	Regression equation	r^2	To	K : DD
Egg	Y = 0.0145 t – 0. 1462	0.678	30.11	67.50
I instar	Y = 0.0327 t – 0. 3280	0.539	20.10	64.71
II instar	Y = 0.0512 t – 0.6410	0.611	21.49	71.60
III instar	Y = 0.0659 t – 0.6337	0.742	25.7	63
IV instar	Y = 0.0996 t – 0.6517	0.832	31.63	44.0
V instar	Y = 0.071 t – 0. 8315	0.851	30.38	44.26
I – Adult	Y = 0.0511 t – 0. 0528	0.721	34.28	284.67
Total	0.0062 x – 0. 0678	0.764	25.3	316.03

Figure 1. Adult longevity of freshly moulted *R. marignatus* (a), *R. fuscipes* (b) adults maintained at various temperatures (^{o}C)

(a)



(b)

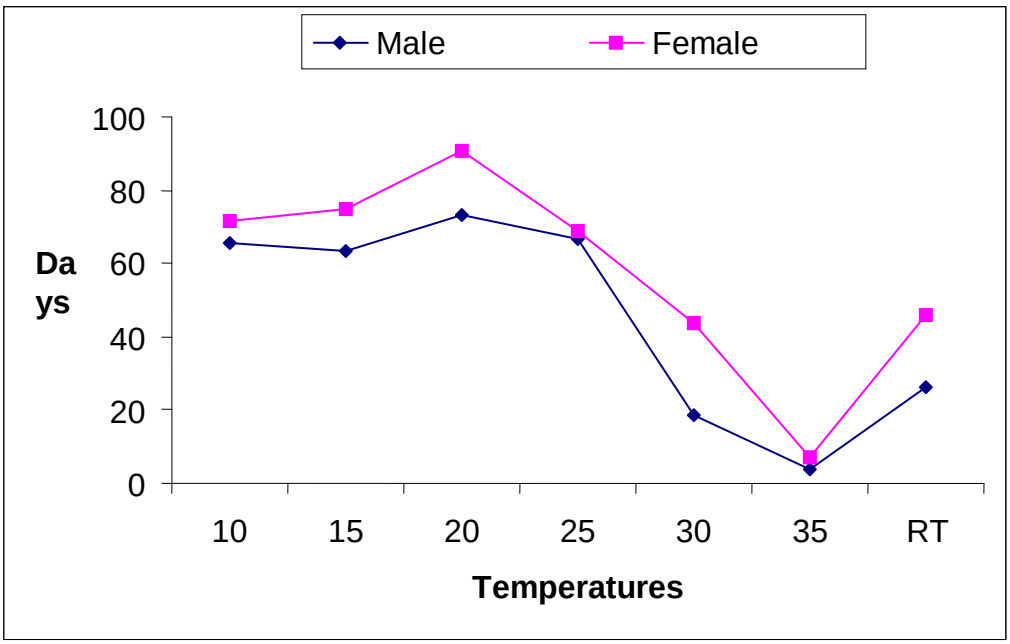
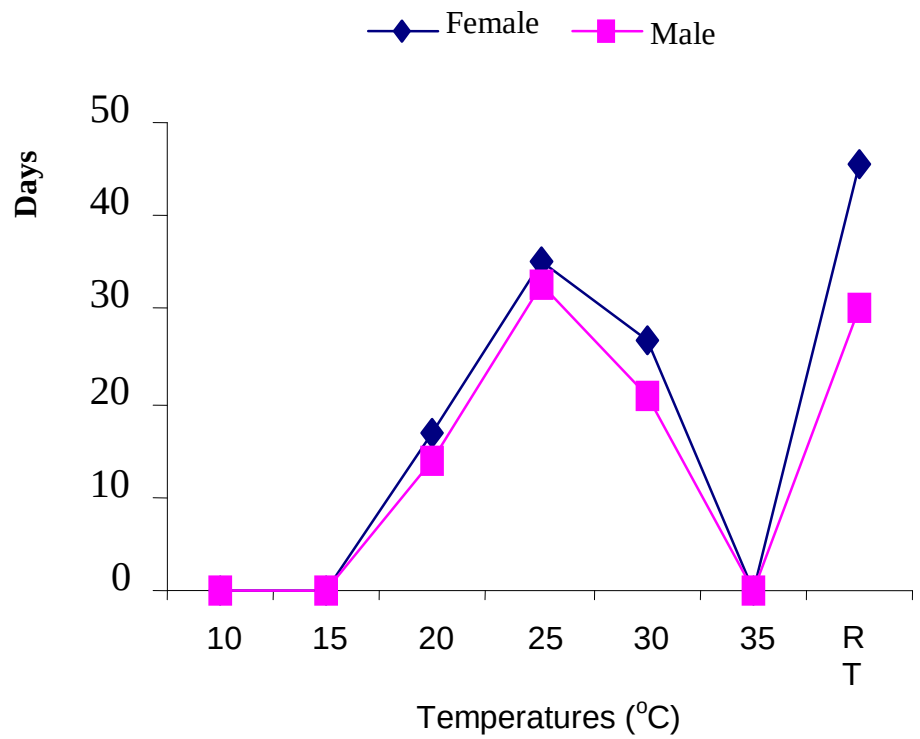


Figure 2. **Impact of various temperatures (°C) on the adult longevity (in days) of *R. marginatus* (a), *R. fuscipes (b)* male and female**

(a)



(b)

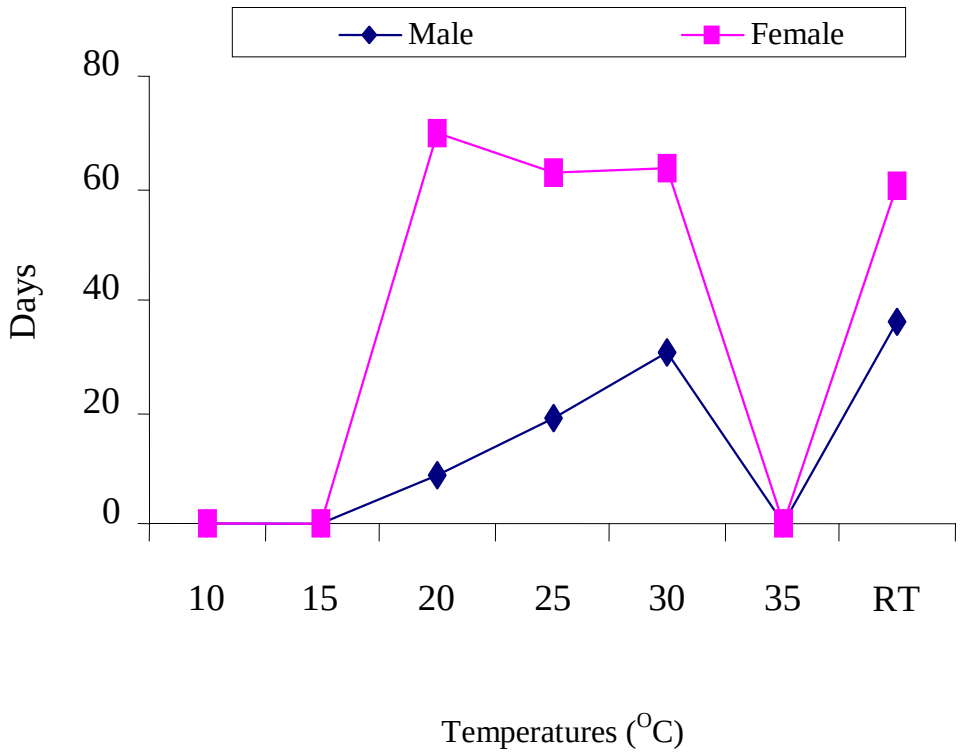


Figure 3. **Impact of various temperatures (^{o}C) on the body weight loss (-) and gain (+)) (in mg.) of *R. marginatus* nymphs and adults**

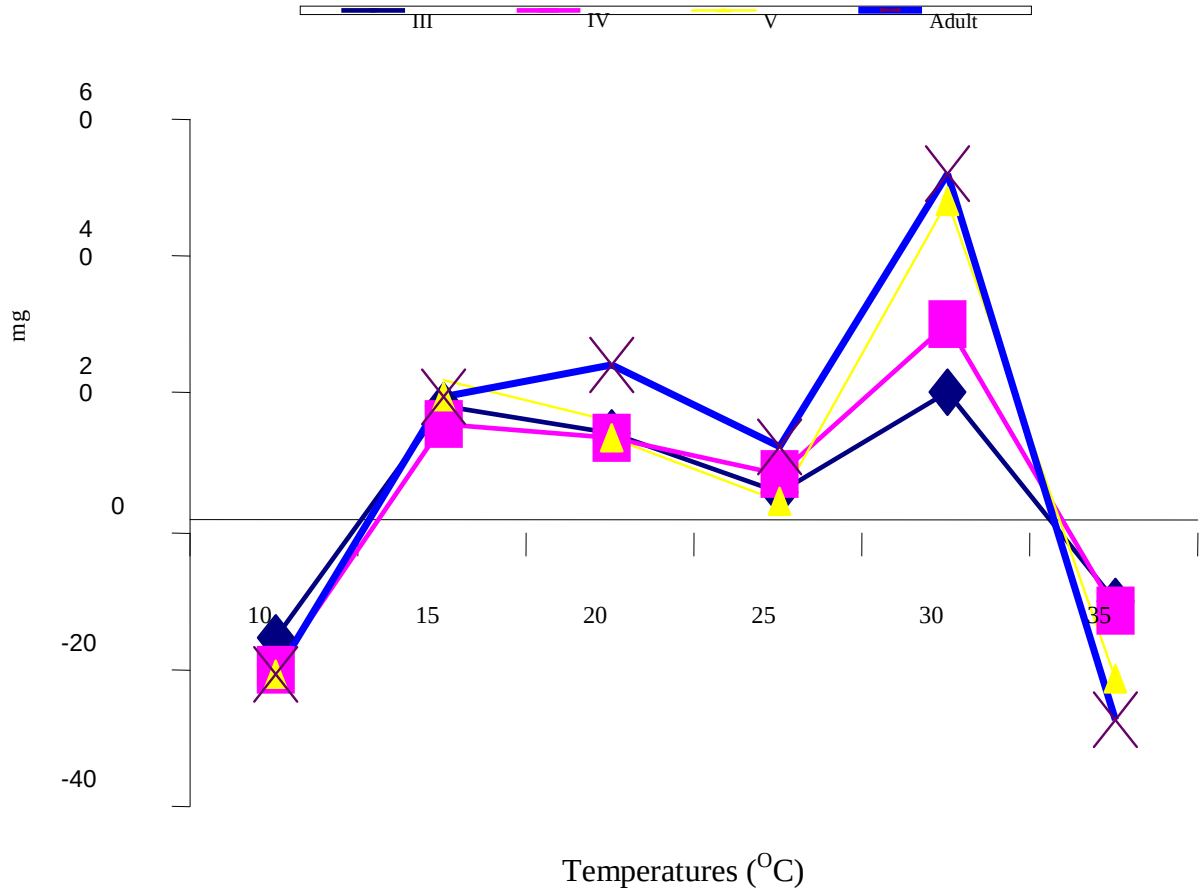


Figure 4. **Impact of various temperature (oC) on the body weight loss (-) and gain (+) (in mg.) of *R. fuscipes* nymph and adults**

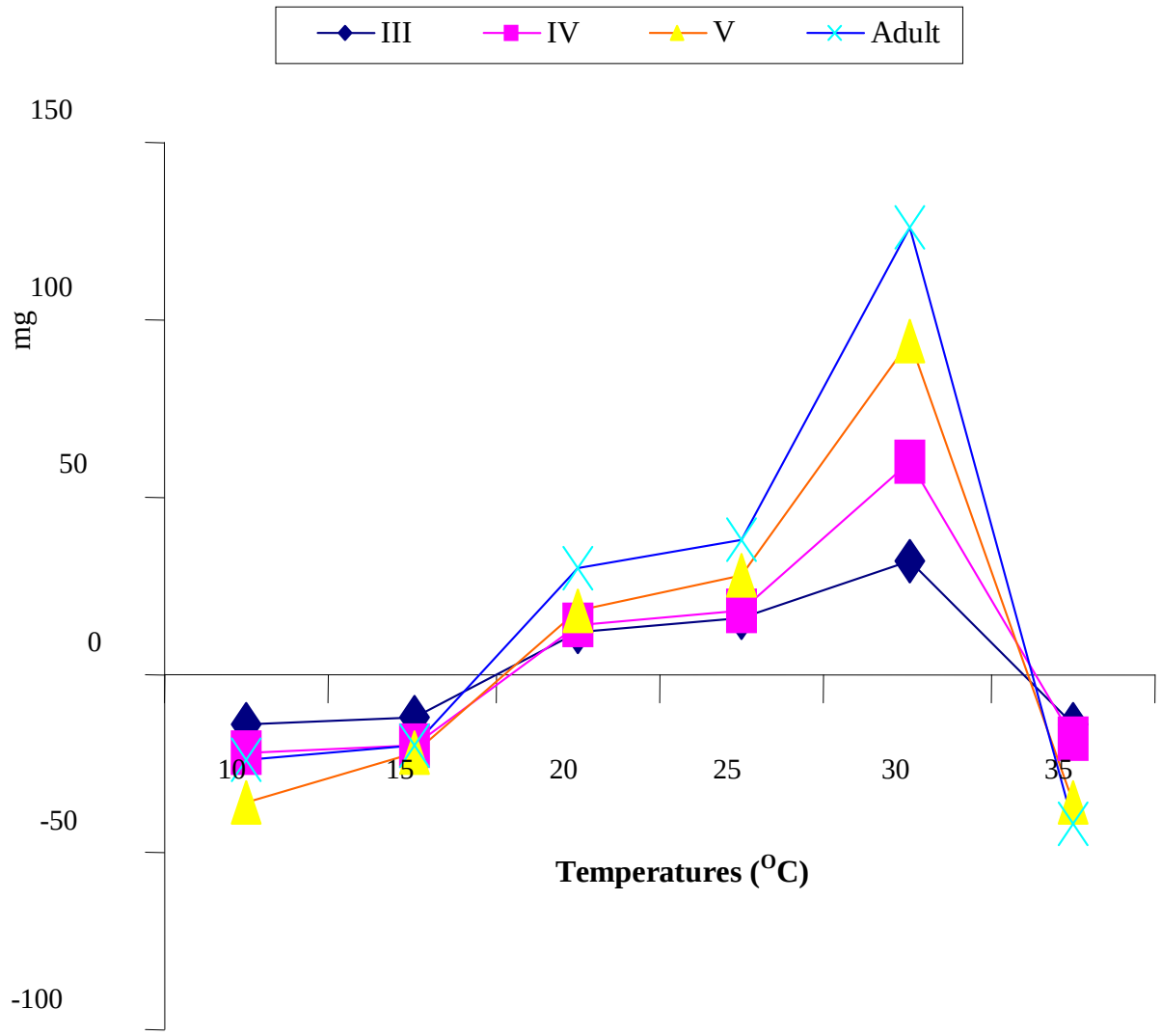


Table 12. Morphometric analyses (in cm) at different temperature maintained adult of *R. fuscipes*

T (°C)	Antennae	Rostrum	Head	Thorax	Abdomen	Fore Leg	Mid leg	Hind leg	Fore wing	Hind wing	Abdominal width	Thoracic width	Total Length
20	0.46 ± 0.073	0.27 ± 0.011	0.29 ± 0.02	0.36 ± 0.05	0.81 ± 0.06	0.76 ± 0.04	0.60 ± 0.05	0.86 ± 0.05	0.88 ± 0.08	0.68 ± 0.05	0.47 ± 0.25	0.23 ± 0.02	1.46
25	0.46 ± 0.06	0.22 ± 0.01	0.25 ± 0.48	0.35 ± 0.78	0.67 ± 0.05	0.68 ± 0.053	0.57 ± 0.74	0.71 ± 0.12	0.74 ± 0.07	0.62 ± 0.05	0.42 ± 0.03	0.23 ± 0.02	1.27
30	0.25 ± 0.10	0.25 ± 1.08	0.24 ± 0.73	0.35 ± 0.82	0.61 ± 0.12	0.68 ± 0.15	0.60 ± 0.15	0.70 ± 0.26	0.54 ± 0.12	0.46 ± 0.07	0.42 ± 0.08	0.20 ± 0.02	1.23
R T	0.25 ± 0.10	0.24 ± 0.17	0.25 ± 0.21	0.35 ± 0.74	0.65 ± 0.23	0.69 ± 0.03	0.59 ± 0.20	0.70 ± 0.09	0.54 ± 0.06	0.45 ± 0.05	0.43 ± 0.03	0.21 ± 0.07	1.25

Table. 11. Morphometric analyses (in cm.) of *R. marginatus* adults maintained at different temperatures.

T (^{o}C)	Antennae	Rostrum	Head	Thorax	Abdomen	Fore Leg	Mid leg	Hind leg	Fore wing	Hind wing	Abdominal width	Thoracic width	Total length
20	0.60 ± 0.09	0.29 ± 0.23	0.26 ± 0.04	0.47 ± 0.04	0.58 ± 0.039	0.79 ± 0.04	0.57 ± 0.08	0.83 ± 0.08	0.61 ± 0.01	0.54 ± 0.087	0.50 ± 0.11	0.34 ± 0.05	1.30
25	0.66 ± 0.17	0.37 ± 0.06	0.31 ± 0.60	0.83 ± 0.12	0.38 ± 0.06	1.02 ± 0.08	0.75 ± 0.10	1.11 ± 0.19	0.94 ± 0.15	0.71 ± 0.12	0.54 ± 0.10	0.35 ± 0.05	1.52
30	0.56 ± 0.20	0.71 ± 0.09	0.35 ± 0.05	0.832 ± 0.18	0.375 ± 0.068	1.19 ± 0.14	0.79 ± 0.07	1.13 ± 0.17	0.97 ± 0.02	0.73 ± 0.149	0.54 ± 0.09	0.34 ± 0.07	1.56
R T	0.56 ± 0.21	0.71 ± 0.08	0.34 ± 0.19	0.84 ± 0.19	0.86 ± 0.062	1.20 ± 0. 22	0.79 ± 0.08	1.15 ± 0.19	0.95 ± 0.053	0.77 ± 0.13	0.55 ± 0.07	0.35 ± 0.01	2.08

Figure 21. **Mean protein content of *R. marginatus* fed with *S. litura*, *C.cephalonica* and *D. cingulatus***

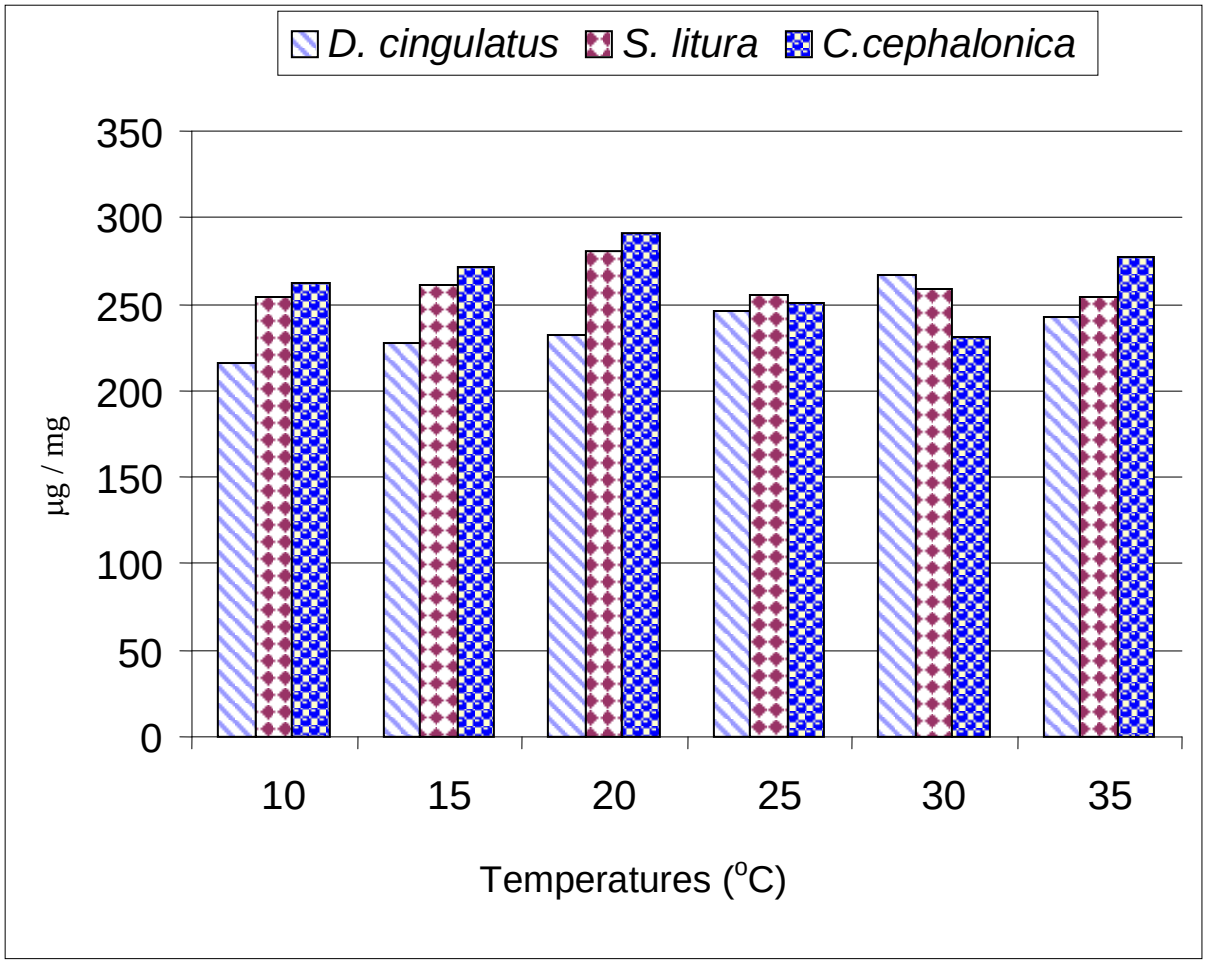


Figure 22. **Mean protein content of *R. fuscipes* fed with *S. litura*, *C. cephalonica* and *D. cingulatus***

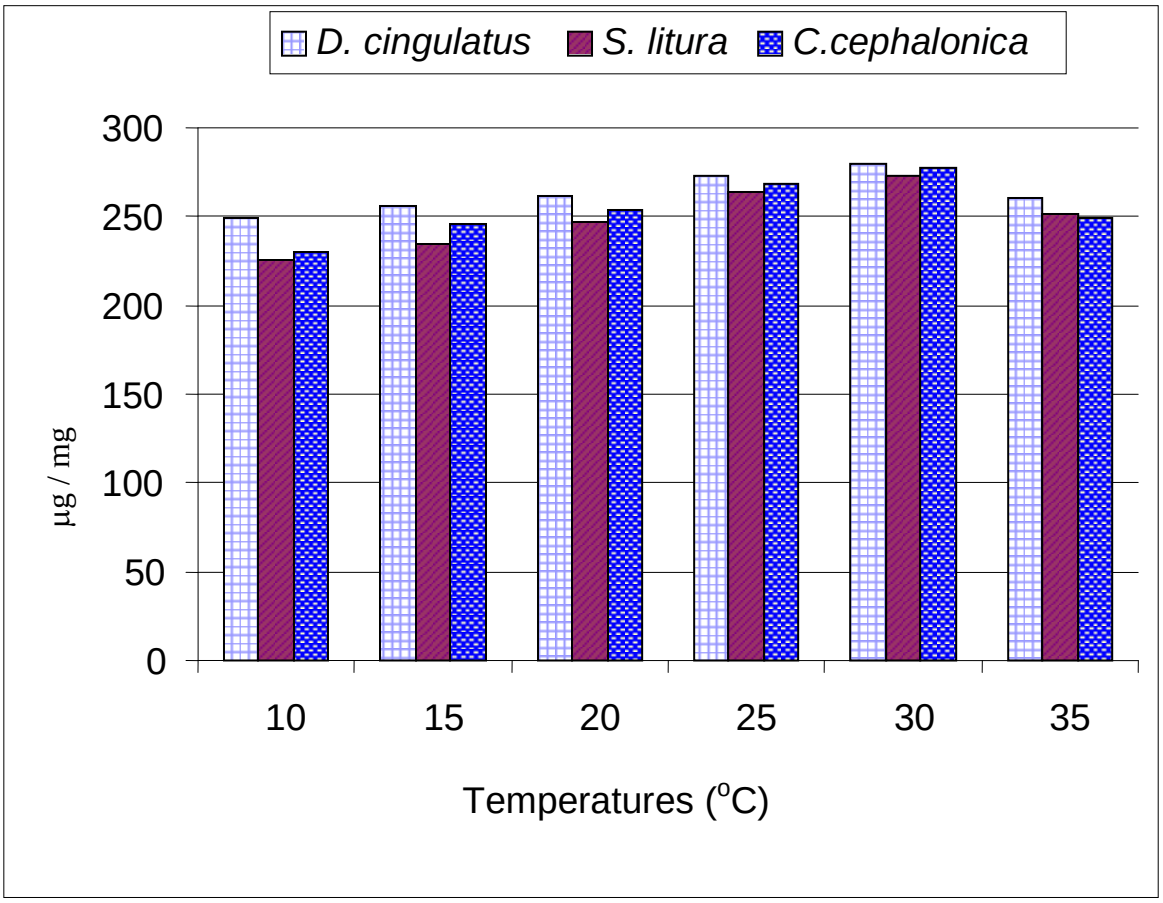


Figure 23. **Qualitative ELISA absorbance analyses of *R. marginatus* (gut) feed with *C. cephalonica* (CC), *S. litura* (SL), and *D. cingulatus* (DC) at various temperature (^{o}C)**

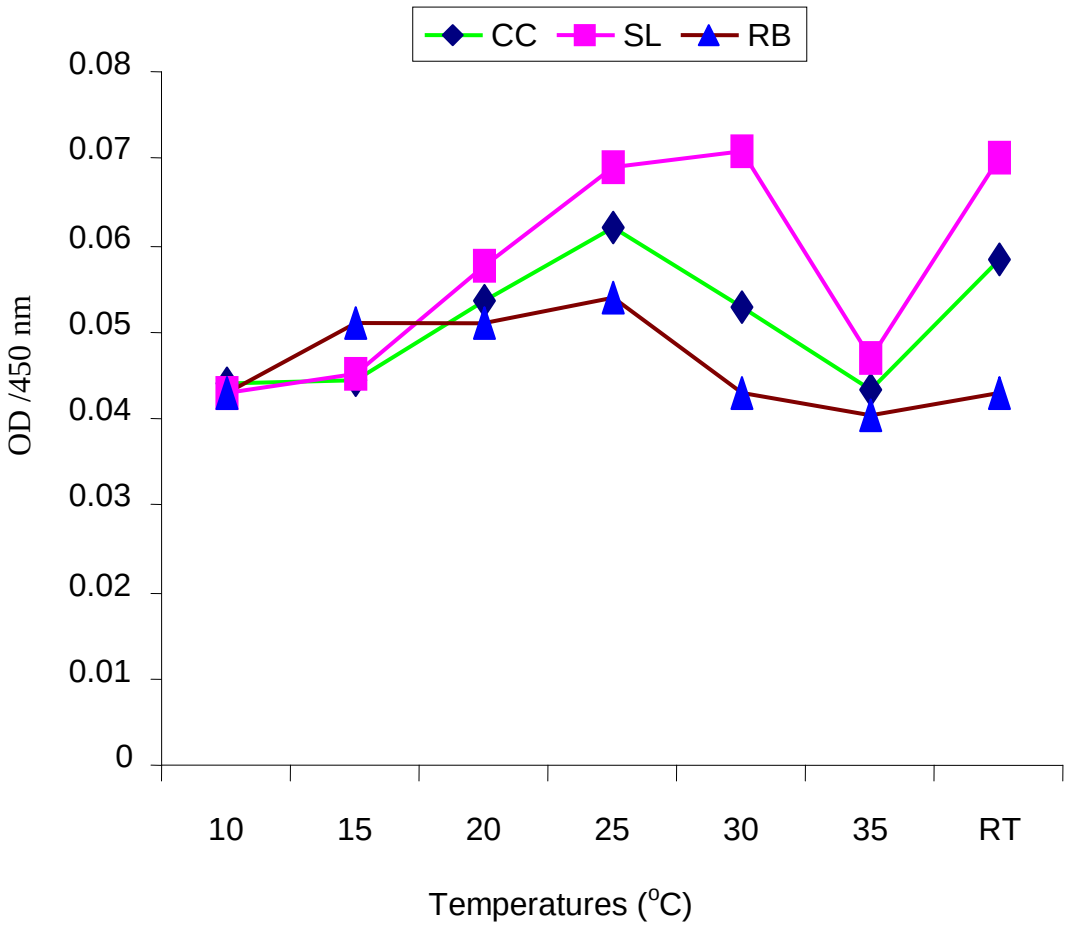


Figure 24. **Qualitative ELISA absorbance analyses of *R. fuscipes* (gut) feed on *C. cephalonica* (CC), *S. litura* (SL), and *D. cingulatus* (DC) at various temperatures (^{o}C)**

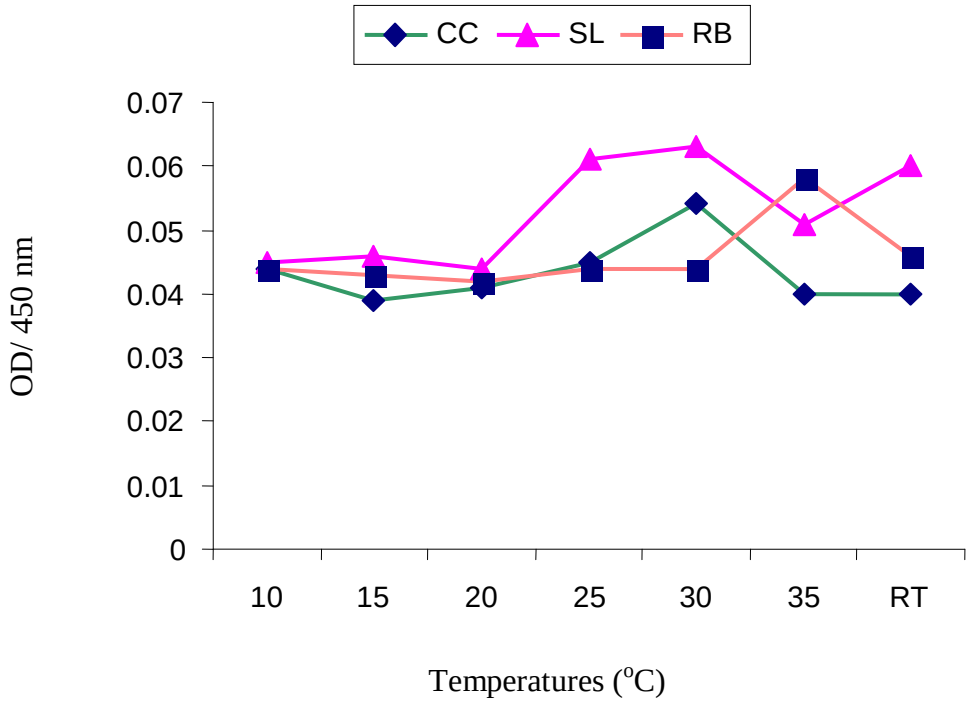


Figure 12. Total heterotrophic bacterial population (THBP) (CFU/g) of *R. marginatus*, *R. fuscipes* at different temperatures

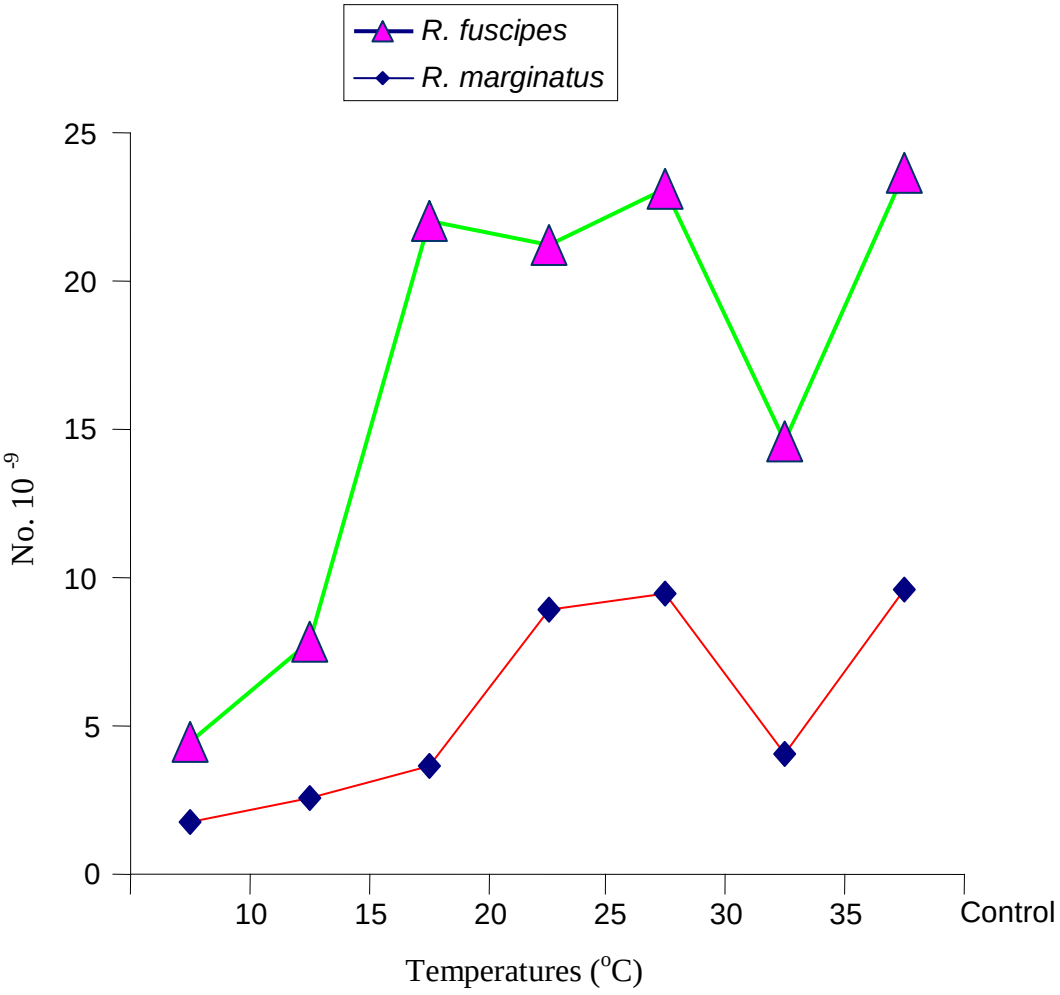


Figure 11. **Gut wet weight of the *R. marginatus* and *R. fuscipes* at six different temperatures (^{O}C)**

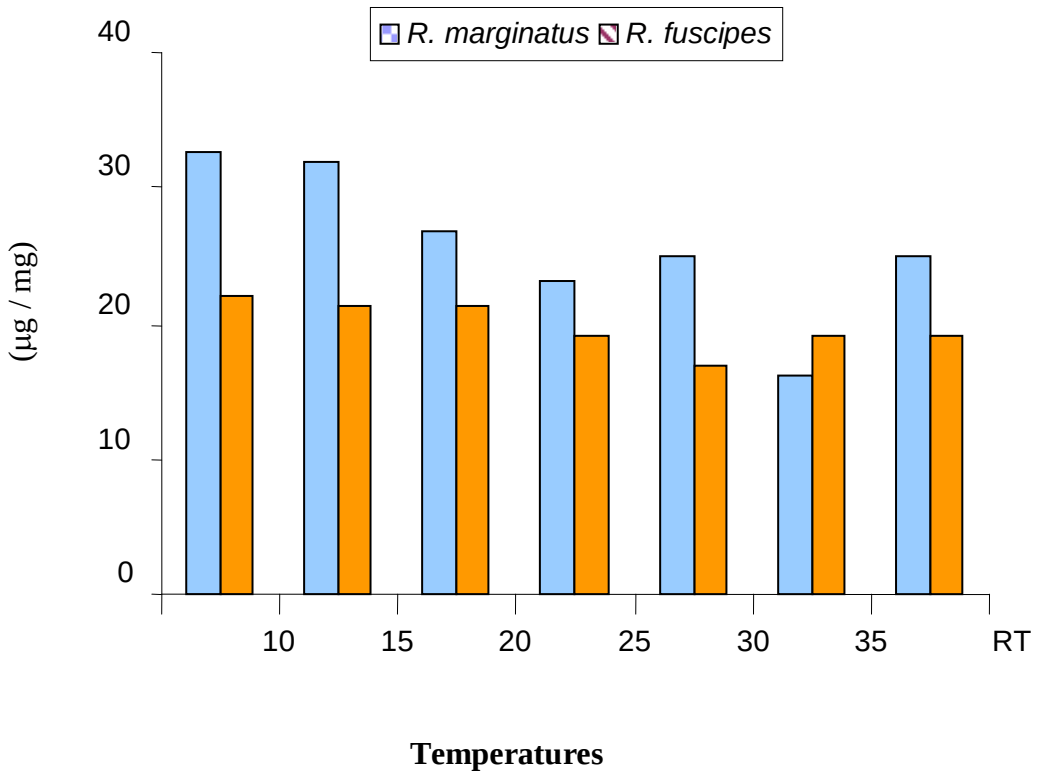


Figure 13. **Whole body biochemical composition (in μg/mg) of *R. marginatus* and *R. fuscipes* at various temperatures**

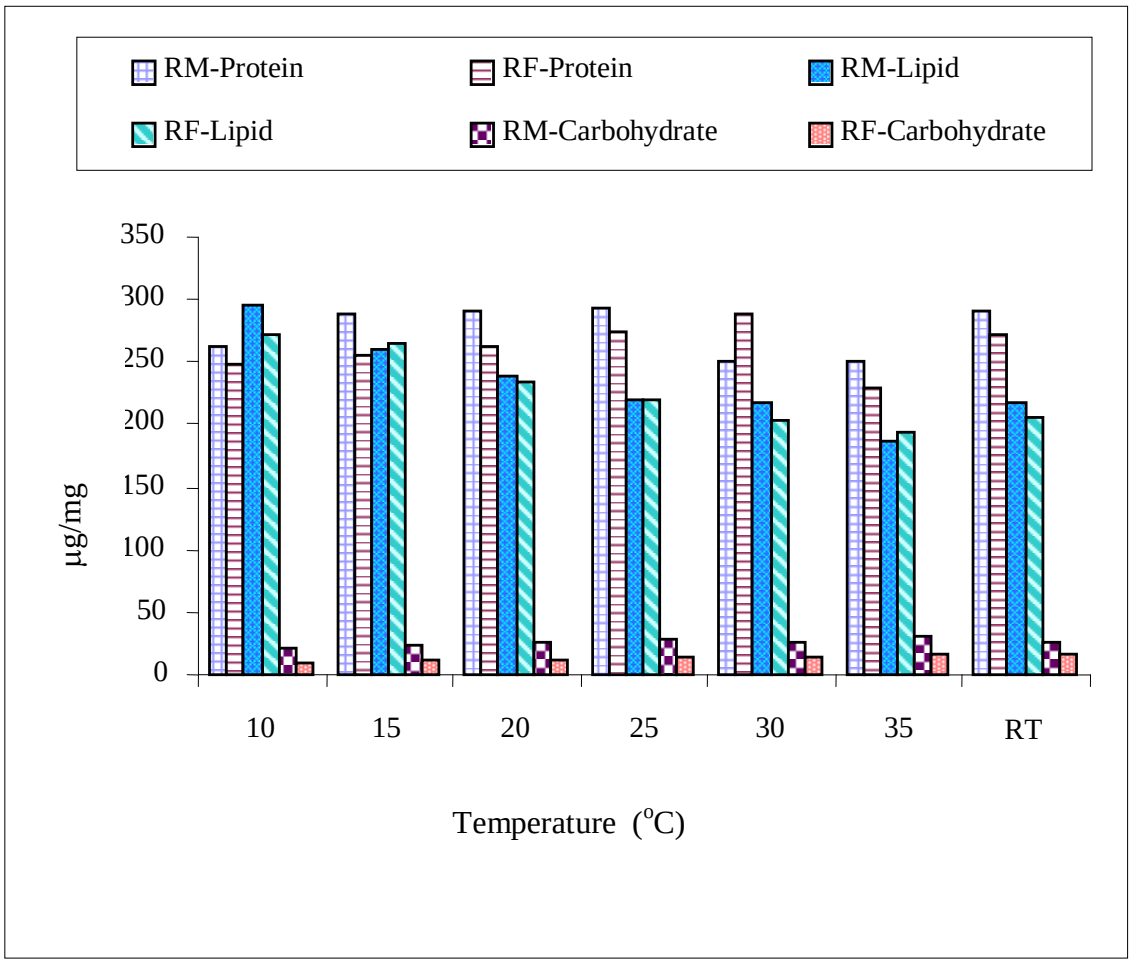


Figure 14. **Protein, carbohydrate and lipid (in mg/100mg) of *R. marginatus* and *R. fuscipes* eggs at various temperatures**

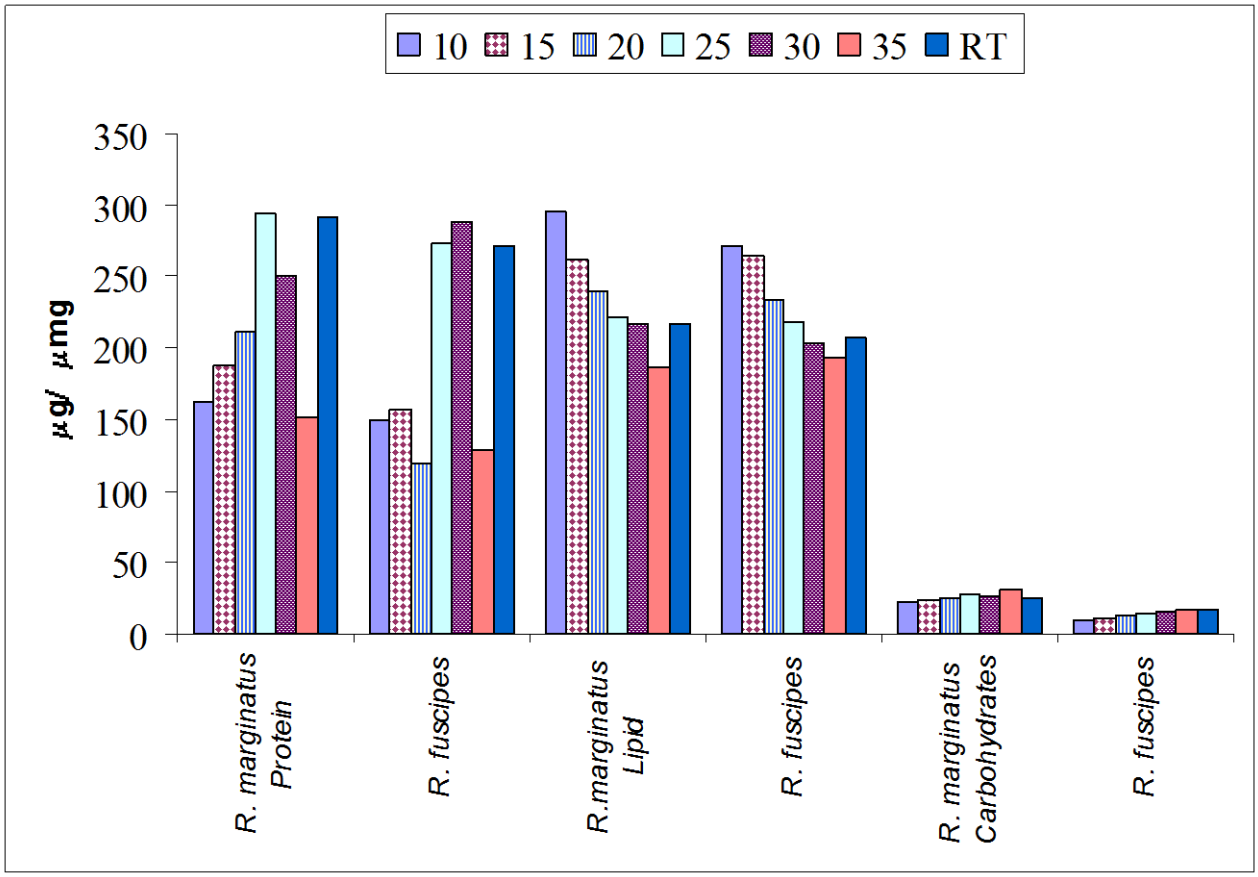


Figure 9. Influence of temperatures (^{o}C) on *R. marginatus* foregut (a) and hindgut (b) enzyme activity (in µg/mg)

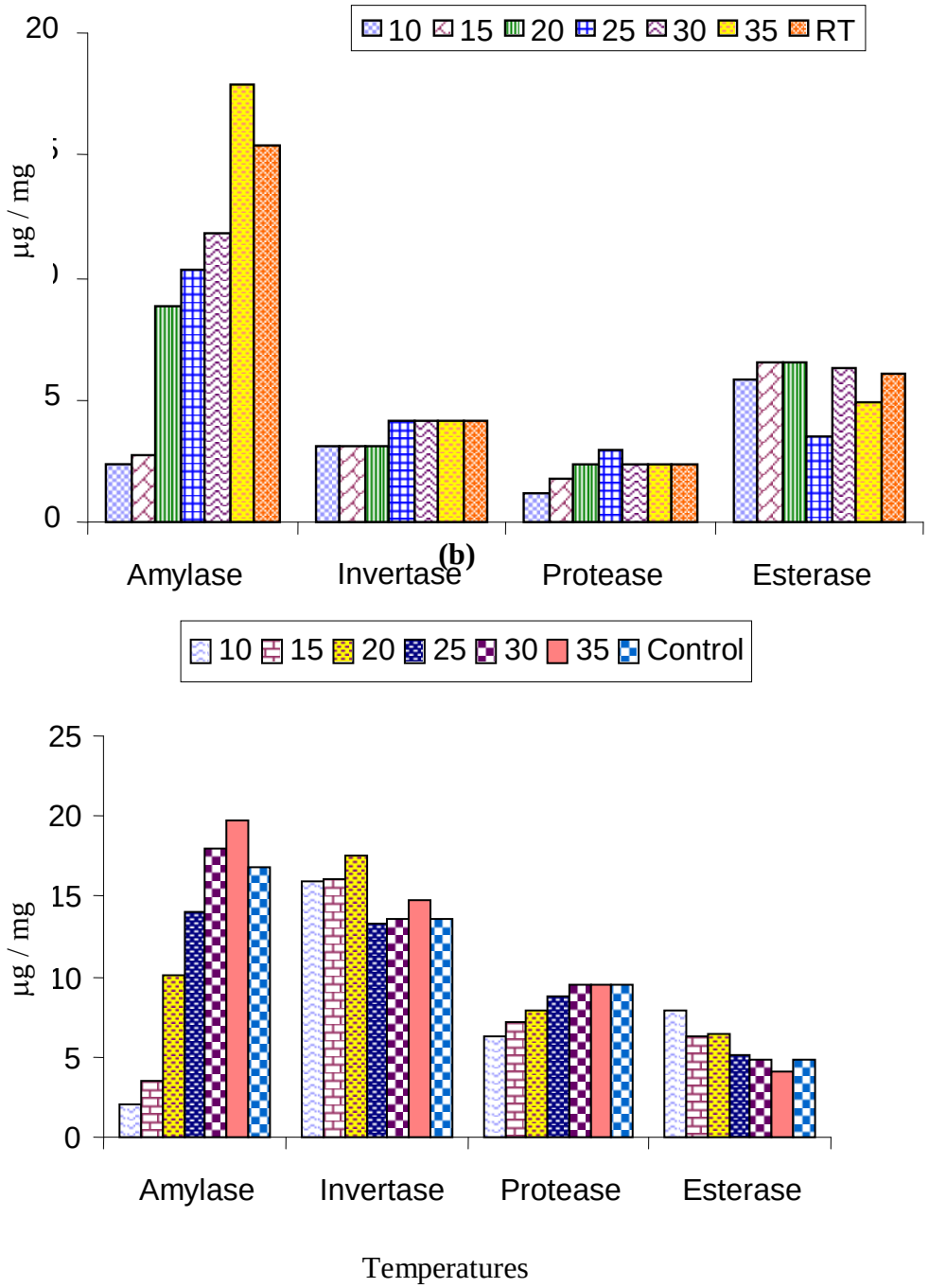
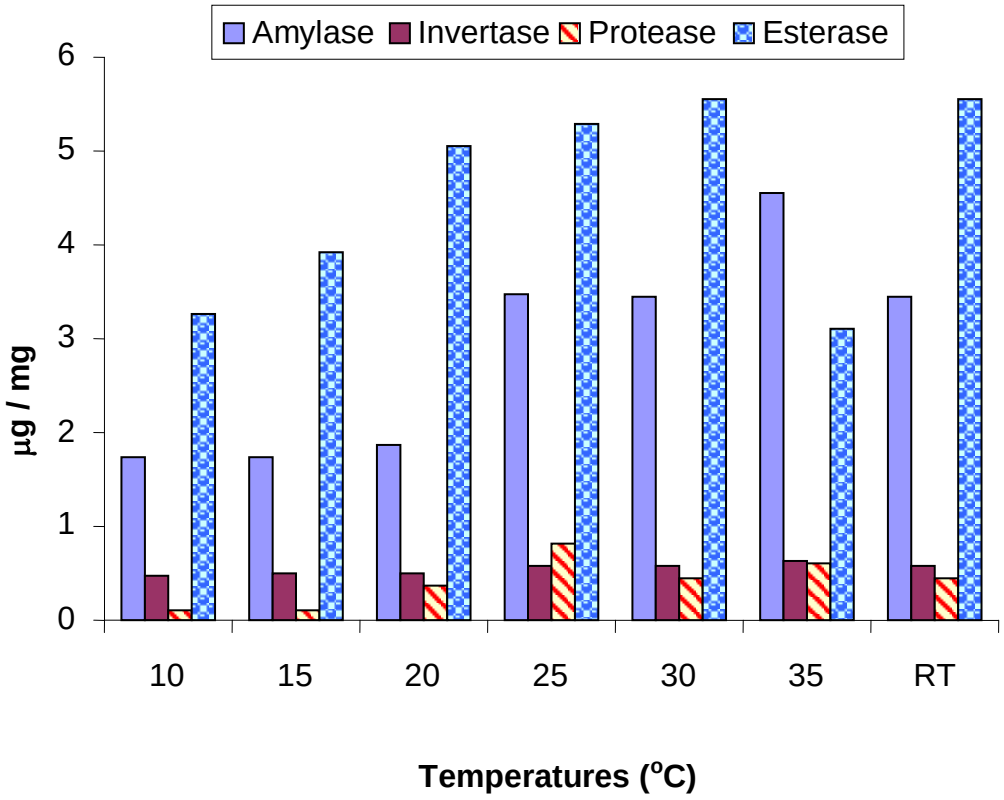


Figure 10 . Influence of temperatures (^{o} C) on *R. fuscipes* foregut (a) and hindgut (b) enzyme activity (in µg/mg)

(a)



(b)

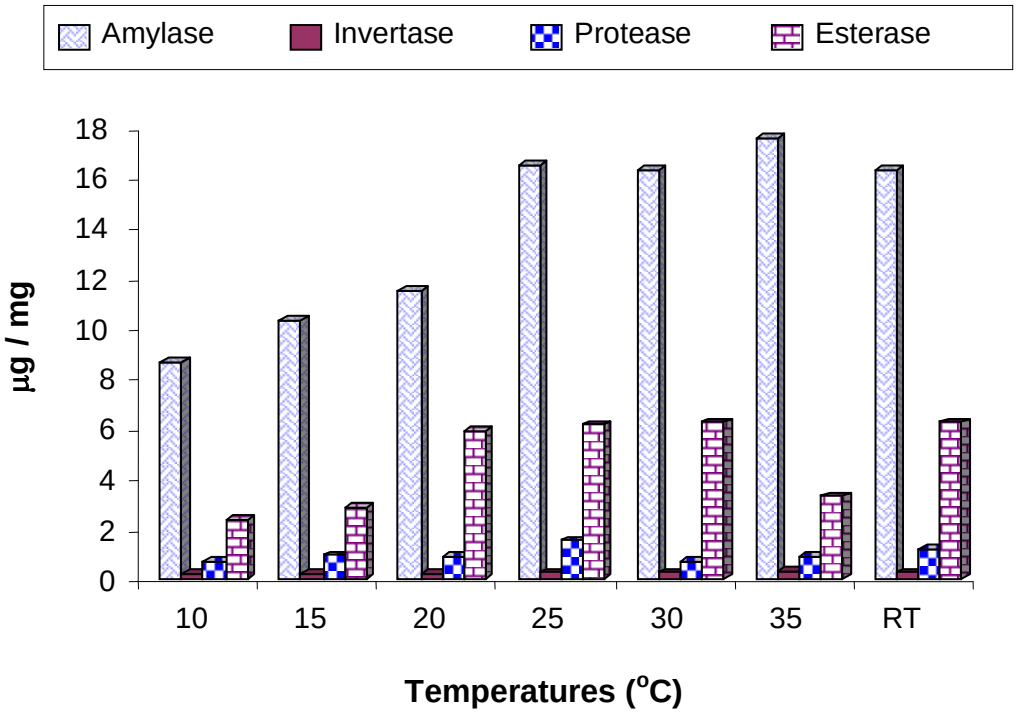
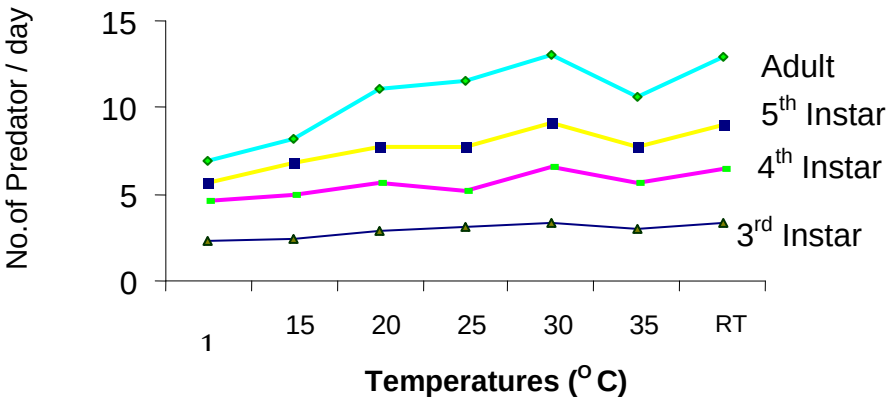


Figure 5. Predatory rate of *R. marginatus* life stages on *D. cingulatus* (a) and *S. litura* (b) at various temperatures

(a)



(b)

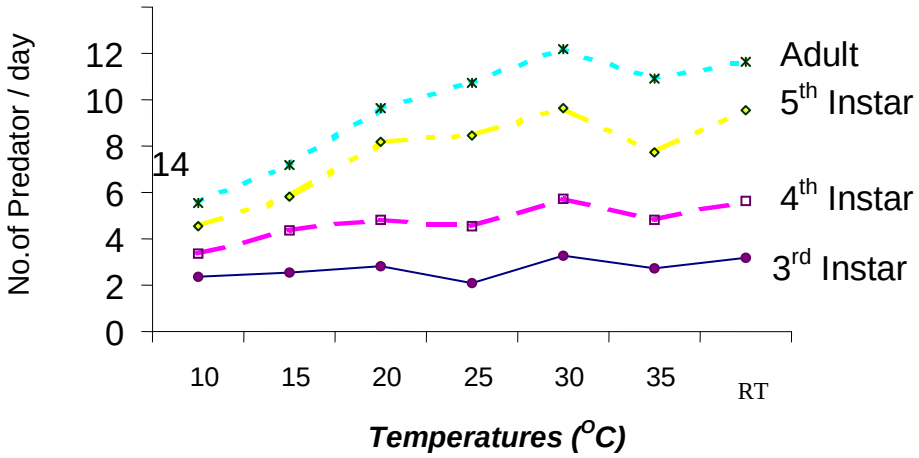
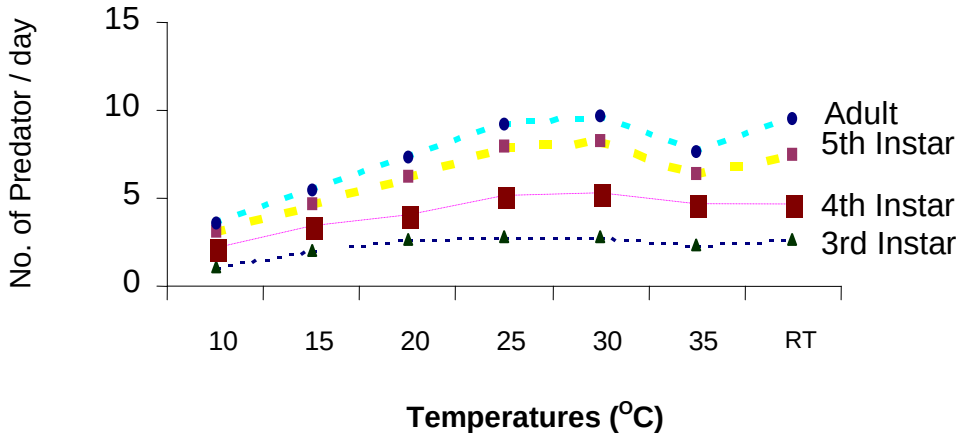


Figure 6. Predator rate of *R. fucipes* on *D. cingulatus* at various *D. cingulatus* (a) and *S. litura* (b) temperatures

(a)



(b)