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

Thesis submitted to

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Doctor of Philosophy in Zoology

By

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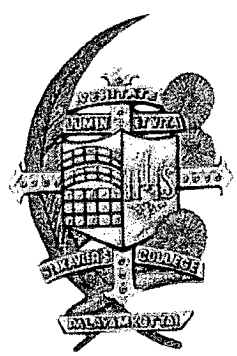

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CERTIFICATE

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 It is not the plagiarism of any other work either published or unpublished without acknowledgement.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 I also assure that no part of the thesis is a reproduction from any other sources either published or unpublished without acknowledgement.

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MR.R.BALASUBRAMANIAN

Abbreviations

AD	-	Artificial Diet
AT	-	Approaching time
Bp	-	Base pairs
BSA	-	Bovine Serum Albumin
CBR	-	Cost Benefit Ratio
CC	-	*Corcyra cephalonica*
CT	-	Consumption time
CW	-	*Corcyra cephalonica* weekly once with water
DASE	-	Day after seedling emergence
dH_2O	-	deionized water
DNA	-	deoxyribonucleic acid
dNTP	-	deoxyribonucleoside triphosphate
EDTA	-	Ethylene Diamine Tetra Acetic acid
ELISA	-	Enzyme Linked Immuno Sorbent Assay
EtBr	-	Ethidium Bromide
EtOH	-	Ethanol
FPI	-	Food preference index
IAA	-	Iso Amyl Alcohol
M	-	Molar
MAb	-	Monoclonal Antibody
mg	-	milligram
ml	-	milliliter
mM	-	millimolar
NA	-	Nutrient agar
OD	-	Oligidic diet
OC	-	Oligidic Diet + *Corcyra cephalonica*
OD	-	Optical density
PBS	-	Phosphate buffered saline
PCR	-	Polymerase Chain Reaction
RAPD	-	Randomly Amplified Polymorphic DNA
Rpm	-	rounds per minute

SDS	-	Sodium Dodecyl Sulfate
TBE	-	Tris-boric acid EDTA buffer
TE	-	Tris-EDTA buffer
TEN	-	Tris – EDTA NaCl
THBP	-	Total Heterotrophic Bacterial Population
TSA	-	Trypticase soy agar
UV	-	Ultra Violet
µg	-	microgram
µl	-	microliter
Mm	-	micromolar

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CHAPTER - 2 MATERIALS AND METHODS

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Abstract

ABSTRACT

The entomophagous reduviid predator *Rhynocoris marginatus* (Fab.) (Heteroptera: Reduviidae) is an effective biological control agent of various insect pests which belongs to many orders. Feeding behavior of insect hosts as well as artificial diet reared *Rhynocoris marginatus* (Fab.) life stages on *Spodoptera litura* (Fab.), *Dysdercus cingulatus* (Fab.) and *Corcyra cephalonica* (Stainton) in terms of approaching time, handling time, and predatory rate (weight gain and number of prey consumed) were evaluated under laboratory conditions. Results revealed that particular stage of the predator preferred desired stage of the pests studied. Moreover, younger reduviid preferred younger prey *vise-versa*. Irrespective of the preys offered, females consumed more number of prey than the male. Among the three preys tested, *S. litura* was the most preferred prey followed by *D. cingulatus* and *C. cephalonica*. Both the handling time and weight gain were differed among the life stages of this reduviid reared with artificial diet and insect prey.

Biology of *R. marginatus* was monitored on five diets: *C. cephalonica* (T_1), *C. cephalonica* + weekly once with water (T_2), Oligidic diets (4, 5 and 6) (T_3), Oligidic diets weekly once with *C. cephalonica* (T_4) and *S. litura* (T_5). Nymphal development period, survival rate and reproduction of *R. marginatus* results revealed that Oligidic diets reduced the size, delayed developmental period and reduce the reproduction. However, significant improvement was observed over the three generations: percent of egg hatching in OD was ranging from 94 to 100%, incubation period was ranged from 8-12 days and 6-8 days for OD and natural diet respectively. Females biased sex ratio was observed in all the categories.

The effect of two conventional diets (prey) and oligidic diets with antimicrobial agent like streptomycin and formaldehyde (40%) shows that THTP was varied in insect fed and OD reared *R. marginatus*. A comparison of digestive enzyme activities in oligidic diet and insect fed categories did not reveals not much differences in enzymes and suggested that microbial derived enzymes are not an essential component of the digestive process in this reduviid. Among all the categories, the bacterial population was found to be higher in *S. litura* fed (114.32 CFU) (T_5) category followed by *C. cephalonica* (103.8) (T_1), *C. cephalonica* + weekly once with water (T_2) (98.55 CFU) OD + with *C. cephalonica* (68.5) (T_4) and oligidic diet (40.4) (T_5). In contrast addition of more number of bacteria was isolate in oligidic diet (14 species) reared predator.

Both qualitative and quantitative profiles of protease, lipase amylase and invertase of foregut, hindgut and salivary gland enzymes were tested. Among the five diets, OD reared reduviid hindgut and salivary gland have the maximum activities followed by *C. cephalonica* and *S. litura*. In general *S. litura* fed reduviid foregut showed maximum lipase amount than other categories. Salivary gland showed meager amount of lipase activity. The results of *R. marginatus* showed that production of enzyme depends upon location in the gut and prey specific.

Indirect ELISA was examined to detect the influence of *S. litura, C. cephalonica,* and OD in adult *R. marginatus*. The *S. litura* (0.062) and OD reared predator yield the highest frequency of positive immuno assay followed by *C. cephalonica* reared predator.

Six primers KTG-3, KTG-5, KTG-4 and OPE-8 OPE-13 OPE-16 were tested to amplify the DNA of the predators population. The size of the amplified DNA ranged from 200 to 2000 bp. A total of 52 amplicons resulted from four primers in five population of *R. marginatus* analysed. The number of amplifications was 24, 16 and 7 for KTG-3, KTG-5 and KTG-4 respectively. The lowest number of amplicons polymorphism was produced with the primer OPE-8. The electrophoretic pattern reveals polymorphism was present among *R. marginatus* populations.

Among all predators recorded, *R. marginatus* was the most abundant in the groundnut field. During the study period five pests such as *Aphis crassivora* Koch, *Helicoverpa armigera, S. litura, Mylabris indica* and grosshoppers were recorded both in summer (January-May) and kharif (June to August) 2006 and 2007 respectively. During this period, the most abundant pest was *A. crassivora* followed by jassids, grasshoppers. From the results, it was very clear that *R. marginatus* greatly suppressed the population of *S. litura, A. craccivora, H. armigera* and *Pericalia ricini.* Among the two-diet regime, OD reared *R. marginatus* highly reduce the pest populations. Both in summer 2006 and Khariff 2007, groundnut production was maximum in the artificial diet reared predator. Similarly the cost benefit ratio was maximum in the OD predator released field (1:2) followed by T_1 (CC) reared predator (1:1.8).

Introduction

CHAPTER - 1 INTRODUCTION

Predatory insects like reduviids are an important group of biological control agents. As the use of chemical insecticides gained currency, every farmer has been adapting pest management. Today, however, people are becoming increasingly aware that in the long run, the harmful effect of the insecticides far outweighs the advantage of temporary pest control. Reduviids are important predators of cotton, rice, brinjal, groundnut and various other crops and plantations crop pests. Currently the reduviids are not commercially available any where in the world. However, very little efforts have been made to rear few reduviids in small scale under laboratory conditions by larval card method, (Sahayaraj, 2002a).

1.1. Hemipteran as Biological Control Agents

The important hemipteran bugs used as biological control agents are *Platymeris laevicollis* Distant (Antony *et al.,* 1979); *Nabis spp.* (Anonymous, 1987); *Geocoris puntipes* Say (Cohen, 1984, Cohen and Bryne, 1992); *Zelus renardii* Kolenati (Cohen, 1993); *Podisus maculiventris* (Say) and *P. sagitta* (Fab.) (De Clercq and Degheele, 1994); *Eocanthecona furcellata* (Wolff) (Usha Rani and Wakamura, 1993); *Cyrtopeltis tenius* (Torreno, 1994); *Cardiastethus exigus* Poppius and *Buchananiella sodalis* (Sujatha and Singh, 1999); *Rhynocoris marginatus* Fabricius (Sahayaraj, 1998, 1999 b, 2002 b; Ambrose and Claver, 1999 b; Sahayaraj and Martin, 2003); *Rhynocoris kumarii* Ambrose and Livingstone (Claver, 1998; Ambrose, 2000; Ambrose and Claver, 2001a, b) and *Pristhisancus plagipennis* Walker (Grundy and Maelzer, 2000). Seven hemipteran predators were included as biological control agents (Navarajanpaul, 2003). Ballal *et al.* (2003) also included *Blaptostethus pappescens* Poppius as a biological control agent.

1.2 Reduviids

Reduviids are generalist predators that mainly feed on lepidopteran, coleopteran, hemipteran and isopteran insects and are considered a potential biological control agent against various pests (Schaefer and Ahmad, 1987; Schaefer, 1988; James, 1994; Ambrose, 1980, 1991, 1995, 1996; Sahayaraj, 1999b, 2002b; Sahayaraj and Martin, 2003; Sahayaraj *et al.,* 2006, 2007). The incidence of reduviid predators in diverse cropping system and their biological control potential is documented elsewhere (Werner and Butler, 1957; Whitcomb and Bell, 1964; Altieri and Whitcomb, 1980; Mc Pherson *et al.*, 1982; Ambrose, 1987, 1988, 1991, 1995, 1996, 2000; Sahayaraj, 1991, 1998 a, 1999 b, 2001, 2002 b; Claver, 1998; Claver and Ambrose, 2001a, b; Sahayaraj *et al.,* 2004). The quality and quantity of nutrients of the prey influence not only the growth rate and survival of the predator (Ambrose and Subbarasu, 1988; Ambrose *et al.,* 1990; O'Neil and Widenman, 1990; Ambrose and Rani, 1991; Cohen, 1990, 1993; George *et al.,* 2002) but also increase the fecundity and life table characteristics such as generation time and intrinsic rate of population increase (Awadallah *et al.,* 1986; George, 1999, 2000 a). The nutritional quality of the prey has a high influence over the whole physiological process of a predator (Ananthakrishnan, 1996).

1.3 *Rhynocoris marginatus* (Fab.) (Heteroptera: Reduviidae)

Rhynocoris marginatus (Fab.), a harpactorine reduviid predator, redescribed by Ambrose and Livingstone (1986), is generally present in agroecosystems, semiarid zones, scrub jungles and tropical rain forests (Ambrose and Livingstone, 1986; Vennison and Ambrose, 1990; Sahayaraj, 1991; Kumar, 1993; Kumaraswami and Ambrose, 1994; Ambrose and Rajan, 1995; Edwin and Ambrose, 1996). Its biology (Ambrose and Livingstone, 1987 a; Claver, 1998); mating behaviour (Ambrose and Livingstone, 1987);

the saliva spitting behaviour (Vennison, 1988; Vennision and Ambrose, 1990, Sahayaraj *et al.,* 2006) and predatory behaviour (Ambrose and Claver, 1996); nymphal cannibolism (George, 2000 b) were studied extensively. Ambrose and George (1996) reported the effect of flooding on the incubation and hatchability of *R. marginatus* eggs. Five types of haemocytes were observed in the haemolymph of this bug (Ambrose and George, 1996; George, 1996). The insecticidal impact on the postembryonic development (George, 1996; George and Ambrose, 1999 a); and biochemical modulations by insecticides (George and Ambrose, 1999 b) were studied. The impact of antennectomy and eye blinding on the predatory behaviour (Claver and Ambrose, 2001 d) was also studied.

Rhynocoris marginatus has been reported as a potential predator on various economical important agricultural pests such as *Spodoptera litura* (Kumaraswami, 1991; Sahayaraj, 1994; Ambrose and Claver, 1995, 2001a; Sahayaraj and Siva kumar, 1995; Claver and Ambrose, 2001 a); *Papilio demoleus* L. (Kumaraswami, 1991) *Spodoptera litura* and *Dysdercus cingulatus* (Sahayaraj and Balasubramanian, 2008); *Dysdercus cingulatus* Fabricius, *Earias vitella* Fabricius, *Euproctis mollifera* Walker, *Oxycarneus hyalipennis* Costa, *Earias insulana* Boisdual (Kumaraswami, 1991, Ambrose, 1995); *Earias fraterna* Moore (Ambrose, 1995); *Achea janata* Linn. (Ambrose and Claver, 1995; Ambrose, 1996); *Ergolis merione* Costa (Ambrose, 1995; Ambrose and Claver, 1995); *Patanga succinata* L. (Ambrose, 1995); *Anomis flava* Fabricius (Ambrose and Claver, 1995; Ambrose, 2000); *Pectinophora gossypiella* Saunders (Ambrose, 1995; Ambrose and Claver, 1995); *Calacoris angustate* Lethiery (Ambrose and Claver, 1995; Ambrose, 1996); *Mylabris pustulata* Thunberg (Ambrose, 1995) and *Helicoverpa armigera* (Ambrose, 1995, 1996, 2000; Claver and Ambrose, 2001 b). In the laboratory, it feeds on stored product pests such as *Tribolium confusum* Duv. (Ambrose, 1988) and larvae of *Corcyra cephalonica* (Kumaraswami, 1991; Ambrose, 1996; Claver, 1998).

Biological control potential related aspects like host preference (Kumaraswami, 1991); searching behaviour (Claver and Ambrose, 2001a); functional and numerical response (Ambrose and Claver, 1996) were available. Bioefficacy of this Reduviid under the laboratory (Kumaraswami, 1991; Sahayaraj, 1994; Claver, 1998), field cages (Ambrose, 2000; Claver and Ambrose, 2001 b) and field such as cotton and groundnut (Sahayaraj and Paulraj, 2001, Sahayaraj and Martin 2003, Ravi, 2004) were investigated. Moreover, the development and life table on *S. litura, E. vitella* and *C. cephalonica* (George, 2002); *S. litura* and *H. armigera* was reported (Sahayaraj and Sathyamoorty, 2002). However, the role of *Rhynocoris marginatus* as natural pest control was greatly neglected and even forgotten.

Rearing and mass multiplication of the reduviids in the laboratory is an important requirement for the successful biological control programme (Schaefer, 1988; Cohen, 1993; Ambrose, 1995, 2001; Sahayaraj *et al.*, 2006). Attempts were made by several workers to mass multiply the reduviids in the laboratory (Lakkundi, 1989; Sahayaraj, 1991; Kumaraswami, 1991; Claver, 1998; Grundy and Maelzer, 2000; Sahayaraj and Paulraj, 2001; Sahayaraj, 2002 b). Its high labor cost, laborious process (Sahayaraj, 2002) and nymphal cannibalism (Ambrose, 1999; George and Ambrose, 2000; George, 2000 b) are the major constraint. The reduviids are reared in the laboratory mainly on the rice moth *Corcyra cephalonica* alive larvae (Lakkundi and Parshad, 1987; Sahayaraj, 1991, 2001, 2002 b; Claver, 1998; George, 2000 a; Ambrose, 1999; Sahayaraj and Paulraj, 2001; Sahayaraj and Martin 2003, Sahayaraj *et al.*, 2006) and other prey such as *S. litura, Earias vitella* Fabricius, (George *et al.*, 1998, George, 2000 a; Sahayaraj and Paulraj, 2001); *Helicoverpa armigera* Hubner and *Nezara viridula* L. (Grundy and Maelzer, 2000); frozen larvae of *C. cephalonica* (Sahayaraj and Jayalakshmi, 2002) and *H. armigera* (Grundy and Maelzear, 2000) and oligidic diet (Sahayaraj *et al.*, 2006, 2007,

2008). In India, a few reduviids like *Platymeris laevicollis* (Antony *et al.,* 1979); *Acanthaspis quinquespinosa* Fabricius (Lakkundi, 1989; Sahayaraj, 1991, 2002); *Brassivola hystrix* Distant, *Coranus sp., Endochus parvispinus* Distant, *Irantha armipes* Stal, *Isyndus heros* (Fabricius) (Lakkundi, 1989); *Acanthaspis pedestris* Stal (Sahayaraj, 1991); *Cyndocoris gilvus* Burm (Venkatesan *et al.,* 1997); *R. kumarii* (Claver, 1998); *R. marginatus* (George, 2000b; Sahayaraj, 2002b); *Neohaematorrophus therasii* Ambrose and Livingstone (Sahayaraj, 2001, Sahayaraj *et al.,* 2004, 2006 and 2007) were mass reared in the laboratory and a little success was achieved.

Although the technology required for the large-scale production of reduviids is relatively straightforward, it is not necessarily available to small laboratories and at present it appears that there is meager information available in the literature on small-scale reduviid production. Cohen (1993); James (1994); Ambrose (1995); Schaefer (1988); Vandekerkhove (2006); Sahayaraj *et al.,* (1999a, 2002a, 2004, 2006, 2007) felt that there is an urgent need for evolving the strategies to mass rear the potential hunter reduviids. Furthermore, one of the basic requisites for a biological control agent is the availability of a sound, low cost rearing and mass multiplication technique, which is not available for the reduviids particularly *R. marginatus*.

A required number of bioagents can be obtained by rearing them either on their natural host or on oligidic diets. Rearing of this predator on natural and/or factitious host is entirely impracticable owing to non-availability of host throughout the year, (Sahayaraj, 1998, 2002a; Sahayaraj and Sathyamoorthi, 2002; Sahayaraj and Jeyalakshmi, 2002). Later, importance and applicability of the oligidic diet in reduviid rearing was reported by Sahayaraj *et al.,* 2006. Sahayaraj *et al.,* (2006) studied the feeding behavior of four reduviids on meat and insect-based oligidic diets and they

reported that irrespective of the reduviids, all the four studied reduviids preferred to feed meat-based diet. It was also reported that in general, oligidic diet reared *R. marginatus* consumed more number of preys than those reared on natural diet and/or factitious hosts (Sahayaraj and Balasubramanian, 2008). Literature survey reveals that no published work is available about the effect of oligidic diet on the biology of this reduviid or any other reduviid predators.

Diet quality can be measured in terms of growth rate, development, reproduction, mortality, longevity and occurrence of abnormalities (Singh, 1977). The development of effective and economically profitable technologies for mass rearing of entomophagous insects is at present a key goal for successful biocontrol programmes. Conditions for the nutrition of insect predators are of special value in the success of insect mass rearing programme. Inadequate nutrition usually results in great changes in the metabolism, behaviour and other insect vital activities. These changes inevitably depreciate subsequent insect release. The ability of providing optimal nutrition will greatly affect both expenditures for entomophage production as well as colony quality and sometime determines economic expediency and indeed the possibility for mass culture (Yazloveltsky, 1986). The main development prospects for entomophage mass production for inundative release are, therefore, associated with the development of cheap and adequate artificial diets (Waage *et al.,* 1985, Yazlovetsky *et al.,* 1992). Successful development of such technologies based on artificial media will, however, requires a thorough knowledge of entomophage physiology, and an understanding of the peculiarities of their interactions with insect hosts and prey (Thompson and Hagen, 1999).

1.4. Oligidic Diet

The greatest barrier for the mass production of predatory insects is the lack of suitable artificial diets. Singh (1977) noted that 754 species of arthropod had been reared on artificial diets, of these 27 were arachnids. The remaining are insect species spanning 10 orders consisting of 19 families of Coleoptera, 24 Diptera, 11 Homoptera, 8 Hymenoptera, and 27 Lepidoptera. Sikorowski and Goodwin (1985) also reported similar numbers. However, Waage *et al*, (1985) pointed out that no suitable artificial diets had been developed for predators. An oligidic diet free of insect components (Cohen, 1985) was developed and sustained (says Cohen, 1985). The status of *in-vitro* culture of parasitic insect has been reviewed by House (1967), and Thompson and Hagen (1999).

In spite of some promising results obtained in the development of artificial diet for entomophage, the use of artificial diets in mass propagation programmes is currently limited to only a few species of predators and parasitoids (Ridway *et al.,* 1984, Slansky and Rodriges, 1987, Waage *et al,* 1985, Yaloveltsky *et al.,* 1992). Predatory insects such as, *Geocoris punctipes* Say (Cohen, 1984, Cohen and Bryne, 1992); *Zelus renardii* Kolenati (Cohen, 1993); *Podisus maculiventris* (Say) and *P. sagitta* (Fab.) (De Clercq and Degheele, 1994); *Rhynocoris marginatus* Fabricius (Sahayaraj *et al.,* 2006, 2007 and 2008); *Hylobius transversovittatus* (Carruthers, 2007) have been reared with Oligidic diets. Some artificial diets have been proposed for rearing mired bugs (Cohen, 2000). A simple diet based on beef and liver was successfully developed for *Geocoris punctipes* Say. (Heteroptera: Lygaeidae) and *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae) (Cohen, 1985a; Cohen and Smith, 1998) this diet was also suitable for rearing *Podisus maculiventris* Say and *P. sagitta* Fabricius (Heteroptera: Pentatomidae) after some adjustments (De Clercq and Degheele, 1992,

). These meat-based diets were cheap and easy to prepare. Liver-based artificial diets were developed for the production of *Orius laevigatus* Fieber (Heteroptera: Anthocoridae) (Arijs and De Clercq, 2001, 2002, 2004). With reference to Mirid bugs, these diets have been successfully used for rearing *Dicyphus tamaninii* Wagner, a Mediterranean species with similar habits and a similar distribution to *M. caliginosus*. Iriarte and Castane (2001) reared *D. tamaninii* on the diet described for *P. maculiventris* in De Clercq and Degheele (1992).

1.5. Microbial Diversity in Insect Gut

The diversity of the insect is reflected in the large and varied microbial communities inhabiting the gut (Dillon and Dillon, 2004). The indigenous gut bacteria is regarded as a valuable metabolic resource to the nutrition of the host by improving the ability to live on suboptimal diets, improved digestion efficiency, acquisition of digestive enzymes and provision of vitamins (Douglas, 1992; Tanada and Kaya, 1993; Breznak and Brune 1994; Biggs and Mc Grego, 1994; Bignell *et al.,* 1997; Brauman *et al.,* 2001; Broderick *et al.,* 2004). The contribution of gut microbiota to nutrition and disease suppression was also studied by Dillon and Charnley (1986, 1988 and 1996).

The symbiotic bacteria harbored in the midgut caeca of the Heteroptera caused Aposymbiotic nymphs of several heteropterans were reported to exhibit retarded growth and/or nymphal mortality (Abe *et al.,* 1995) suggesting that the symbionts play some important roles for the host insects. The microbiological nature of symbiotic bacteria has been poorly understood. Although several bacteria have been isolated from the gut of some heteropterans in general (Dasch *et al.,* 1984) and reduviids in particularly it has scarcely been confirmed whether the isolates are identical to the predominant bacteria harbored in the midgut caeca.

Insects dependent on restricted diets, such as plant sap, Heamolymph feeder commonly carry symbiotic microorganisms that are thought to provide nutritional supplements for their hosts (Dasch *et al.,* 1984). In this respect the Heteroptera are an interesting research subject for understanding the diversity and evolution of insect-microbe symbiotic associations, because a number of heamolymph feeders and predators of other arthropods are found in this well-defined insect group. Certainly, symbiotic relationships in the Heteroptera correlate reasonably well with diet; symbiotic bacteria tend to be found in heamolymph feeders. Among the Haemolymph-sucking groups of the Heteroptera, the family reduviidae shows the most remarkable behavioral and anatomical arrangement for transmission of the symbiont. Large-scale food processing machinery is available for making the hundreds or thousands of kilograms of diet per day that may be enquired in a mass rearing facility (Rothrock, 1996; Cohen, 2000). The insectary worker is the major source of microbes in a rearing facility (Sikorowski, 1984; Sikorowski and Goodwin, 1985), and once the worker is removed microbes from as much of the diet production and rearing process as possible, the contamination problem can be solved.

Advancement of augmentative biological control would also greatly profit in post-rearing distributional technology, field release and field evaluation systems. A number of arthropod natural enemies have been reared with variable success on artificial diet. Several predatory heteropterons, Chryospids, Reduviids and Coccinellids have been reared for consecutive generations on diets devoid of insect materials. In the present study microbiological aspects, such as microbial diversity, localization in-vivo and fitness effects on the host insect, of the bacteria contained in the gut were investigated.

1.6. Enzyme Activity

Several insect families contain omnivores. However, some predatory insects exclusively predate and feed on other animals (Alomar and Wiedenmann 1996). The origin of feeding habits among the Heteroptera remains controversial (Sweet 1979; Cobben, 1979; Cohen, 1990; Schaefer, 1997; Wheeler, 2001). The diverse trophic habits of bugs make the reduviidae ideal for studies of feeding strategies, including digestive enzyme composition employ macerate (or lacerate) and flush feeding (Miles, 1972; Hori, 2000; Wheeler, 2001) that incorporates piercing/sucking mouthparts and watery saliva from the salivary gland complex. Reduviids feed in a manner that is typical of heteropterans, piercing and cutting tissues with their stylets while injecting digestive enzymes through the salivary canal to liquefy food into nutrient rich slurry. The food slurry is ingested through the food canal and passed into the alimentary canal where it is further digested and absorbed (Cohen, 2000). Digestive enzymes are produced and distributed in various regions of the gut and salivary gland different proportion and quantity. Wide ranges of digestive enzymes were recorded in the alimentary canal of insects and their level varies in relation to diet. It is a well-known fact that the digestive enzymes play a major role in insect physiology by converting complex food materials into micro molecules necessary to provide energy and metabolites for growth, development and other vital functions. A consumers ability to use plant or animal materials for food is indicated by the presence of specific digestive enzymes (Miles 1972; Cohen 1990, 1995, 1996, 1998a, 1998b, 2000; Agusti and Cohen, 2000; Hori, 2000; Zeng and Cohen, 2000a, 2000b) which includes proteases, hyaluronidase, and lipase (Cohen 1998b, 2000) and amylase (Cohen 1996; Sahayaraj, 2007). The chemical composition of the watery saliva of hemipteran insect is crucial for effective feeding because there

insects very heavily on saliva for extra oral digestion (Cohen, 1995, 1996, 1998a). Salivary and gut enzyme compositions were studied for predatory heteropterans (Cohen, 1995, Cohen and Wheeler, 1998).

The digestive physiology of reduviid predators solicits greater attention in view of its economic importance. The nutritional need and the knowledge of the functional organization of digestive system of reduviid predators may be useful in designing Oligidic diet for mass production (Sahayaraj *et al.,* 2007). Moreover this information could be useful to understand how reduviids adopt to its natural or factitious prey or artificial diets. Among the digestive enzymes, amylase, invertase, lipase and protease, activities are of great importance in the digestion of food. Utilization of macronutrients from the available prey food depends on the digestive enzymes. Digestive enzymes of alimentary canal and the salivary gland of *Sophrorhinus insperatus* Faust (Hori, 1969; Ravikumar *et al.,* 2002) were investigated. Studies on digestive enzyme profile of Indian reduviids were not available except the preliminary works of Ambrose and Maran (2000) and Sahayaraj *et al.* (2007).

1.7. Gut Content Assay with ELISA

Progress in quantifying predation in agricultural systems has been hampered by the difficulty of studying predation in the field. Unlike parasitism, evidence of predation is seldom preserved in the field and researchers must generally rely on indirect and often less precise measures of activity (Luck *et al.,* 1988; Sunderland, 1988; Naranjo and Hagler, 1998). Many other factors, such as,small size, nocturnal activity, cryptic behavior, and pre-oral digestion, contribute to the difficulty of observing and measuring predation under natural conditions (Hagler and Naranjo, 1994 a, b; Hagler and Naranjo, 2005).

Techniques to study the interactions between predator and prey communities have become increasing complex as they attempt to address the imbalance created by visual identification. These include radio-isotope labeling, the application of stable isotopes, electrophoretic detection of prey isozymes, the detection of prey pigments by chromatographic analysis and the detection of prey proteins using polyclonal antibodies (Sunderland, 1988; 1996; 2000; Pierce and Boyle, 1991; Greenstone, 1996; Greenstone *et al.,* 2005). Current predator–prey studies, however, tend to rely on monoclonal antibody and/or DNA-based technology, which allow accurate and rapid detection of prey remains within predator guts or faecal samples. Immunological assays using prey-specific protein antibodies (Greenstone and Hunt, 1993; Powell *et al.*, 1996; Hagler and Naranjo, 1997; Hagler, 1998; Symondson *et al.*, 1999a, b; Shapiro and Legaspi, 2006) was widely used to identify predator gut contents. These assays are used to determine absence or presence of prey in the gut. The accuracy of the assay depends on several factors, including temperature, meal size, time since feeding, resistance of the target protein to digestion, and predator species (Hagler and Naranjo, 1997; Symondson 2002; Symondson *et al.*, 1997, 1999b, Hagler, 1998).

The works were carried out on *Lygus hesperus* (Hemiptera: Miridae) predators (Hagler et al., 1992) *Bemisia tabaci* (Homoptera: Aleyrodidae) and *P. sagitta* (Hagler and Naranjo, 1994a); *Pectinophora gossypiella* (Lepidoptera:Gelechiidae), *P. maculiventris* (Hagler and Naranjo, 1994a, 2005); *Collops vittatus* (Coleoptera:Melyridae) (Hagler and Naranjo, 1994b), *Hippodamia convergens* (Coleoptera:Coccinellidae) (Hagler and Naranjo, 2004), *Otiorhynchus sulcatus* (Coleoptera:Curculionidae) (Crook and Solomon, 1997), *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae), *Orius* sp. (Heteroptera: Anthocoridae) (Sansone and Smith, 2001, Ruberson and Greenstone, 1998), *Pterostichus melanarius* (Coleoptera:Carabidae) (Bohan *et al.*, 2000); (Araneae:

Linyphiidae) (Harwood *et al.* 2005); *Helicoverpa armigera* eggs and larvae (Lepidoptera: Noctuidae) (Sigsgaard *et al.* 2002): *Nilaparvata lugens* (Homoptera: Delphacidae) (Zhao *et al.*, 2004), Lim and Lee, 1999), Homoptera:Aphididae *Pachygnatha degeeri* (Araneae:Tetragnathidae) Harwood *et al.* (2004 and 2005a), Homoptera: Aphididae (Coleoptera: Carabidae) Winder *et al.* (2005).

In the context of biological control, increased prey biodiversity would be predicted to enhance the ability of populations of generalist predators to achieve sustainable levels of pest control. However, pest species are often poor on terms of nutritional quality (Toft, 1999, 2005) and are avoided by some predators (Toft, 1997). Hence, increased dietary diversity has the potential of the predators from feeding on pests and ultimately reduce levels of biological control (Halaj and Wise, 2002; Madsen *et al.,* 2004; Koss and Snyder, 2005; Wise *et al.,* 2006). Elucidating possible shifting preference of Oligidic diet (OD) for alternative non-pest prey in complex food webs, and uncovering the strength of those tropic linkages, are therefore essential for incorporating biological control by predators reared with Oligidic diet in integrated pest management (IPM).

Of the many methods used for studying predation, postmortem approaches are among the most direct and least likely to introduce bias through unintentional experimental disruption (Luck *et al.,* 1988; Sunderland, 1988). Postmortem methods include gut dissection and chromatographic, electrophoretic, PCR, and immunological analysis of predator gut contents. Depending on the type of antibody and assay system used, immunological methods can be species or stage specific, highly sensitive, and rapid enough to facilitate screening of thousands of predators in a shortest period (Sunderland, 1988; Greenstone, 1996).

Predator digestion rate, prey size, predator size, diet and their physiological state of the prey can affect the outcome of a gut content immunoassay (Sunderland, 1996). Before a precise estimate of predation can be made, these factors must be considered. Despite these characteristics, immunoassays remain a qualitative method that provides direct evidence of predation by specific species but rarely provides quantitative estimates of the number of prey killed. However, previous studies (Sopp and Sunderland, 1989; Symondson and Liddell, 1993; Shapiro and Legaspi, 2006), suggest that gut content ELISAs vary in efficacy among pest species. One of the fundamental parameters for qualitatively or quantitatively estimating predation using immunoassays is the period of time the prey antigens remain detectable in a predator's gut. The detection interval is a key parameter in most indices that have been developed to assess predation using immunoassays (Sopp *et al.,* 1992) and is very important in comparative evaluations of different predator species feeding on the same prey.

1.8. DNA Quantification

Rapid PCR-based screening systems for the study of the prey diversity of generalist predators have been developed to expand the potential of molecular detection into various areas of food-web research. The techniques described by Harper *et al.,* (2005) using a PCR to simultaneously amplify DNA from a range of prey species. The retention time for DNA within the gut of a predator during digestion is influenced by factors including the size of the target DNA molecule. Predation by the coccinellid beetle *Coleomegilla maculate* De Geer (Coleoptera: Coccinellidae) upon the eggs of the European Corn Borer *Ostrinia nubilalis* (Hubner) (Lepidoptera: Crambidae) has been characterized by PCR amplification of four fragments of prey genomic DNA of different sizes from predator guts (Hoogendoorn and Heimpel 2001, 2002). Use of PCR in the gut

contents analysis of predator (Agusti *et al.*, 1999, 2000; Zaidi *et al.*, 1999; Chen *et al.*, 2000; Hoogendoorn and Heimpel, 2001) was studied by many workers.

Amplifying prey DNA from predator gut contents is used increasingly to elucidate predator-prey relationships, and is comparable to serology in many aspects (Symondson 2002, Dodd *et al.*, 2003). For instance PCR amplified DNA of noctuid larvae by mirids (Agusti *et al.*, 1999) aphid predation by a spider, ladybird and lacewing larvae (Chen *et al.*, 2000, Greenstone *et al.*, 2005), European corn borer predation by a Coccinellid (Hoogendorn and Heimpel 2001), mosquito predation by a dragonfly (Morales *et al.*, 2003) were available in the literature.

Identification of the gut contents of predatory insects can provide information on trophic relationships and the dynamics of predator-prey interactions. Several problems may be encountered in determining the diet of predatory insects in the field. Direct observations of predation (Legaspi, 1996; Munyaneza and Obrycki, 1998) can be complicated by the fact that both prey and predator are often small and cryptic. Microscopic analysis of gut contents (Aussell and Linley, 1994; Powell *et al.*, 1996; Sleaford *et al.*, 1996; Triltsch, 1997) was possible for predators that ingest relatively large prey fragments. This work builds upon a number of recent studies on the use of PCR in the analysis of predator gut contents (Agusti *et al.*, 1999; Agusti and Cohen, 2000; Zaidi *et al.*, 1999; Chen *et al.*, 2000; Hoogendoorn and Heimpel, 2001).

1.9. Augmentative Field Release and Biocontrol Potential Evaluation

Biological control is often viewed as a promising alternative or complement to pesticides in IPM programme (Bengtsson *et al.*, 2005; Hole *et al.*, 2005). The successes and failures of biological control have been extensively reviewed (van Driesche and

Bellows 1996; Cardinale *et al.,* 2003; Aquilino *et al.,* 2005; Byrnes *et al.,* 2005; Wilby *et al.,* 2005). Factors that can influence the effectiveness of biological control agents include agent specificity (generalist or specialist), the type of agent (predator, parasitoid, or pathogen), the timing and number of releases, the method of release, synchrony of the natural enemy with the host, field conditions, and release rate. Augmentative, or inundative, biological control is the release of large numbers of natural enemies to augment natural enemy populations or inundate pest populations with natural enemies, Snyder *et al.,* 2008).

Augmentative release of the predators is a main component in the IPM and especially the reduviids play a major role in the suppression of various pests of economic importance (Schaeffer, 1988; Sahayaraj, 1999b; 2002b and Sahayaraj and Martin, 2003, Sahayaraj and Ravi, 2007). 120 pests attack groundnut both at crop stage and storage (Ramaraju *et al.,* 1998). The pests are classified as defoliators, borers and sucking pests. The important defoliators are the larval forms of *Amsacta albistrigia* Walker, *Aproarema modicella* Dev., *Spodoptera litura* Fab., and *Helicoverpa armigera* Hubner etc. and they cause severe damage to groundnut (Amin, 1983; Panchabhavi and Nethradhaniraj, 1987 and Wightman and Rao, 1993). The irrigated groundnut in Tamil Nadu is severely attacked by *S. litura* and *H. armigera* (Peter and David, 1998). The other major pests of groundnut are jassids (Singh *et al.,* 1993), thrips, white flies, bugs, beetles and grasshoppers (Jayanthi *et al.,* 2000 and Sridhar and Mahto, 2000). The extent losses of groundnut by feeding and transmitting virus disease of aphids are well reported (Nandagopal and Gunathilagaraj, 2008). Grasshopper causes leaf damage and yield loss in groundnut. It was controlled by both biological and chemical agents in groundnut field (Peveling *et al.,* 1999).

In India, groundnut cultivation is mostly monoculture in major groundnut growing states likes Andhra Pradesh, Gujarat, Tamil Nadu, Karnataka, Maharashtra and Orissa. However, there is a great diversity in the cultural practices followed in different agro climatic regions. Extensive cultivation in groundnut with introduction of new varieties and adoption of modern agro techniques has brought a great deal of changes in the abundance of cosmopolitan and regional pests and their associated natural enemy complexes.

In India, an exotic reduviid predator *P. laevicollis* was colonized and the laboratory reared bugs were released in large numbers on the coconut palm in Kerala, Lakshadeep and Karnataka and they controlled the beetle *Oryctes rhinoceros* Linn. (Antony *et al.,* 1979). After a long time Sahayaraj (1999 b) Sahayaraj and Martin (2003), Claver and Ambrose, 2001b; Sahayaraj, 2002b mass reared *R. marginatus* in the laboratory and released in the groundnut field and reported that greatly suppressed the population of *Spodoptera litura*, *Helicoverpa armigera* and *Aproaerema modicella.* Initially *Platymeris laevicollis* was released in the coconut field to reduce the grubs and adults of *O. rhinoceros* (Antony *et al.*, 1979). Sahayaraj (1999 b) released *R. marginatus* in the groundnut field and observed the suppression of lepidopteran pests and reported high groundnut yield. Grundy and Maelzer (2000) evaluated biological control of *P. plagipennis* in the pigeon pea field and reported the control of various pests in Australia. Sahayaraj (2002b) integrated certain botanicals along with *R. marginatus* in the groundnut field and obtained a good groundnut yield. Ambrose (2000) and Claver and Ambrose (2001b) released *R. kumarii* in cotton and pigeon pea field cages and reported the pest suppression by the predator. The present study was undertaken to find out the impact of the different prey reared *R. marginatus* separately on groundnut pest infestation, yield and cost benefit ratio in groundnut fields.

Carpenter and Greany (1998) showed that medium-reared *D. introita* searched for and parasitized host pupae in field-cage experiments. These authors suggested that the ability to rear *D. introita* on an inexpensive artificial medium significantly enhanced the possibility of mass rearing parasitoid for use in inundative releases against *Spodoptera* spp. Host location and host acceptance by female parasitoids can be influenced by maternal factors such as age (Doutt, 1959), physiological state, and previous experience (Morrison and King, 1976). However, until the development of effective artificial rearing systems, laboratory-reared parasitoids completely lacking, both as developing immatures and as adults, were not available.

The objectives of the current study was to assess the effect of factitious, natural prey and meat based artificial diets on the development, survival and reproduction of *R. marginatus*

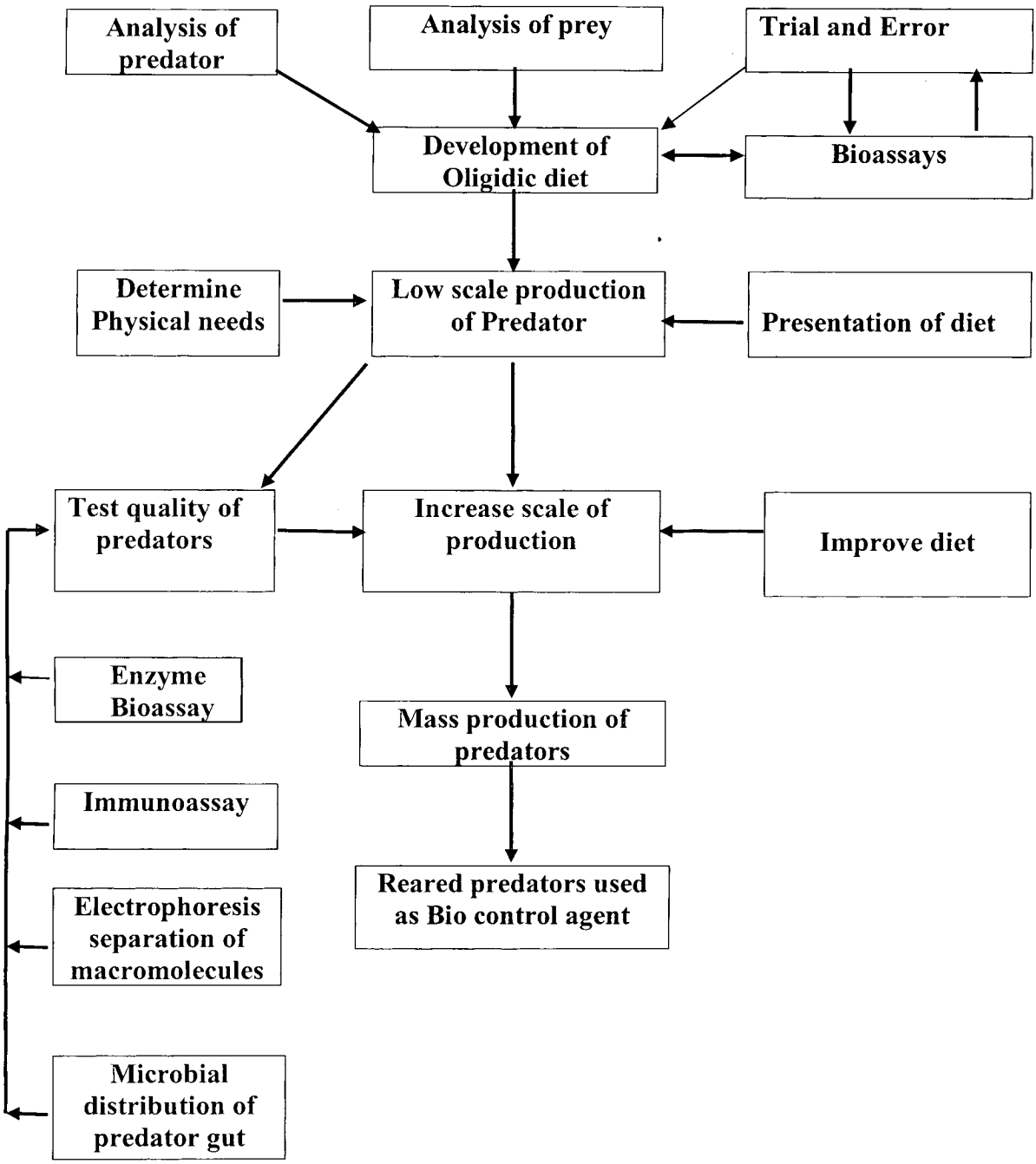
1. To study the life table of *R. marginatus* on Nymphal development of *R. marginatus* was monitored on five diets: fourth instar larvae of *C. cephalonica* (T_1), *C. cephalonica* weekly once with water (T_2), Oligidic diets (OD), (4, 5 and 6) (T_3), OD with weekly once with *C. cephalonica* (T_4) and *S. litura* (T_5).

2. To record the prey preference (visual observation and ELISA) and evaluate the biological control potential of *R. marginatus* both in laboratory and field conditions for two subsequent seasons at summer 2006 and khariff 2007, Tamilnadu, India.

3. To find out the impact of OD on the qualitative and quantitative enzyme profiles (salivary gland and alimentary canal). The activity values of the main digestive enzymes of *R. marginatus* to evaluate the nutrition specialization to other food substrates. Autothonomous gut bacterial populations and their hydrolytic enzyme activities of *R. marginatus.*

4. To record the genetic similarities and variability of *R. marginatus* using three common primers.

Materials and Methods



Flowchart for experiment

CHAPTER - 2 MATERIALS AND METHODS

2.1. Collection and Maintenance of Pests and Reduviids

A laboratory colony of *R. marginatus* was maintained with individuals collected from the agricultural and nearby scrub jungle ecosystems of Tirunelveli District, Tamil Nadu, India. The stock colony were cultured under laboratory conditions (30 $\pm 1^{O}$C and 80% Rh and 11L: 13D) on *C.cephalonica,* as well as natural prey *S. litura. S. litura* was collected from cotton agroecosystem at Sivanthipatty, Alangulam, Thalapathysamuthram, Pavoorchatram and Killikulam agricultural college agroecosystem, Tamilnadu, India, and mass reared on their natural host. Laboratory emerged *R. marginatus,* and pests were used for this experiment. *C. cephalonica* was also maintained under same laboratory conditions using the methodology of Sahayaraj (2002a).

2.2. Oligidic Diet ingredients and preparation

The meat-based oligidic diet used in this study was prepared following the method used by Sahayaraj *et al.* (2006) with some modifications. The source and other ingredients present of the oligidic diets are presented in the table 1. Source ingredients such as pig liver and pig blood were dried in hot air oven at 60^{O}C for 25 to 30 minutes. They were ground well by mortar and pestle and stored in refrigerator for further use within a month. Hundred milliliters of distilled water was boiled at 100^{O}C for 20 minutes. Ten milliliters of the boiled water was taken for dissolving the milk powder (Lactogen, Nestle, Mumbai, India) with boiled water and it was allowed to cool. Water-soluble yeast extract (Fine chemicals Ltd, Mumbai), dried egg yolk, liquid honey (Dabur Narendrapur,

West Bengal, India), and acetic acid (Glaxo, Gujarath, India) were added to the remaining 90 ml water and in desired quantity and boiled at 100°C. After 10 minutes, the temperature was reduced to 40°C and then the source ingredients, multivitamin, vitamin C and E and streptomycin (Sarabairaman, Vadodara, India) were added and stirred for thorough mixing. Then the milk powder solution was added and stirred well. After the thorough mixing the prepared diet was allowed to cool at room temperature and then it was filtered through Whatman No.1 filter paper. Filtered liquid diet was stored in 125ml reagent bottles in refrigerator for the future use on longer than 2 to 3 weeks.

2.3. Behavioral Studies

Preliminary behavioral studies were conducted in reduviids to understand their approaching behavior towards the oligidic diets. Field-collected and laboratory emerged adults as well as nymphs of *R. marginatus* were used for the study.

2.3.1. Choice and Non-Choice Test

To know the suitability of the oligidic diets, feeding behavior of *R. marginatus* was determined by both the choice and non-choice test. For the laboratory testing, 1 to 3 concentrations of the oligidic diets (AD) (1 to 3) were placed in the olfactometer along with water (refere annexure figure 1). Then 24 h starved *R. marginatus* adults were introduced into the olfactometer. Fifteen replications were made for each experiment. The approaching time (AT) and consumption time (CT) and choice of feeding by reduviid were recorded for one hour continuously by visual method in each experiment (Sahayaraj and Paulraj, 2001). The equipment was cleaned with 0.2% Sodium hypochloride between each experiment to prevent cross contamination between replications.

2.3.2. Stage Preference

Stage preference study of *R. marginatus* with different life stages of *D. cingulatus, S. litura* and *C. cephalonica* separately was carried out by a choice experiment as described by Holling (1966). *R. marginatus* was introduced into a petridish and *D. cingulatus* first, second, third, fourth and fifth instar nymph were released and the predatory behavior was observed consecutively for 6 hrs by visually. Successfully captured, killed and consumed prey stage was recorded as preferred stage of the reduviid. Fourth, fifth and adult predators were provided for the third, fourth, fifth nymphal instars and adults of *D. cingulatus.* For both *S. litura* and *C. cephalonica* preference. Life stages of *R. marginatus* were provided with all the five nymphal instars of the prey separately. Ten replications were maintained for each life stage of the predator separately. The preferred life stages of the pests were used for the biological control potential evaluation studies.

2.3.3. Feeding Behavior

The laboratory emerged *R. marginatus* life stages (except first instar) were used for evaluation of their biocontrol potential. Preferred stages of *D. cingulatus* (5/ container) was introduced into the container containing cotton twig and it was allowed to acclimatize for 10 minutes. Then a *R. marginatus* was introduced in to the same container and the feeding events like approaching time, handling time, and sites preferred by the reduviid for feeding were recorded continuously for 6 hours. After 24 hrs, weight gained and number of prey consumed by a predator was recorded. Ten replications were maintained for each life stage of the predator separately.

2.4. Development

Nymphal development of *R. marginatus* was monitored on five diets: fourth instar larvae of *C. cephalonica* (T_1), *C. cephalonica* weekly once with water (T_2), Oligidic diets (OD), (4, 5 and 6) (T_3), OD with weekly once with *C. cephalonica* (T_4) and *S. litura* alone (T_5). For each treatment, 100 newly hatched first instars *R. marginatus* was randomly taken from laboratory culture and place individually in plastic vials (6 cm height and 4.5 cm diameter). Water and oligidic diet were soaked in cotton (5 mg) and provided to the reduviids. For T1-T5 categories nymphs (except T_3), reduviids were also provided one or two preys per day. Fresh weight of *S. litura*, *C. cephalonica* larvae were 200 mg and 150 mg respectively. Larvae of both prey species were made partly defenseless by crushing their head capsules, this also prevents the larvae from web spinning. Biological parameters like nymphal developmental periods, weight, survival rate and sex ratio (♀/(♂+♀) of *R. marginatus* were recorded upon emergence for each category. The nymphs and adults were weighed on a Dhona, monopane balance (± 0.1mg). Occurrences of morphological abnormalities were also recorded if any.

2.5. Reproduction

Adults were also maintained on the same diet as in their nymphal instars. For each diet, 25 pairs were collected from cultures and maintained in 500 ml capacity plastic box (5.5 cm length and 12.5 cm diameter). Filter paper was furnished on the bottom of the container. Oligidic diet, natural and factitious hosts were provided and excess and unconsumed prey were replaced every day. Preoviposition, oviposition and postoviposition period, total number of eggs laid per female and egg hatched on each category were monitored on daily basis. The experiments were carried out in

environmental chambers for three generations (Remi, Mumbai) continuously at 28± 1°C, a relative humidity of 75± 80% and a photoperiod of 11:13 (L:D) h.

2.6. Life Table

By using oviposition data, life tables was constructed according to the methods recommended by Birch (1948) and modified by Southwood (1978). In life table statistics, the intrinsic rate of increase has been determined using the equation '$\sum e^{-rm \times lxmx-1}$', where e is the base of natural logarithms, x is the age of the individuals in days, 'l_x' is the number of individuals alive at age x as the proportion of 1 and m_x is the number of female offspring produced per female in the age interval 'x'. The sum of products '$l_x m_x$' is the net reproductive rate 'R_0'. The rate of multiplication of population for each generation has been measured in terms of females produced per generation. The precise value of cohort generation has been calculated as follows:

$$\text{Mean length of generation } T_c = \frac{\sum l_x m_x}{R_0}$$

The arbitrary value of innate capacity for increase 'r_c' has been calculated applying the equation

$$\text{Innate capacity for increase for increase in number } r_c = \frac{\log_e R_0}{T_c}$$

This is an appropriate rm value. The values of negative exponent of 'e^{-rmx}' ascertained from this experiment often lay outside the range. For this reason both sides of the equation have been multiplied by a factor of '$\sum e^{7-rm \times lxmx}$' - 1096.6 (Birch, 1948 and Watson, 1964). The two values of '$\sum e^{7-rm \times lxmx}$' have been then plotted on the horizontal axis against their respective arbitrary 'rm' on the vertical axis. Two points have been

then joined to give a line, which is intersected by a vertical line drawn from the desired value of '$e^{-rm \times lxmx'}$ (1096.6). The point of intersection gives the value of 'rm' accurate to three decimal places. The precise generation time 'T' has been then calculated from the equation

$$T = \frac{\log_e R_0}{R_m}$$

The finite rate of increase (λ) has been calculated as '$e^{rm'}$. This 'λ' represents the number of individuals added to the population per female per day (Siddiqui *et al.,* 1973). The weekly multiplication of predator population has been calculated as 'e^{rm7}'. The doubling time has been calculated as log '2/log λ'.

2.7. Microbiology

2.7.1. Dissection of Predators

Laboratory emerged adult of *R. marginatus* were selected randomly prior to the morning feed when the gut was empty and kept at 4^0 C for 15 min prior to handling to prevent regurgitation, surface sterilized with 0.1% mercuric chloride solution for 2 minutes and thrice washed with sterile distilled water. Under aseptic condition each insect was carefully dissected by using pins, fine forceps and razors in a dissection tray filled with sterile phosphate buffered saline (P^H, 6.9). Isolated guts were individually washed several times with fresh phosphate buffered saline to minimize the possible microbial contamination and used for the study and the dry weight of the gut was recorded

2.7.2. Enumeration of THMP of Gut Content

The gut was homogenized with sterile insect ringer's solution (IRS) in mortar and pestle. The homogenate was filtered through Whatman filter paper no. 1 and the pH was measured using pH meter (Mic Datal and Co., Chennai). 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 NA plates were incubated at 37^0 C for 24 - 48 hours whereas the SDA plates were incubated at 28^0 C for 24-72 hrs respectively. Microbial colonies appeared after the incubation period was enumerated and the numbers of colony forming units were expressed as dry weight of the gut.

2.7.3. Identification of Bacteria

Different morphological microbial colonies were selected, subcultured and stored at 4^0 C on respective agar slants. Bacterial strains were identified using the criteria suggested by Buchanon and Gibbons (1979) based on morphological, cultural and biochemical characteristics.

2.7.4. 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 milk powder (protease) as substrates. Pure culture of each bacterial isolate was streaked on respective media and utilization of these substrates was determined by observing the clear zone around the colonies (Buchanon and Gibbons, 1979). The screening experiments were performed in triplicates.

2.8. Enzymology

2.8.1. Preparation Enzyme Source

Rhynocoris marginatus adults were asphyxiated in a deep freezer for 30 minutes and their guts were carefully dissected out in sterile phosphate buffered saline (P^H 7.4). From the isolated digestive tract, fore and hind guts were separated individually, washed several times with fresh phosphate buffered saline to minimize possible microbial contamination and used for enzyme bioassay. Similarly entire salivary gland was removed carefully by teasing the head and used for this study. Wash the salivary glands and alimentary canal in distilled water thrice and once with 0.15 M NaCl solution. Transfer the salivary glands and alimentary canal separately in to a small test tube containing 1 ml of distilled water and homogenize with tissue Homogenizer (Remi 8000 RPM, Mumbai). Then centrifuge the homogenise at 15000 rpm for 15 minutes at 4^0C. The resulted supernatant was used as enzyme source for this experiment.

2.8.2. Qualitative and Quantitative Enzyme Profile

Invertase, amylase, lipase (Nigam and Omkar, 2003), pepsin (Tonapi, 1996), protease and trypsin (Balogun and Fisher, 1970) qualitative profiles were performed using sugar, olive oil emulsion, acid casein, peptone, casein, alkaline casein, as substrates respectively. Based upon the color intensity, the enzyme quantities were expressed as less (+), moderate (++) and maximum (+++) activities.

2.8.3. Quantitative Enzyme Bioassays

2.8.3.1. Amylase

Amylase was measured using 3,5-dinitrosalicilic acid reagent prepared (DNS) according to Bernfeld (1955) and Baker (1991) methods. Take 200 µl of enzyme source in a test tube (5 ml capacity) and add substrate with buffer solutions each of 100 µl (0.2 % soluble starch in 50 Mm $CaCl_2$ and 20 Mm NaCl). The assay was terminated by the addition of 500 µl DNS. The mixture was incubated at 100^0C for 5 min, cooled and after the addition of 500µl of distilled water. The color development was read at 575 nm and composed with standard maltose hydrate. All assays were carried out at 30°C.

2.8.3.2. Invertase

Sumida *et al.* (1994) method was adapted to estimate the invertase activity using 0.2% sucrose as substrate in the reaction mixture, consists of 10 Mm phosphate buffer of P^H 6.8 and by measuring the glucose at 30°C. Moreover the glucose estimation was done using the dinitrosalicylic acid reagent with dextrose for this standard at 540 nm.

2.8.3.3. Lipase

Lipase enzyme activity was estimated using Olive oil emulsion as a substrate in the reaction mixture with 10 mM phosphate buffer (P^H 7.2) and measuring the P^H drops by about 0.2 units. 0.1N Na OH was added to bring the PH back to the original level. The amount of Na OH added was measured and it is equivalent to the fatty acids (Jeyaraman, 1985) formed during the reaction.

2.8.3.4. Protease

60 µl of diluted enzyme was added to 200 µl of 1% azocasein (in 0.2 M glycine–NaOH, pH 10.0) and incubated at 37^0 C for 30 min. The reaction was terminated by the addition of 300 µl of 5% trichloroacetic acid (TCA). After centrifugation at 15000 g for 10 min, an equal volume of 1 M NaOH was added to the supernatant and absorbance was measured at 450 nm. Determinations were always carried out in triplicate. Azocasein and tyrosine were used as substrate and standard (Brock *et al.,* 1982).

2.9. Macromolecules

2.9.1. Extraction of Protein and Sample Preparation

Each insect was homogenized in ice hold phosphate buffered saline (PBS 500 µl) for one minutes with a homogenizer (Remi homogenizer, Mumbai) in 0.9 ml of Phosphate buffered saline containing 0.1 ml of protease inhibitors (Genei, Bangalore). The extracted aliquots were stored at a -20 ^{0}C (INLAB, Equipments pvt. Ltd. Chennai) until assayed. Total protein was determined using the Lowry's method discussed earlier. Gut particles were removed from aqueous homogenates by centrifugation for 10 minutes at 15000 rpm. Protein was quantified form a standard curve by using bovine serum albumin as a standard. Color was read by a spectrophotometer at 580 nm and results were discussed.

Individuals of *R. marginatus* were homogenized in 500 µl of PBS. An aliquot from each sample from the first feeding trial was assayed by the Immuno assays by the indirect ELISA described below. The optimum sample (500 µl) volume used for immunoassays was *S. litura, D. cingulatus* and *C. cephalonica* and Oligidic diet.

2.9.2. Total Macromolecule Content

10 uniform sized preys *C. cephalonica* and *S. litura* and 5 g pig liver were used for the macromolecules profile analyses. Total carbohydrates was estimated using glucose as a substrate (Nigam and Omkar, 2003). Total protein was determined using the Lowry's method. Gut particles were removed from aqueous homogenates by centrifugation for 10 minutes at 15000 rpm. Protein was quantified form a standard curve by using bovine serum albumin (BSA) as a standard at 580 nm. Lipid was estimated olive oil using as a standards (Jeyaraman, 1985).

2.9.3. SDS-PAGE for body Proteins

The protein profiles were studied by SDS-PAGE according to the method of Laemmli (1970). A 7.5% slab gel was cast between the glass plates supplied by Bangalore GENEI. A 5% stacking gel (P^H 6.8) was also cast over the resolving gel. The protein samples were mixed with an equal volume of sample buffer (0.125 M Tris-HCL P^H 6.8, 4.6% SDS, 20% sucrose, 10% B-mercaptoethanol and 0.1% bromophenol blue) and boiled for 2-3 min then centrifuged at 10,000 g for 5 minutes and 25µl each of marker and samples were loaded on to the well. In each lane the samples containing 100-125 µg protein concentration were loaded. Initially, a current was applied at a constant voltage of 50 V till the samples reached the resolving gel and then increased to 100V till the completion of run using electrophoresis power pack. Standard protein molecular weight markers (low molecular weight, Gene, Bangalore) were also run in one lane parallel to the samples. The gels were fixed in staining Comassine Brilliant Blue (CBB) (ethanol: acetic acid: water 40:50:10) solution for overnight and destained using ethanol: acetic acid: water(40:50:10) till the bands were clearly visible.

2.9.4. Extraction of DNA

For DNA extraction, insects stored in ethanol were successfully extracted by drying the samples prior to extraction. Sambrook *et al.,* (1989) method were followed for DNA extraction with some modifications as described below.

R. marginatus was taken in a test tube and kept on ice. Add 500 µl of TEN (Tris: EDTA: NACL) extraction buffer / 2% SDS (9:2) and 0.1 volume (50 µl) Proteinase – K. Mix the solutions well and the total volume was made up to 550 µl. Using the homogenizer the sample was gentle homogenize. Incubate the homogenate at 55-60°C for one hour. Add 500 µl of phenol (pH 8.00) and 448 µl of chloroform and 2 µl of Iso Amyl Alcohol (25:24:1) (IAA) and shake for 10 min. Centrifuge the samples at 13000 rpm for 10 minutes using a microcentrifuge. The lower (organic) phase was disposed of in suitable phenol waste bottles. 500 µl of chloroform and Iso amyl alcohol (1:1) preparation was added to the sample and shaked gently for 10 minutes. Remove the upper aqueous phase and place this into a new eppendroff tube. Take 50 µl of 2M sodium acetate (P^H 5.6) and 1ml of ice cold ethanol (100%) to the aqueous solution. The solution is inverted several times. Kept the samples at -20°C overnight. Centrifuge the sample at 13000 rpm for 15 minutes. Discard the supernatant is blotting out with what mann paper and the precipitate is washed in 1 ml of 70% ethanol. The washing step involved centrifuged at 13000 rpm for 5 min with the pellet of DNA being on the inner surface during the spin. The alcohol was tipped out after each spin and blotted on a clean tissue and then the remaining supernatant was removed using a fine pipette attached to a vacuum suction. Thereafter, the DNA pellet was air dried for 15-30 minutes in room temperature. When the tubes were seen to be dry the pellet was resuspended in 20µl TE buffer and stored at -20 °c up to one month.

2.9.4.1 Quantification of DNA

The quantification of DNA was performed in UV-Visible spectrophotometer (Eppendroff, Bangalore), in order to find out the purity as well as the quantity of the DNA. The purity of DNA was calculated by using the formula:

Purity of DNA = OD at 260nm/OD at 280nm

The amount of DNA was calculated by using the formula:

Amount of DNA = OD at 260nm x 50 x Df/1000

1 OD = 50µg of DNA.

2.9.4.2. Procedure for PCR

0.2 ml PCR tubes for each genomic DNA were labeled and tubes were arranged in the rack provided to hold the 0.2 ml tubes. 2 µl of template DNA (Calf thymus) having the concentration of 50 ng was added to the tubes already labeled and kept in the PCR tube rack. The following reaction cocktail was prepared in a 1.5 ml micro centrifuge tubes for required number of reactions plus two reactions to compensate the pipeting loss. 23 µl of the reaction mixture was added to the 0.2 ml PCR tube, which was already loaded with 2 µl of template DNA making the final volume to 25µl. The content was mixed and performs the PCR reaction was performed in a thermocycler following the program given below.

For checking the amplified product we run the product in 1.5 % agarose gel and stained with Ethidium bromide. 1.5% agarose gel was casted with suitable gel electrophoresis system along with Ethidium bromide. 2 µl of 6X loading dye was added

to 10 µl of the PCR product and mixed well. The gel was loaded with PCR product. The gel was run in 1X TBE at 50 V till the Bromophenol Blue dye front reach the end of the gel. The gel was viewed using UV transilluminator (Genei, Bangalore) and the gel was documented using photo-documentation system (Biotech, Yarcad, Tamilnadu). Photograph document was preserved as hard copy or the image was stored in the hard disc as a softcopy. The banding pattern was analysed.

The extracted DNA from the experimental predators was subjected to PCR analysis using 6 primers 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 containing 1 mM dNTP's 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 Taq polymerase enzyme (5U / µl) and sterile distilled water. Above mentioned 25 µl of reaction mixtures were placed in PCR tubes in two layers. The bottom layer consists of all reagents except Taq, sterile distilled water and template DNA. PCR was carried out in a programmable thermal controller (Master cycler ep's ependroff) for 40 cycles (refer table 1 and 2). Amplification products were separated on a 1.4% agarose gel, and the banding profiles visualized in UV Transilluminator GeNei^{TM}Image system Bangalore (Carezza Booto *et al.,* 2005).

2.10. Immunology

2.10.1. Effect of Meal on Antigen and Antibody Interaction

Predators were deprived of prey for 48 h prior to testing. After starving, individual predators were placed into a small box (500 ml capacity) and allowed to feed *S. litura, D. cingulatus,* and *C. cephalonica* and Oligidic diet separately. Predators were then placed in

Table 1. Reagents for RAPD analysis

Sl. No	Constituents	Quantity	Final concentration
1.	Sterile Double distilled water	18.5 µl	As required to make up to 25µl
2.	dNTPs	1 µl	200µM of each dNTP
3.	$MgCl_2$	1 µl	1.2 µM
4.	10X PCR Buffer	2.5µl	1X
5.	Primer	1 µl	50ng
6.	Taq polymerase	1 µl	1.5Units
7	Total	25 µl	

Table 2. Program set up for RAPD analysis

Activity	Temperature	Time	Number of cycles
Initial denaturing	94°C	2 Min	One
Denaturing	94°C	1 Min	40
Annealing	37 °C	1 Min	
Extension	72°C	1 Min	
Final Extension	72°C	5 Min	One
Storage	4°C	Upto end	

laboratory conditions on the rearing diet described above for two weeks continuously Predators were removed from the respective diet and immediately for the presence of *S litura, D. cingulatus* and *C. cephalonica* and Oligidic diet remains in the gut of the predator was analysed with ELISA methods.

2.10.2. Indirect ELISA

An indirect ELISA was performed on the predators from all three feeding trails. A 100 µl aliquots of each macerated predator was placed in an individual well of a 96 well assay plate (round-bottom wells of polystyrene micro titer plates, Nunc, Moxsorp, UK). Each plate was incubated at 4°C overnight. Following incubation, the insect macerates were discarded from each plate and free reactive sites were blocked with 1% Bovine Serum Albumin (BSA) in distilled water (blocking solution) was added to each well for 30 min to block any unoccupied antigenic sites in the wells. The blocking solution was emptied from each plate and a 50-µl aliquot of *S. litura, D. cingulatus, C. cephalonica* and pig liver was added with Rabbit monoclonal Antibody (MAb) (Mab was purchased from Omega diagnostics, UK) using acetic fluid it was mixed with diluted 1:10000 in BSA and was added to each well of the ELISA plate (Hagler *et al.*, 1994b). Both positive control (known sample) and negative control (phosphate buffered saline solution) were also used in this experiment. The ELISA plates were then incubated for 1 h at room temperature. The contents from each plate were discarded and the plates were briefly rinsed three times with PBS-Tween 20 (0.05%) and twice with phosphate buffered saline (PBS). Goat anti-mouse IgG conjugated to alkaline phosphatase diluted (1:500) in blocking solution (BSA) was added to each well (50 µl) of the plates for 1 h. 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 reagents supplied in an alkaline phosphatase substrate kit (Omega diagnostics, UK) following the addition of 50 µl

µl of 2N H_2 SO_4. After 4 h, the absorbance of each well was measured with a SLR 36 ELISA microplate reader at 450 nm.

2.11. Bioefficacy evaluation by Augmentative Release

2.11.1. Plot Description

The experiment was carried out in farmer's field from mid December 2006 to May 2007. Groundnut (variety - TMV 7) was cultivated under well irrigation. The farmer was advised not to use pesticides or any other pest control practices during the experimental time. Total area of 4050 m^2 (36×45m) was chosen for the present study. It was divided into 9 plots.

2.11.2. Predator Release

Three treatments were performed in this study. They were

T_1 - *R. marginatus* reared with *C. cephalonica*

T_2 - *R. marginatus* reared with OD

T_3 - Control (Plot without predator)

In plots T_1 and T_2 *R. marginatus* life stages except V instar (360 predator/sub-plot) were released (60 each) on the 40^{th} day after seedling emergence (ASE). Totally 1080 *R. marginatus* were released during the study period. The release was done during the early morning hours (6:30 A.M. to 8:30 A.M.). One of the plot was served as control where no predators were released. On the release day, the laboratory reared 24 hrs starved *R. marginatus* were released in to the field using a camline brush (20 cm) in to the topmost five compound leaves.

2.11.3. Sampling of Pests and their Infestations

The sampling of the pest was done by visual observation and expressed in number of pests/plant. For the infestation of the pests, the uppermost 10 leave were considered (Amin. 1983) and 90 plants were reared randomly in each plot. The sampling was done four days before and after the release of the predators.

2.11.4. Cost Benefit Ratio (CBR) and Percent Avoidable Loss Analyses

On the harvest day, 90 plants were selected randomly from each plot and one pod, two pod and three pods in each plant were recorded. After the harvest, the production of groundnut from each plot was estimated and expressed in $Kgha^{-1}$. The CBR was also worked according to Kalyanasundaram *et al.* (1994).

$$\textbf{Cost benefit ratio} = \frac{\text{Total gain}}{\text{Total cost of cultivation}}$$

Percent avoidable losses was calculated using the formula of Krishnaiah (1977)

Percent avoidable loss =

$$\frac{\text{Mean yield from Protected plots} - \text{Mean yield from unprotected plots}}{\text{Mean yield from protected plots}}$$

2.12. Statistical Analyses

The effects of the different diets on developmental times and adult weights of *R. marginatus* were analysed using paired sample t-test and their significance was express at 5% level. Diet effects on reproductive parameters of *R. marginatus* were also tested using Student's't' –test. Chi-square't' test was used to find out the significance for sex ratio. For the field experiment, multivariate ANOVA was used to compare pest population at different comparisions between facititious host and oligidic diet reared reduviids released fields using SPSS 13, 2003 version.

The effectiveness of a predator under controlled and field conditions were depends up on the number and type of prey consumed by them. Moreover before utilizing a natural enemy for biological control. It is important to assess its ability to capture and consume relevant stages of the target pest. Such assessment can identify the limitation of the predator and its potential impact before being costly experiment is conducted. However, information was not available about the comparative feeding behavior of reduviids reared on different prey and oligidic diets. This study was undertaken to study stage preference and feeding efficiency of this reduviid on three important pests such as *D. cingulatus, S. litura* and *C. cephalonica.*

3.1. Behaviour

3.1.1. Approaching time (AT) and consumption time (CT)

Field collected reduviids were used to study the feeding behavior on oligidic diets. At 24 hrs starvation, field-collected *R. marginatus* adults oriented towards the oligidic diets. 48 hrs of field-collected adults were also exhibited 30 per cent responses towards milk - based oligidic diets and then it was increased to 60 percent approach within one hour. At 72-hour hunger level, 70 percent of the predators approached the diet. When five per cent insect source such as *Mylabris indica*, *M. pustulata* and *D. cingulatus* were added in the milk-based diet, reduviids failed to approach the diet within two hours. During feeding, following sequential events were observed, antennal stretching-cat walking towards the direction of diet-restless movement resulted in flight antenna brushing, leg brushing and repeated movement towards the diet source. But the predators

failed to approach the cotton containing milk-based diet with insect source. Further, from the table 3, it was very clear that *R. marginatus* food consumption was gradually decreased when the starvation period was increased from 24 to 72 hrs. Similarly approaching and sucking time were also decreased. Between the two sexes female consumed more milk-based oligidic diet than male. Significantly ($p<0.05$) female spent more time to suck the diet than male.

3.1.2. Stage preference

Stage preference studies of *R. marginatus* to the life stages of *S. litura, C. cephalonica* and *D. cingulatus* is presented table 4. Prey stage preference studies showed that life stages of *R. marginatus* preferred different stages of the pests tested. The result also suggests that both fifth instar and adult predators were more successful in encountering the large size *S. litura* (iv and v instars) and *C. cephalonica* (v instars) larvae. Though the different nymphal instars of *R. marginatus* preferred life stage of lepidoptera larvae, second and third instar reduviid preferred second instar *D. cingulatus* nymphs and the remaining life stages of the reduviid preferred only adult *D. cingulatus.*

3.1.3. Food preference index (FPI)

The oligidic diet was provided with additional combination with different foods such as oligidic diet (T3), oligidic diet + *C. cephalonica* (T4), *C. cephalonica* + water (T2). One ml of OD was provided once in two days. After two days, cotton ball was removed and discarded in order to maintain the hygienic conditions. Moreover, before introduce the insect in the container (100 and 500 ml capacity), they were washed with 0.2 % sodium hypochloride. Thirty male and female *R. marginatus* from each treatment were randomly selected, weighed and introduced in olfactometer covered with muslin

Table 3. Cumulative approaching behaviour of field-collected *R. marginatus* adults on oligidic diets

Sex	Animal Weight(mg)	Approaching Time (M)	Sucking Time(Minutes)	Weight Gain(mg)
24 hrs Starvation				
Female	199.2 ± 11.7	1.2 ± 0.9^{NS}	59.6 ± 9.7*	30.6 ± 4.8*
Male	110.3 ± 12.4	5.6 ± 2.1	06.5 ± 3.0	08.1± 2.5
48 hrs starvation				
Female	197.1 ± 8.3	12.7 ± 3.0^{NS}	11.8 ± 2.4*	24.2 ± 2.8*
Male	102.8 ± 7.1	08.5 ± 2.1	12.3 ± 2.3	06.3 ± 3.2
78 hrs starvation				
Female	151.4 ± 0.5	5.5 ± 1.7*	26.1 ± 0.3*	21.0 ± 2.8*
Male	085.0 ± 0.5	2.7 ± 0.6	20.8 ± 0.1	04.7 ± 1.1

*NS-Not significant

Table 4. Stage preference of *Rhynocoris marginatus* on *C. cephalonica* (CC), *S. litura* and *D. cingulatus*

Predator life stages	Preys		
	D. cingulatus	*S. litura*	*C.cephalonica*
II	II	II	II
III	II	II	II
IV	Adult	III	IV
V	Adult	IV	V
Male	Adult	V	V
Female	Adult	V	V

cloth. Among the tested insects female took more time (59.6 min) for sucking and also gained more (30.6 mg) weight in all the testes (Table 3).

3.1.4. Feeding behavior

During the feeding time, *R. marginatus* oriented towards the prey with facing antenna, after setting a perfect orientation position, the reduviid palpated its antenna then aroused and subsequently showed the approach rostral probing, injection of toxic saliva for paralysing, sucking the prey content and post-predatory behavior observed in this study.

3.1.4.1 Capturing

At close proximity of the prey, the predator extended its rostrum and captured the prey preferably in the abdominal region. If the prey is small and less active, the predator captured the prey with its forelegs firmly kept over the prey. If the prey was agile, the predator used to raise its antennae and extended its rostrum and pinned the prey at a preferred site (table 5). The predator was found probing the motionless prey with its antennae presumable testing the prey with its inserted rostrum. But the acts did not affect the subsequent predatory activities such as pinning and paralysing. The extended rostrum was inserted into the captured prey to test the suitable site for sucking. The fed predators took more time for capturing and pinning when compared with prey deprived predators.

3.1.4.2. Paralysing

After the successful capturing of the prey, the predator paralysed the prey by injecting its toxic salivary secretion. The fed predators took more time for paralysing the prey when compared to the prey deprived predators.

Plate -1. *Rhynocoris marginatus* nymphs (a) and adult (b) infected with *Aspergillus flavus* contaminated oligidic diet.
Feeding behaviour of *Rhynocoris marginatus* on *Spodoptera litura* (c), *Dysdercus cingulatus* adult (d) and *Corcyra cephalonica* (e)

Table 5. Site preference of *Rhynocoris marginatus* during feeding on three pests

Predator life stages	*D. cingulatus*	*S. litura*	*C. cephalonica*
II	Thoracic pleural membrane	Thoracic pleural membrane, tergum	Sternum, neck membrane
III	Eye, thoracic pleural membrane	Thorax pleural membrane, tergum	Neck membrane, sternum
IV	Thoracic and abdoman pleural membrane	Thoracic and abdoman pleural membrane	Neck and abdoman pleural membrane
V	Thoracic and abdoman pleural membrane	Thoracic pleural membrane, tergum	Neck and abdoman pleural membrane
Male	Head neck muscle, tergum	Thoracic and abdoman pleural membrane	Neck and abdoman pleural membrane
Female	Neck membrane, tercum	Thoracic pleural membrane, tergum	Neck and abdoman pleural membrane

3.1.4.3. Rostral probing and sucking

After the paralysing of prey, the predator sucked the predigested body fluids of the prey by inserting the rostrum at different regions of the prey body. *R. marginatus* also frequently changed the sucking sites during feeding. While sucking one of the antennae was kept towards the prey in an upright position while other antenna towards the prey in drooping but in extended position, presumably to test various rations of the prey. It was further accomplished by rostral probing. The predators often selected the sucking sites from the abdominal region and less often from the cephalic and thoracic regions (Table 6). At the time of sucking, the forelegs of the predator were found kept on the prey. The fed predator took less sucking time. When compared to the prey deprived predators.

3.1.4.4. Post-feeding behavior

The satiated predator after sucking the prey at all possible sites started cleaning rostrum and antennae, by drawing and grooming in between the forelegs followed by cleaning the fore tibial pad, antenna and hind tibia.

3.1.5. Feeding behavior on three pests

First attacking site of *R. marginatus* on three pests is presented in table 6. It was very clear from the table 7-9 that life stages of the reduviid invariably preferred particular site for paralysing and sucking the prey content. Both for paralysing and feeding the victim, reduviid significantly preferred thoracic pleural membrane of the pests followed by the abdominal pleural membrane. In general approaching time gradually diminished from the second instar to fifth nymphal instars (7.0, 2.2 and 2.4 minutes for *C. cephalonica,S. litura* and *D. cingulatus* respectively) (Tables 6-8). OD reared *R. marginatus* took more time for approaching the prey *C. cephalonica, D. cingulatus* than

Table 6. Feeding behavior (in min) and weight gain (in mg.) of *C. cephalonica* (CC) and oligidic diet reared *R. marginatus* on *D. cingulatus* life stages

Instar	Approching time	Handling time	Weight gain	No. of site selected
D. cingulatus				
II	7.0 ± 1.7	131.3 ± 9.5*	08.1 ± 0.7	2.0 ± 0.2
III	4.7 ± 0.7	167.2 ± 9.9	18.1 ± 1.1*	2.3 ± 0.1
IV	4.4 ± 0.6	199.5 ± 10.3	31.0 ± 4.5*	2.4 ± 0.2
V	1.7 ± 0.2	141.6 ± 8.3	41.5 ± 0.0	2.6 ± 0.2
Male	4.1 ± 0.9	128.5 ± 3.7	25.6 ± 3.1	1.8 ± 0.3
Female	5.0 ± 1.4	138.7 ± 2.5	35.2 ± 2.8*	1.6 ± 0.2
Oligidic Diet				
II	7.3 ± 0.8	88.0 ± 08.6	1.9 ± 0.0*	11.6 ± 0.1*
III	7.3 ± 1.7	259.5 ± 8.0*	11.1 ± 1.6	3.2 ± 0.2*
IV	3.5 ± 0.5	282.8 ± 24.3*	15.8 ± 3.2	2.2 ± 0.2
V	4.3 ± 1.0	214.5 ± 12.2*	31.2 ± 4.6*	2.3 ± 0.1
Male	4.3 ± 0.8	144.2 ± 26.4*	35.2 ± 4.3*	3.2 ± 0.5*
Female	5.4 ± 0.7	125.0 ± 20.8	25.6 ± 3.1	2.6 ± 0.5 *

* Significant at 5% level

natural host *S. litura* reared predator. Statistical analysis between OD reared and prey reared predator approaching times were insignificant at 5% level. OD reared reduviid approached *S. litura* faster than other prey.

Irrespective of the preys, the oligidic diet reared *R. marginatus* significantly handled maximum time (270.5 minutes) than insect hosts reared predator (Table-7). The results indicate that, there was positive correlation between the handling time (250.10 min.) and weight gain (48.10 mg) ($p<0.05$). *R. marginatus* second, third, fourth and fifth instar nymphs and adult reared with insect hosts handled maximum time in *D. cingulatus* and *S. litura.* Minimum consumption time was observed in third instar *R. marginatus* feeding on *C. cephalonica* (Table 7). *R. marginatus* female took more time for handled *C. cephalonica* and *S. litura* than male. But male took less time when *R. marginatus* was provided with *D. cingulatus* (Table 6). Irrespective of the preys, the weight gain was gradually increased when *R. marginatus* grew older (except adult).

It is revealed from tables 6, 7 and 8 that maximum weight gain was recorded in adult female when OD reared *R. marginatus* was provided with *S. litura* (76.1 mg.) (Table 8). But an opposite trend was observed on other pests for instance when *D. cingulatus* was provided to this reduviid predator. In general, OD reared *R. marginatus* consumed more number of prey than those reared on *C. cephalonica* and *S. litura.* Similarly, in OD reared *R. marginatus,* maximum predatory rate was observed on *S. litura* adult female (2.40 prey/predator/day) and was very low in *D. cingulatus* third instar (1.36 prey/predator/day). In this experiment *R. marginatus* nymphs and adult (except second instar larvae and adult female) have the capacity to consume more number of *S. litura* larvae (76.1 mg/predator), when the predator was reared on oligidic diet (Table 8). Similarly insect hosts were reared by *R. marginatus* second and fifth nymphal instars and

Table 7. Approaching time, handling time (in min.) and weight gain (in mg.) of *R. marginatus* life stages on *C. cephalonica* and oligidic diet

Predator stage	Approaching time	Handling time	Weight gain	No. of site selected
C. cephalonica				
II	2.2 ± 0.4	130.6 ± 29.1*	7.3 ± 9.0*	1.3 ± 0.1*
III	2.0 ± 0.4	120.0 ± 25.5*	11.7 ± 0.3*	1.1 ± 0.0
IV	1.5 ± 0.2	134.8 ± 11.3*	11.4 ± 0.2*	2.1 ± 0.2*
V	1.4 ± 0.2	157.2 ± 09.1*	22.9 ± 8.2*	2.2 ± 0.2*
Male	1.3 ± 0.2	165.0 ± 20.4*	34.3 ± 3.5	1.8 ± 0.3*
Female	1.6 ± 0.4	163.3 ± 20.6*	33.2 ± 0.3*	1.6 ± 0.2*
Oligidic Diet				
II	4.6 ± 1.0	227.5 ± 14.4*	8.0 ± 0.6*	1.5 ± 0.1
III	4.7 ± 0.8	215.0 ± 18.1*	20.5 ± 2.4*	1.8 ± 0.1*
IV	4.4 ± 0.8	250.1 ± 10.3*	48.1 ± 2.8*	1.6 ± 0.1
V	3.0 ± 0.8	270.5 ± 12.9*	43.9 ± 8.6*	1.2 ± 0.1
Male	4.6 ± 0.8	159.0 ± 19.9*	34.6 ± 6.5	2.2 ± 0.2*
Female	4.0 ± 1.6	198.0 ± 21.9*	53.0 ± 9.8*	2.4 ± 0.2*

* Significant at 5% level

Table 8. Approaching time, handling time (in min.) and weight gain (in mg.) of *R. marginatus* life stages on *S. litura* and oligidic diet

Life stage	Approaching time	Handling time	Weight gain (in mg)	No. of site selected
S. litura				
II	24.3 ± 0.8	126.5 ± 9.9*	8.6 ± 0.7*	1.6 ± 0.1*
III	19.5 ± 2.4	148.0 ± 8.7*	19.0 ± 2.2*	1.8 ± 0.1
IV	13.7 ± 2.7	129.0 ± 9.8	21.5 ± 2.3	2.0 ± 0.2*
V	10.1 ± 1.6	159.0 ± 21.0*	54.0 ± 7.6*	2.1 ± 0.2*
Male	11.2 ± 4.7	188.7 ± 18.7	34.5 ± 5.5	1.5 ± 0.2
Female	9.6 ± 2.0	175.0 ± 11.6*	29.0 ± 3.4*	1.5 ± 0.2*
Oligidic Diet				
II	7.4 ± 1.1	230.5 ± 11.1*	6.7 ± 0.5	2.1 ± 0.2*
III	4.5 ± 1.2	207.0 ± 11.2*	13.3 ± 1.2	1.8 ± 0.2
IV	3.3 ±0.8	241.5 ±13.1*	60.7 ± 1.9*	1.5 ± 0.6
V	3.8 ± 0.8	241.5 ± 13.1*	63.7 ± 1.9**	1.5 ± 0.1
Male	5.5 ± 1.6	225.0 ± 19.3*	63.7 ± 8.3*	2.0 ± 0.4*
Female	4.5 ± 1.2	227.5 ± 19.5*	76.1 ± 1.1*	1.8 ± 0.3*

* Significant at 5% level

adult consumed by more number of *S. litura* and other life stages consumed *D. cingulatus* second instar. But life stages of either insect host or oligidic diets reared *R. marginatus* consumed minimum number of *C. cephalonica.* Hence this reduviid can be used as a biological control agent in crop where *S. litura* and *D. cingulatus* are present.

3.2. Preparation of oligidic diets

With trial and errors so far we have prepared more than 17 diets. But listed out the composition/ constituents of the oligidic diets which are in favour for the development and fecundity of *R. marginatus* (refer-table 9) (plate 2). All the listed diets contain pork liver as a source ingredients. Other ingredients were present in different concentrations at different proportions. Initially we tested four objects (cotton, capsule, cavity slide and foam) for providing the OD to *R. marginatus* life stages. They preferred cotton (Plate 2 b, d, g, h) and hence we have used to it for providing OD to *R. marginatus*.

3.3. Biology of *R. marginatus*

3.3.1. Nymphal development

Reduviid predator nymphs were successfully reared to adult on meat - based diet and these adults were subsequently able to reproduce. This result indicates that it is possible to rear this predator on a diet completely excludes insect material. Although the average fecundity of a female was low for the oligidic diets, nymphal survival rate and longevity of female were good. The oligidic diet was sufficient for sustain continuous generation of *R. marginatus*. Three generation have been obtained through continuous cultures of this reduviid fed meat diet. The meat-based diet tested was able to sustain the rearing of *R. marginatus* for several generations without supplying any insect material, showing a good nymphs survival rate.

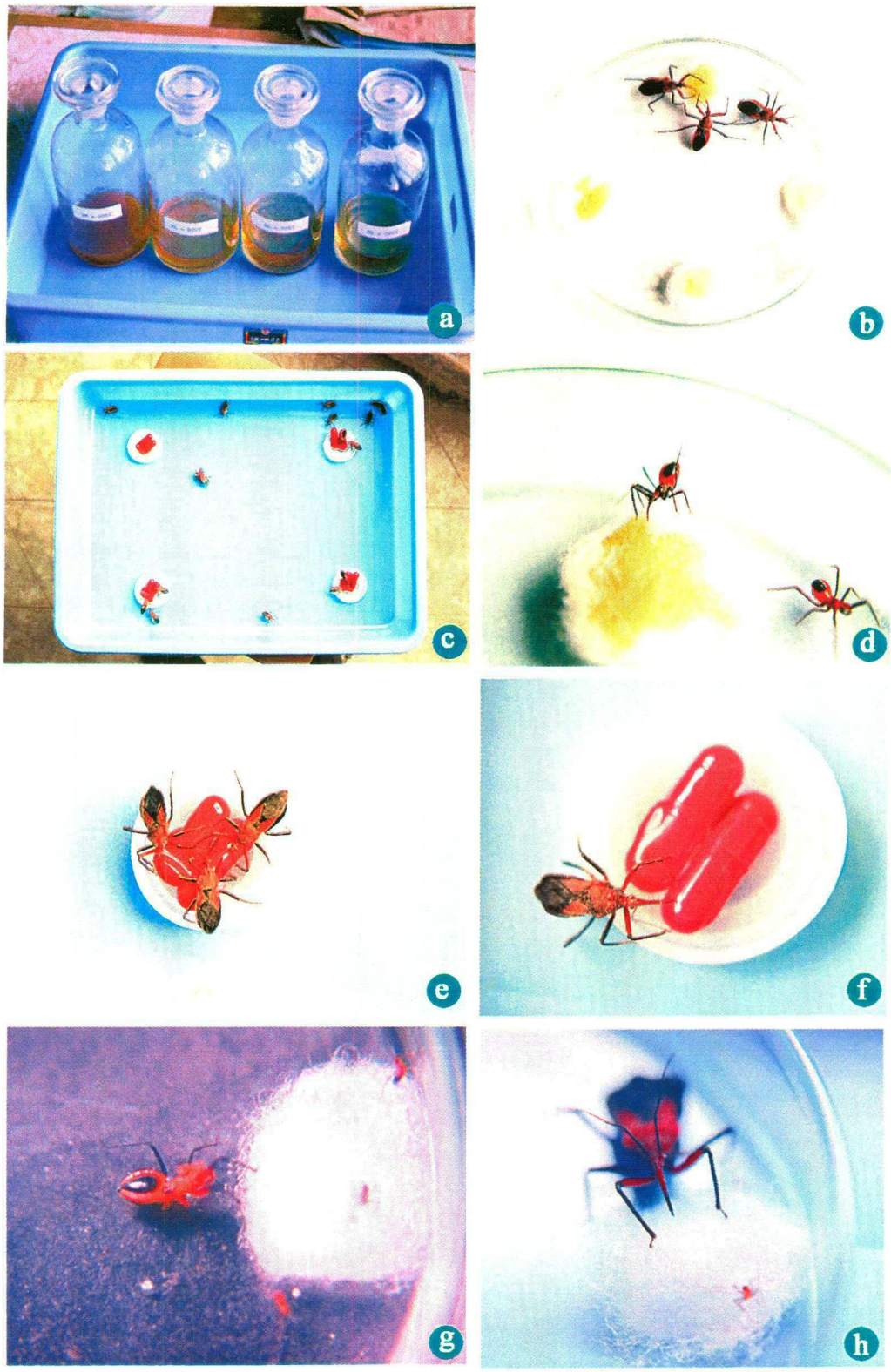


Plate 2. Oligidic diets (a), rearing methods (b&c), feeding behaviour and object preference (d, e, g and h) of *Rhynocoris marginatus* life stages

Table 9. Composition of various ingredients of oligidic diets (for 100 ml)

Components (in mg./ml.)	Diet					
	1	2	3	4	5	6
Source ingredient (g)	60	5	5	5	5	5
Pork blood (g)	-	-	-	5	5	-
Blood serum(ml)	-	-	-	-	20	20
Sucrose (mg)	200	200	-	500	500	500
Yeast Extract (water soluble)	-	-	-	-	5	5
Yeast (g)	-	-	2	2	-	-
Milk Powder (g)	-	-	5	5	5	5
Egg yolk (g)		4.0	4.0	4.0	5	5
Honey (ml)	5.0	5	5	5.0	5.0	5.0
Vitamin (multivit. mg)	10.	200	200	200	200	300
Vitamin E	-	-	-	200	200	200
Vitamin C	-	-	-	2.5	2.5	2.5
Casein (mg)	-	-	5	4	4	4
Cholesterol (mg)	-	-	200	200	200	200
Acetic Acid (10%) (ml)	3.7	-	-	2.5	2.5	2.5
Nacl (mg)	5.0	5.0	5.0	-	-	-
Streptomycin (mg)	7.5	100	100	100	-	-
Formaldehyde 40% (ml)	-	-	-	-	1	1

Table 10. Nymphal developmental period (in days) of *R. marginatus* reared with oligidic diets

Diet	Developmental (days)					
	I	II	III	IV	V	Total
3	9.2 ± 0.5	16.6±1.0	15.9 ± 1.3	-	-	-
4	12.8 ± 0.6	17.6 ± 1.0^{a}	18.1 ± 1.3^{ab}	17.3 ± 1.1^{abc}	16.5 ± 0.9^{a-cd}	80.7^{a---d}
5F1	13.0 ± 0.9	15.7 ± 1.8^{a}	20.2 ± 1.8^{ab}	12.2 ± 3.0^{a-c}	17.8 ± 1.7^{a-cd}	75.1^{a---d}
5F2	9.5 ± 0.3	12.2 ± 0.5^{a}	16.8 ± 0.9^{ab}	13.0 ± 0.8^{a-c}	20.1 ± 1.1^{a--d}	71.9^{a---d}
6F1	6.6 ± 0.2	13.9 ± 0.9^{a}	12.9 ± 0.9^{ab}	14.8 ± 0.7^{a-c}	21.8 ± 0.7^{a--d}	70.1^{a---d}
6F2	7.8 ± 0.4	7.1 ± 0.4^{a}	13.8 ± 0.9^{ab}	15.7 ± 1.9^{a-c}	24.2 ± 1.5^{a--d}	68.8^{abcd}
6F3	8.1 ± 0.3	12.1 ± 0.6^{a}	12.9 ± 0.5^{ab}	13.1 ± 0.6^{a-c}	18.8 ± 1.3^{abcd}	65.1^{abcd}

F1, F2 and F3 stands for first, second, and third filial generations respectively.

Means ± means within a column and between a instar followed by the same letter are not significant $p>0.05$; 't' test

R. marginatus was fed with five different diets such as *C. cephalonica* (T1), *C. cephalonica* weekly once with water (T2), oligidic diet (T3), oligidic diet and weekly once *C. cephalonica* (T4), and *S. litura* (T5). The nymphal developmental period of *R. marginatus* reared on oligidic diet (OD), natural host, *S. litura* and factitious host, *C. cephalonica* are presented in tables 10-12. Results revealed that rearing of *R. marginatus* with oligidic diet prolonged nymphal developmental period. For instance, the total nymphal developmental period of *R. marginatus* on OD was ranged from 80.7 - 65.1 day (Table 11) when compared with 45.2 to 46.8 days for *C. cephalonica* (Table 11). Initially *R. marginatus* was reared on oligidic diets such as Diet-1, 2 and 3 different source of ingredients. In these diets *R. marginatus* was not proceeds from first instar to third instars even after 50, 40 and 30 days respectively. Similarly Diets 4 and 5 did not showed satisfactory results. Hence their composition was modified and prepared diet 6. In diet 5 ingredients such as pork blood, Vitamin E, and C, then Acetic Acid were added. In this diet, the total nymphal developmental period was too longer (80.7 days). Hence, we included blood serum (2 ml) and considered as diet 5. Addition of blood serum reduced *R. marginatus* nymphal development period was 75.1 days. In diet 6, multivitamin content was increased from 200 mg to 300 mg. It was further reduced to 70.1 days in diet (table 11) when *R. marginatus* was reared on the same diet, the total nymphal developmental period was reduced to 68.8 and 65.1 days in F2 and F3 filial generations.

Shortest (6.6 days for 6F1G) and longest (24.2 days for 6F2G) nymphal developmental period were observed in the first and fifth instars respectively (t= -7.584, df 25, P=0.000). In T2 category total nymphal period was ranged from (CWF1) 55.7 to 51.6 days (F3G) (t= 7.726, df - 28, P=0.000). In control category, total developmental period was ranged from 46.8 to 43.9 days (CF2) (Table 11). Moreover we observed

Table 11. Nymphal developmental period (in days) of *R. marginatus* reared on *S. litura* (T_5) *C. cephalonica* alone (C) (T_1) and *C. cephalonica* once with water (CW) (T_2)

Diet	Developmental stages					
	I	II	III	IV	V	Total
	C. cephalonica					
F1	6.5 ± 0.1	8.6 ± 0.2^{a}	9.5 ± 0.6^{ab}	9.8 ± 0.3^{a-c}	15.3 ± 0.6^{a-cd}	46.8^{a-cd}
F2	7.6 ± 3.1	7.1 ± 1.1^{a}	7.9 ± 2.4^{ab}	7.8 ± 1.8^{abc}	13.0 ± 0.2^{abcd}	43.9^{abcd}
F3	6.5 ± 0.1	8.0 ± 0.2^{a}	8.0 ± 0.6^{ab}	8.2 ± 0.2^{abc}	13.6 ± 0.5^{abcd}	45.2^{abcd}
C. cephalonica + water						
F1	7.8 ± 0.4	11.0 ± 0.6^{a}	10.5 ± 0.8^{ab}	11.3 ± 0.9^{abc}	15.0 ± 0.6^{abcd}	55.7^{abcd}
F2	7.3 ± 0.1	9.9 ± 0.2^{a}	8.0 ± 0.1^{ab}	11.4 ± 0.2^{a-c}	15.3 ± 0.3^{a-cd}	51.9^{abcd}
F3	7.5 ± 0.1	9.7 ± 0.2^{a}	8.4 ± 0.2^{ab}	10.7 ± 0.3^{abc}	15.2 ± 0.4^{abc}	51.6^{abcd}
S. litura						
F1	6.0 ± 0.2	6.95 ± 0.2^{a}	7.4 ± 0.2^{ab}	6.4± 0.1^{a-c}	14.1 ± 0.2^{abcd}	41.1^{abcd}
F2	5.9 ± 0.1	6.2 ± 0.2^{a}	7.1 ± 0.1^{ab}	7.1 ± 0.1^{abc}	14.6 ± 0.1^{abcd}	41.1^{abcd}
F3	6.0 ± 0.0	6.8 ± 0.2^{a}	6.6 ± 0.5^{ab}	8.8 ± 0.9^{a-c}	14.4 ± 1.2^{abcd}	42.6^{abcd}

F1, F2 and F3 stands for first, second, and third filial generations respectively

Means ± means within a column and between a instar followed by the same letter are not significant p>0.05; 't' test

maximum mortality in the third instar. Nymphal developmental period was too longer than control diet so this diet does not gives satisfactory results for the rearing of this bug.

Student 't' test showed that except between *C. cephalonica* and *S. litura* (df = 3; f = 15.000, t = -1.549, p< 0.030) and Oligidic diet (df = 3; f = 15.000, t = .775, p< 0.030) all other comparisions were insignificant at F1 generation. In addition to OD (df = 4; f = 6.533, t = 0.120, p< 0.063), *C. cephalonica* weekly once with water (df = 4; f = 12.000, t = 0.000, p< 0.026) were also significant in F2 generation. In F3 generation, OD was again significant at 5% level (df = 9; f = 3.705, t = -0.650, p< 0.085) when compared *C. cephalonica*.

3.3.2. Nymphal survival rate

In T_1 category, total nymphal survival rate was 92.17 (ranged from 80.56 to 98.3 %). Nymphal survival rate was maximum in first instar (100%) and minimum survival rate was observed in third generation (80.55). Among three generations, CCF3 generation has the highest survival rate (98.26) and lowest in F1generations, (80.55%). In T2 category average nymphal survival rate was 92.17%. Among the three generation F3 generation has the maximum survival rate (96.49%) and minimum was 85.71%. As, observed in T1 category, among the nymphal instars first instar has the maximum survival rate and minimum in second instar (70.00%). In T3 category, average survival rate was 80.91%. Among the three generations, maximum survival rate was observed as 91.71% and minimum survival rare was in 4A oligidic diet (T3) 37.50%. In individual survival rate (100%) was maximum in first and second instar followed by minimum first instar (62.50%) (table 13).

Table 12. Nymphal developmental period (in days) of *R. marginatus* reared on oligidic diets with *C. cephalonica* (T_4)

Diet	Developmental stages					
	I	II	III	IV	V	Total
	Artificial diets with *C. cephalonica* (T_4)					
3C	8.4 ± 0.2	13.6 ± 0.7	16.3 ± 0.3	18.2 ± 3.4	-	-
4C	7.3 ± 0.4	14.4 ± 0.8^{a}	18.2 ± 1.1^{ab}	12.8 ± 0.6^{a-c}	15.6 ± 0.9^{abcd}	68.8^{abcd}
5CF1	8.6 ± 0.5	11.6 ± 0.6^{a}	12.6 ± 0.8^{ab}	12.0 ± 1.2^{a-c}	18.6 ± 1.2^{abcd}	63.6^{abcd}
5CF2	12.0 ± 0.3	13.8 ± 0.6^{a}	12.7 ±0.7^{ab}	11.5 ± 0.6^{a-c}	16.9 ± 0.9^{abcd}	66.9^{abcd}
6CF1	7.0 ± 0.1	10.2 ± 0.5^{a}	13.4 ± 0.9^{ab}	11.3 ± 0.7^{ab-}	19.1 ± 1.1^{ab--}	61.1^{abcd}
6CF2	9.3 ± 0.3	13.0 ± 0.5^{a}	12.8 ± 0.6^{ab}	13.6 ± 0.7^{ab-}	17.2 ± 1.0^{abcd}	64.3^{abcd}
6CF3	9.2 ± 0.2	13.5 ± 0.5^{a}	10.7 ± 1.1^{ab}	12.8 ± 1.1^{ab-}	17.4 ± 1.2^{abcd}	63.7^{abcd}

F1, F2 and F3 stands for first, second, and third filial generations respectively

Means ± means within a column and between a instar followed by the same letter are not significant $p>0.05$; 't' test

Table 13. Survival rate (in %) of *R. marginatus* reared on Oligidic diet (T_3) and Oligidic diet weekly once with *C. cephalonica* (T_1)

Survival rate						
Diet	**Oligidic diet**					
No	I	II	III	IV	V	Total
4	95.01	65.78	76	84.21	94.75	37.50
5F1	100	100	86.66	76.92	92.3	64.28
5F2	62.5	80.00	100	80.00	100	90.00
6F1	100	100	86.66	76.92	92.3	68.28
6F2	100	80.05	87.52	87.5	87.51	70.00
6F3	93.5	92.00	100	95.62	95.45	77.62
Oligidic diet weekly once with *C. cephalonica*						
4C	61.53	83.33	84.01	66.66	66.66	57.69
5CF1	85.71	100	100	100	90	90.00
5CF2	100	100	88.11	72.72	88.88	81.25
6CF1	82.75	87.55	94.25	100	94.03	87.00
6CF2	100	100	85.03	94.11	100	90.00
6CF3	89.55	94.25	95.45	91.66	96.65	97.42

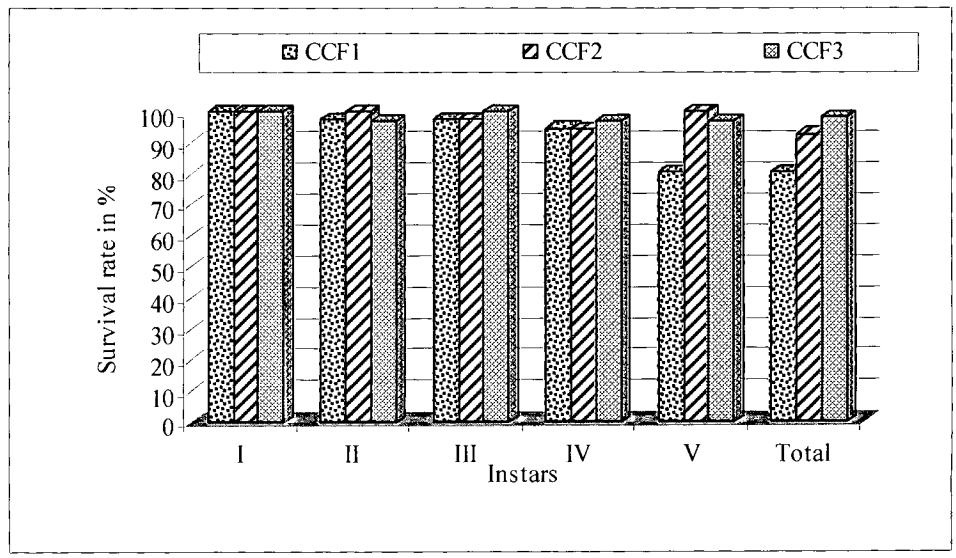


Figure1: Survival rate (in %) of *R. marginatus* reared on *C. cephalonica*

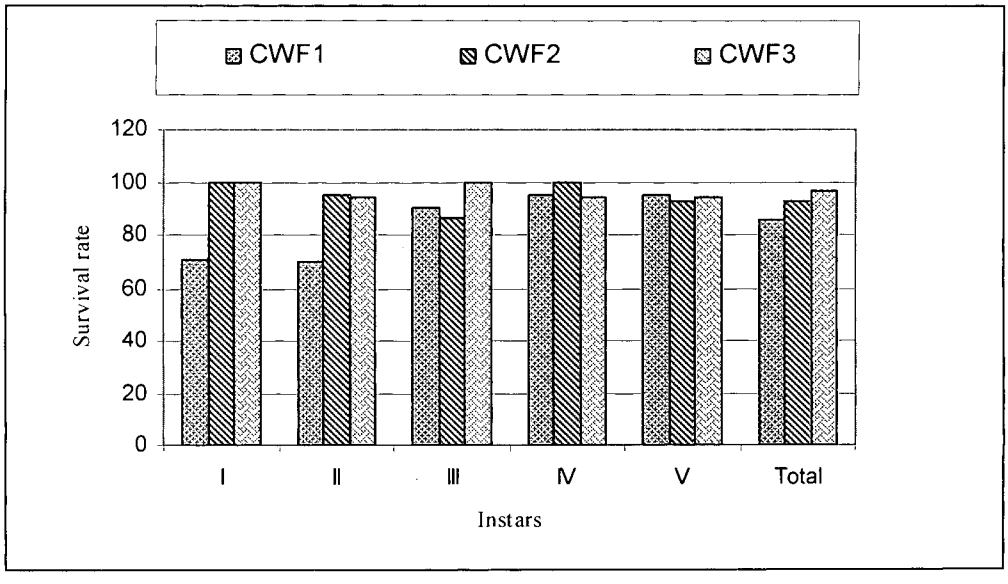


Figure 2: Survival rate (in %) of *R. marginatus* reared on *C. cephalonica* weekly once with water

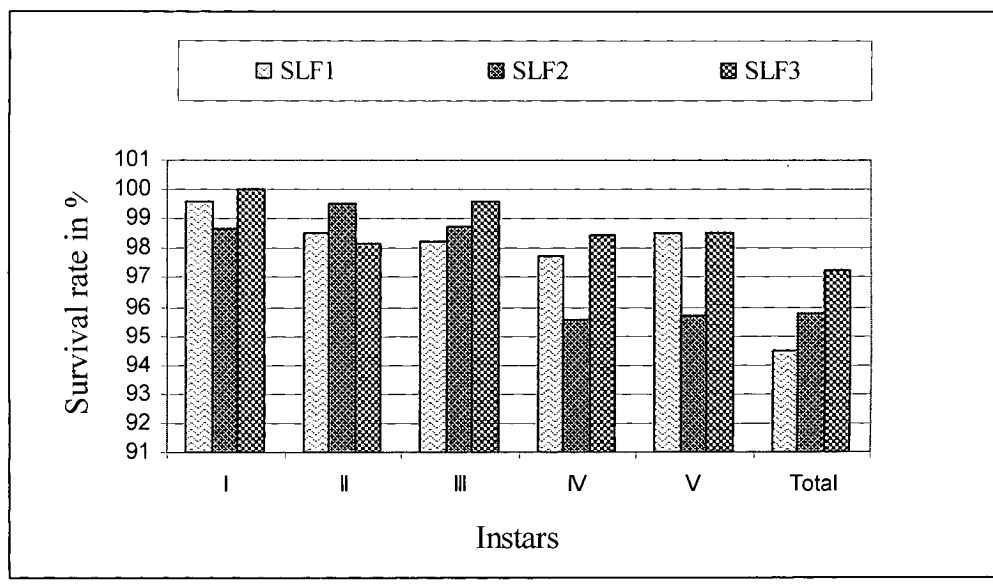


Figure 3: Survival rate (in %) of *R. marginatus* reared on *S. litura*

In T_4 category, the average nymphal survival rate was 85.73%. Among the three generations maximum survival rate was observed in F3 generation and minimum generation observed in diet 4 T_4 generation (57.69%). Individual maximum survival rate was observed in first and second instars (100%) followed by minimum survival rate in fourth instar (72.72 %). Individual maximum survival rate was observed in first and second instars (100%) followed by minimum survival rate in fourth instars (72.72%). In T_5 category, maximum survival rate was observed in first instar 100%. Maximum survival rate observed in SLF2 generation (98.25%). Average nymphal survival rate was maximum in (SL) T_5 category (94.55%).

3.3.3. Weight gain

Fresh weights of the newly emerged *R. marginatus* was affected by diets during their nymphal periods. Among the experimental groups, the weight gain was higher in predators maintained on diet - 5 than those fed with diet-4 (Figure 4). However the weight gain was significantly higher in the control category than *S. litura* (Table 14). In general, the weight gain was gradually increased from the first instar to the adults. But weight gain was reduced when water was provided along with *C. cephalonica* in all the generations (CW T_2) (t=5.921, df -29, P=0.000).

Meat diet produced smaller, as well as lighter weight predator with longer T_4 category, weight gain was increased embryonic and nymphal development times than the control (t=6.6497, df-16, P=0.000) (Figure 5). In the experimental group, *C. cephalonica* was provided along with OD, some of the instars weight gain was maximum for the predator maintained on T3 (5CF2 - 21.66 mg. and 16CF1 - 20.92 mg.) (Figure-6). In among all the diets (t=4.468, df-13, p=0.001).

Table 14.Weight gain (in mg) of *R. marginatus* reared on *S. litura* (T_5) and *C. cephalonica* (T_1)

Life stages					
	I	**II**	**III**	**IV**	**V**
Diets	***S. litura***				
F1	2.1 ± 1.2	19.7 ± 0.2	30.0 ± 0.1 ^{ab}	57.7 ± 3.6 $^{a-c}$	79.3 ± 2.7 $^{a-cd}$
F2	2.6 ± 0.5	19.8 ± 0.1	29.1 ± 0.4 ^{ab}	48.7 ± 1.1 $^{a-c}$	72.2 ± 0.6 $^{a--d}$
F3	1.0 ± 0.2	6.5 ± 0.1 ^{a}	08.6 ± 0.4 ^{ab}	26.1 ± 0.2 $^{a-c}$	45.9 ± 0.1 $^{a--d}$
C. cephalonica					
F1	1.1 ± 0.2	5.2 ± 0.9 ^{a}	16.1 ± 2.1 ^{ab}	27.7 ± 2.1 ^{ab}	32.1 ± 1.3 ^{abcd}
F2	1.4 ± 1.5	11.5 ± 3.1 ^{a}	21.2 ± 5.3 ^{ab}	32.7 ± 6.3 ^{ab}	48.6 ± 1.5 ^{abcd}
F3	1.1 ± 0.1	8.4 ± 0.4 ^{a}	19.7 ± 0.5 $^{a-}$	26.2 ± 0.2 $^{a-c}$	44.9 ± 0.2 ^{abcd}

F1, F2 and F3 stands for first, second, and third filial generations respectively

Means ± means within a column and between a instar followed by the same letter are not significant $p>0.05$; 't' test

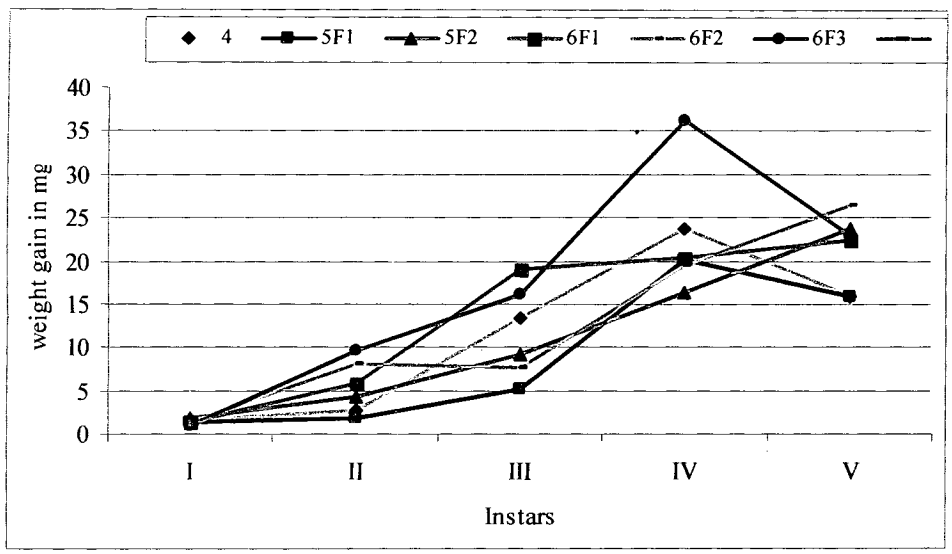


Figure 4: Weight Gain in mg of *R. marginatus* reared on Oligidic Diet

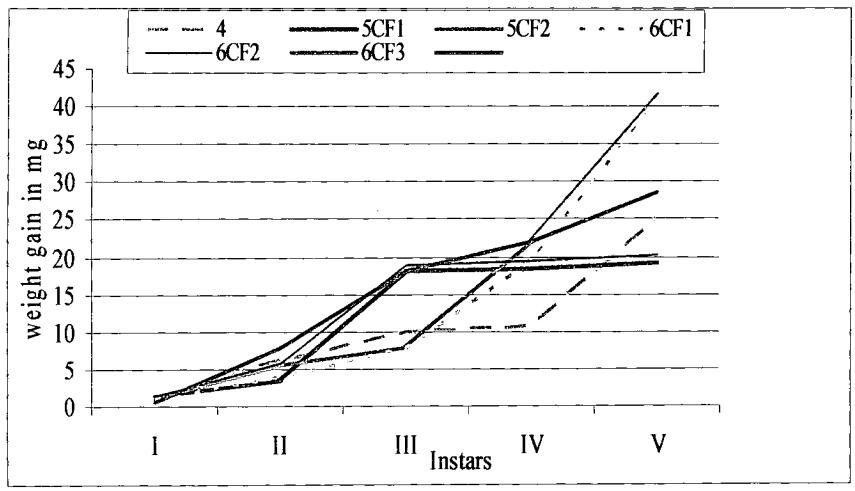


Figure 5: Weight gain in mg of *R. marginatus* reared on Oligidic Diet weekly once with *C. cephalonica*

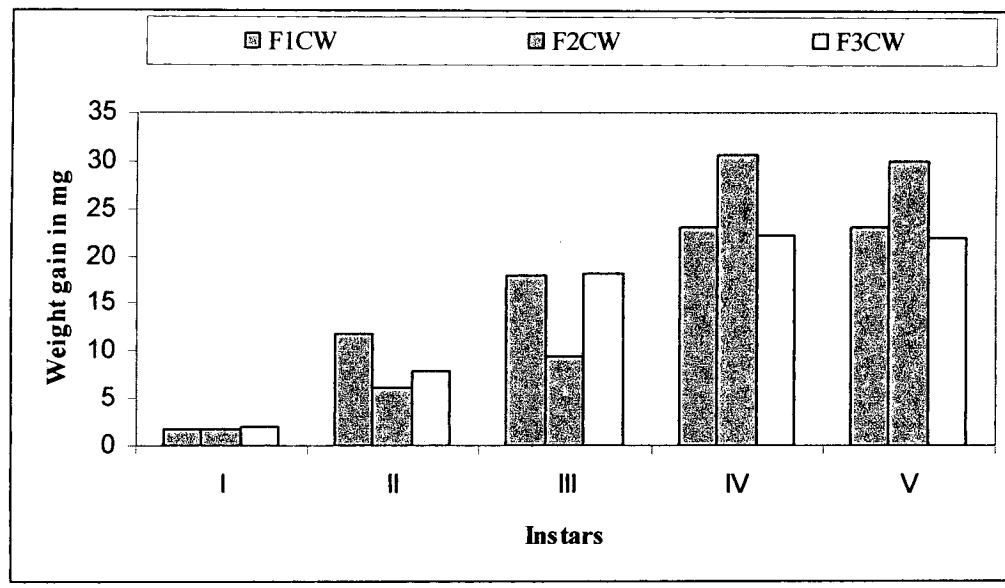


Figure 6: Weight Gain in mg of *R. marginatus* reared on *C. cephalonica* weekly once with water

The weight gain was significantly higher in the control category. The weight gain of predators that reared on the meat-based diet was about lower than control diet, where the average fresh weight of the predator was 30.92 and 38.83 mg respectively. But in oligidic diet with *C. cephalonica* category first, fourth and fifth instars weight gain were also increased (1.24, 15.72 and 20.14 mg.) (Figure 3). The weight gain of the predators was significantly improved when supplied with *C. cephalonica* larvae along with OD (t=6.124, df=13, p=0.000). Compensatory feeding was effective for making up small differences in diet nutrient content, but the lowest nutrient content diet was outside the range of compensatory ability.

3.3.4. Sex ratio and Adult longevity

Irrespective of the categories, the sex ratio was female biased and it was varied from 0.62 to 0.85 (tables 15 and 16). The sex ratio of adults maintained in Oligidic diet 5 and 6 were 0.62 and 0.76 respectively (Chi Square = 0.47, df = 5, p = 0.99), similarly Oligidic diet weekly once with *C. cephalonica* sex ratio was ranged from 0.62 – 0.76. (Chi Square = 0.29, df = 4, p = 0.99) (Table 16). But in control category, the sex ratio was ranged from 0.65 to 0.82 (Chi Square = 0.47, df = 5, p = 0.99) (Table 15). Provision of water along with *C. cephalonica* enhanced the female biased sex from 0.74 - 0.78 (Chi Square = 0.92, df = 4, p = 0.92). Similarly sex ratio was also enhanced in *S. litura* offered *R. marginatus* (0.78-0.85) (Chi Square = 0.85, df = 4, p = 0.95) (Table 15).

The longevity of male and females were not significantly affected by diets with exception of the CW (T_2) male (55.8 days) (t=12.05, df-12, P=0.000) (Table 15). In general females lived a longer than the males. In control (*C. cephalonica*) category females and males lived a maximum days of 122 and 102 (t=25.75, df-12, P=0.000) days respectively. It has reduced when water was provided with *C. cephalonica* average of

Table 15. Sex ratio and adult longevity (in days) of *R. marginatus* reared with different preys

Prey	Adult longevity		
	Male	Female	Sex ratio
S. litura			
F1	86.9 ± 2.9	88.9 ± 2.9*	0.78
F2	82.2 ± 2.1	92.5 ± 1.2*	0.85
F3	71.3 ± 5.9	96.5 ± 2.3*	0.75
C. cephalonica			
F1	109.8 ± 1.8	122.0 ± 3.9*	0.72
F2	102.0 ± 1.2	121.0 ± 0.1*	0.65
F3	108.0 ± 1.2	112.0 ± 0.2*	0.82
C. cephalonica + water			
F1	96.4 ± 2.3	102.7 ± 5.0*	0.74
F2	55.8 ± 2.7	80.5 ± 4.6*	0.77
F3	65.1 ± 5.7	96.1 ± 8.4*	0.78

F1, F2 and F3 stands for first, second, and third filial generations respectively

't' test $p<0.05\%$ level

55.8 days for male and 80.5 days for female). Similar observations were also observed when *R. marginatus* was provided with *S. litura,* and OD + *C. cephalonica* too (Table 16). The adult longevity was slightly longer when compared with natural prey *S. litura* (average of 80.13 days for male, and 92.63 days for female) or factitious host *C. cephalonica* (average 106.6 for male and 118.33 for female) (t=20.96, df=12, P=0.000).

3.4. Fecundity and Hatchability

3.4.1. Oviposition periods

Female adult longevity was divided into three distinct phases namely preoviposition, oviposition and post oviposition periods. In *C. cephalonica* category, preoviposition period was ranged from 20.5 to 26.2.0 days (average 22.93 days) (t =19.75, df =13, p =0.000). It was slightly prolonged (1-3 days) when water was provided along with the *C. cephalonica* from 22.2 to 30 days (t =16.43, df-13, P=0.000) (average 25.26 days) (Table 17). In contrast, preoviposition period was similar for female fed with *S. litura* and *C. cephalonica.* However, the oviposition period of *R. marginatus* was lowered when it was fed with *S. litura* (average 79.8 egg/female for CC T1, 84.63 for CW T2 and 33.53 egg/female for SL T5) (Table 17). It was also revealed from results that oviposition period was more or less similar in T_3 and T_4 reared *R. marginatus* than the control category (Table 17). This was further changed when *C. cephalonica* provided along the OD (78.56. days).

3.4.2. Egg hatchability

The results showed that the mean number of eggs laid by female *C. cephalonica* fed on diets containing egg yolk, vitamin 'C' and mixed yeast extracts mean number of eggs were for OD (T_4). (average for three generations was 33.66%) (t =21.62, df =12,

Table 16. Sex ratio and adult longevity (in days) of *R. marginatus* reared on Oligidic diet with *C. cephalonica* (CC) (T_1) and oligidic diets (OD) only (T_3)

Diets	Adult longevity		Sex ratio
	Male	Female	
Oligidic diets			
4	78.3 ± 4.5	83.0 ± 4.6*	0.76
5F1	85.7 ± 2.0	104.4 ± 6.5*	0.74
5F2	94.2 ± 4.7	108.3 ± 5.9*	0.67
6F1	85.0 ± 3.0	97.2 ± 4.1*	0.62
6F2	63.1 ± 2.2	74.1 ± 7.4*	0.79
6F3	76.5 ± 5.5	109.4 ± 2.3*	0.68
Oligidic Diets + *C. cephalonica*			
4	77.2 ± 5.1	84.6 ± 9.1*	0.62
5F1	81.2 ± 4.9	84.5 ± 3.3*	0.72
5F2	78.0 ± 4.8	87.3 ± 5.9*	0.67
6F1	82.0 ± 2.9	88.9 ± 4.5*	0.76
6F2	75.2 ± 2.8	87.3 ± 6.4*	0.75
6F3	86.2 ± 3.2	104.5 ± 8.2*	0.76

F1, F2 and F3 stands for first, second, and third filial generations respectively

't' test $p<0.05\%$ level

Table 17. Preoviposition (PRE), oviposition (OVI), and post oviposition periods (POVI) (in day), number of eggs laid (NEL), number of egg batches (NEB) and number of nymphs hatched (NNH) of *R. marginatus* reared on *C. cephalonica* and *C. cephalonica* with water

Diets	PRE	OVI	POVI	NEL	NEB	NNH
S. litura						
SLF1	20.8 ± 2.1	28.8 ± 2.1*	15.8 ± 3.3*	131.8 ± 7.5*	3.2 ± 0.1*	94.5*
SLF2	19.8 ± 3.3	32.5 ± 3.5*	14.7 ± 2.2*	122.6 ± 6.8*	3.1 ± 0.2*	95.2*
SLF3	25.3 ± 2.1	39.3 ± 4.2*	12.3 ± 3.4*	108.2 ± 8.2*	3.1 ± 0.5*	91.2*
C. cephalonica						
CCF1	26.2 ± 0.4	50.3 ± 0.8*	24.1 ± 0.3*	177.0 ± 8.6*	3.2 ± 0.1*	85.7*
CCF2	20.5 ± 1.1	95.0 ± 1.5*	29.5 ± 0.9*	88.7 ± 6.5*	2.9 ± 0.1*	88.9*
CCF3	22.1 ± 1.1	94.1 ± 2.2*	32.2 ± 3.2*	92.2 ± 1.2*	2.6 ± 0.2*	96.2*
***C. cephalonica* + water**						
CWF1	30.0 ± 1.0	56.9 ± 1.9*	26.9 ± 0.8*	170.9 ± 12.0*	2.7 ± 0.1*	87.0*
CWF2	22.2 ± 0.9	110.0 ±1.1*	27.3 ± 0.8*	95.5 ± 4.8*	2.7 ± 0.1*	94.7*
CWF3	23.6 ± 1.4	87.0 ± 1.2*	23.0 ± 1.4*	105.4 ± 5.5*	2.1 ± 0.2*	96.4*

F1, F2 and F3 stands for first, second, and third filial generations respectively

't' test $p<0.05\%$ level

P =0.000) and for OC (T_3). (average for three generations was 42.88%) (t =14.54, df =12, P =0.000) and the CW (T_2) (t =11.40, df =12, P = 0.000).

3.4.3. Number of eggs laid

R. marginatus laid 177.0 eggs when *C. cephalonica* was provided as prey. It was reduced (170.9) egg/female when water was provided along with *C. cephalonica.* Further decrease was observed when *S. litura* was provided (131.8 egg/female) (Table 17). When, *R. marginatus* was reared with OD, initially the fecundity was very meager (10- 5 eggs/female) then it was increased to diet 5 (19.0 and 34.6 egg/female for F1 and F2 generation respectively). Further increase was recorded in diet – 6 (53.00 egg/female) (Table 18). The mean number of eggs laid were higher in control categories, whereas fecundity was not significantly different for the prey *S. litura* (T5), *C. cephalonica* (T_1), *C. cephalonica* weekly once with water (T2) respectively.

The incubation period of *R. marginatus* egg was eight days in the control category. The hatching percentage was gradually decreased with increasing the days of incubation. The percentage of hatched eggs was higher (average 93.6 for T_5 and 90.26 for T_1) in the predator reared on natural prey than in the meat - based diet (89.56 and 88.16 for OD and OC respectively). In OD and OD + CC categories, *R. marginatus* laid eggs but they were not hatched. Generally the reproductive rate and hatchability of the predators which reared on OD was less than those reared on factitious or natural prey *S. litura* (Table 17 and 18).

Table18. Preoviposition (PRE), oviposition (OVI) and post oviposition periods (POVI) (in days), number of eggs laid (NEL), number of egg batches (NEB) and number of nymphs hatched (NNH) of *R. marginatus* reared on different oligidic diets (T_3 and T_4)

Diets	PRE	OVI	POVI	NEL	NEB	NNH
Oligidic diet Diets						
3	-	-	-	-	-	-
4	28.1 ± 1.5	-	-	10.5 ± 0.7	1.0 ± 0.0	-
5F1	29.5 ± 1.1	76.0 ± 0.9*	26.3 ± 2.0*	19.0 ± 1.5*	1.5 ± 0.1	89.3*
5F2	25.2 ± 1.5	87.0 ± 0.1*	34.8 ± 1.5*	34.6 ± 3.5*	1.7 ± 0.1*	94.0*
6F1	26.5 ± 1.0	76.3 ± 2.1*	22.8 ± 1.2*	38.9 ± 4.9*	1.5 ± 0.1	92.3*
6F2	27.2 ± 2.2	65.3 ± 4.6*	22.1 ± 2.2*	46.0 ± 7.2	1.3 ± 1.2	76.7*
6F3	47.0 ± 7.3	58.2 ± 2.2*	26.1 ± 4.2*	53.0 ± 1.0*	1.3 ± 0.3*	95.8*
Oligidic Diets + *C. cephalonica*						
3	-	-	-	-	-	-
4	27.0 ± 2.1	-	-	17.6 ± 2.6	1.0 ± 0.0	-
5F1	29.0 ± 1.3	82.1 ± 2.2*	16.4 ± 1.2*	30.2 ± 2.2*	1.5 ± 0.1*	83.8
5F2	25.7 ± 1.4	132 .0 ± 2.5*	36.7 ± 3.0*	41.2 ± 3.1*	2.0 ± 0.1	94.5*
6F1	26.5 ± 1.2	86.0 ± 2.3*	16.4 ± 1.2*	47.7 ± 2.9	1.5 ± 0.1*	93.8
6F2	24.2 ± 3.1	54.4 ± 3.2*	23.0 ±1.4*	61.0 ± 4.5	1.2 ± 0.2	74.6*
6F3	31.2 ± 2.3	38.3 ± 4.2*	13.0 ± 3.2*	59.6 ± 4.7*	1.7 ± 0.2*	94.1*

F1, F2 and F3 stands for first, second, and third filial generations respectively

't' test $p<0.05\%$ level

3.5. Life table parameters

Life table analyses showed that the net reproductive rate (NRR), arbitrary value of innate capacity for increase, innate rate of increase and weekly multiplication were maximum in *C. cephalonica*, *C. cephalonica* + water (Table 19). These parameters were gradually decrease in oligidic diet-6 and 5 and also in oligidic diet and weekly once with *C. cephalonica* (5C and 6C) (Table 19).) precise generation time was minimum (14.58 days for diet 5A) and maximum oligidic diet (6A F3) and doubling time respectively.

The life table parameters of *R. marginatus* on five categories showed that (table 19 and 20) that the net reproductive rate significantly higher in *S. litura* (81.50, average 74.83) reared predators than other diets like *C. cephalonica* (78.00) and OD (22.00 for diet 5) for categories. The corrected rm was higher for *C. cephalonica* (average 0.52) followed by *S. litura* (0.48) and 4A to 6A were 1.00, 0.96, 1.51, 0.90 respectively. The precise generation time (T), finite rate of increase and weekly multiplication time was always greater in *S. litura* than other diets. However more or are less similar results were observed in *C. cephalonica.* The doubling time (C) was ranged from 1.00 to 1.47 for F1 to F3 *C. cephalonica* respectively. The hypothetical F2 female was maximum in T_2 category (4624.00) and minimum in 5A (484.00) category.

3.6. Deformities

When we reared *R. marginatus* with diet 3, 4, and 5, both nymphs (Plate 3 c, d) and adults (Plate 3 e, f, g, and h) were having deformities. They kinds of deformities were common in *R. marginatus*. They were: malformed legs (Plate 3 c, d, e, and f), curved and/or folded wings and exuvia present along with adults. If the OD soaked was not

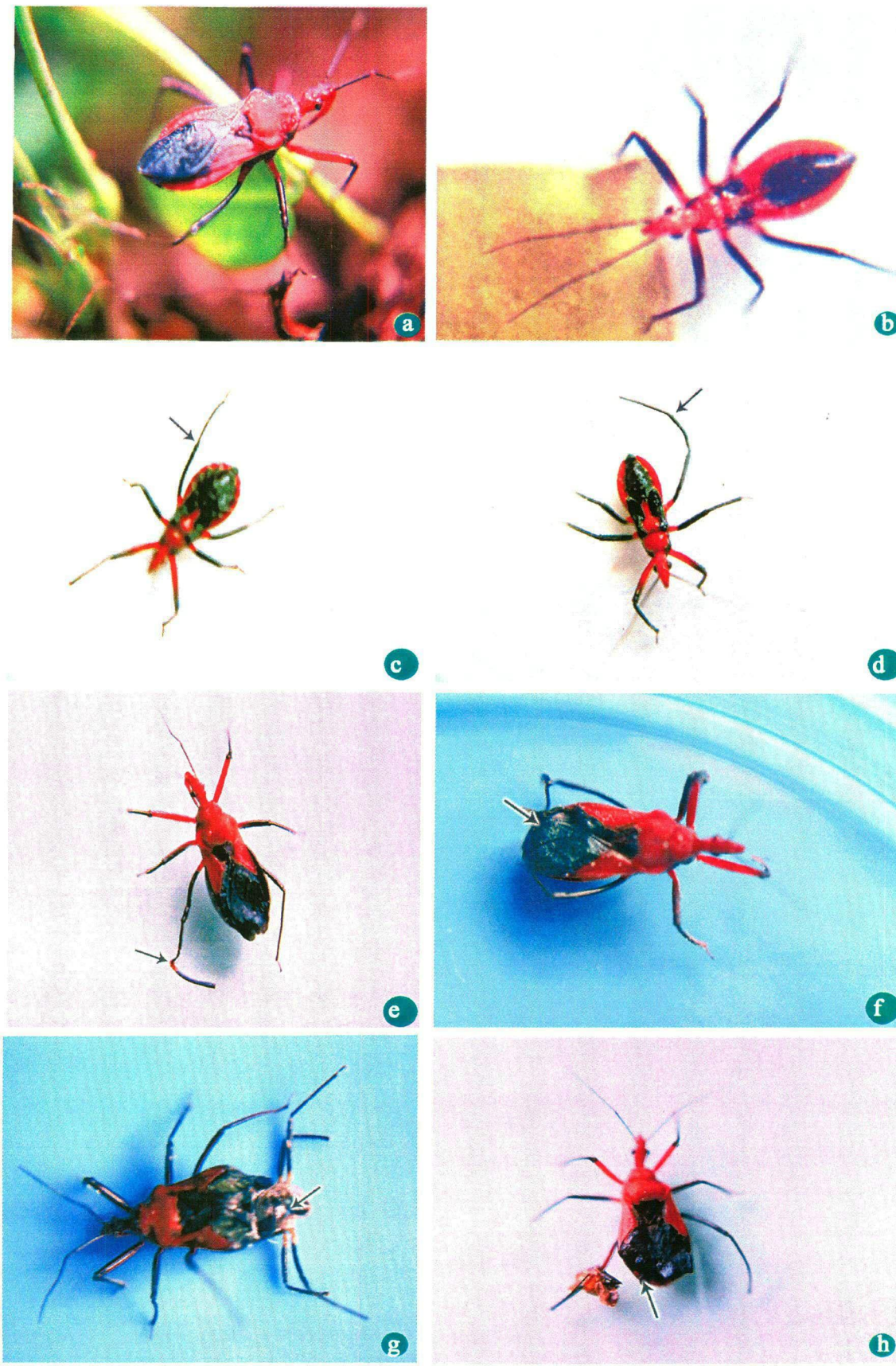


Plate 3. Normal (a & b) and abnormol *R. marginatus* nymph (c, d) and adult (e-h) reared on artificial diet.

Table: 19. Life table parameters of *R. marginatus* on different diets 4 – 6 and *Corcyra cephalonica* with Oligidic diet

Parameters	Oligidic diet						*Corcyra cephalonica* with Oligidic diet					
	4	5F1	5F2	6F1	6F2	6F3	4C	5F1	5F2	6F1	6F2	6F3
Net reproductive rate (NRR)	-	22	23	33.25	48.70	52.25	28	44	60	62.0	64.75	65.75
Mean length of generation (Tc)	32.5	14.63	25.82	46.75	63.25	64.46	30.82	45.72	62	56.06	67.41	88.57
Innate capacity for natural increase (rc)	0.92	1.50	0.89	0.052	0.055	0.062	0.908	0.96	0.96	0.041	0.041	0.044
Corrected rm	0.96	1.51	0.90	0.055	0.045	0.065	0.910	0.97	0.97	0.042	0.042	0.038
Precise generation time (T)	31.25	14.58	25.55	56.75	57.22	58.71	30.76	45.36	61.85	77.28	85.96	86.25
Finite rate of increase (λ)	1.84	3.02	1.79	1.04	1.05	1.07	1.81	1.92	1.92	1.049	1.050	1.046
Weekly multiplication time	1.92	3.76	3.6	12.35	10.02	11.27	3.27	1.94	1.94	4.54	4.26	4.53
Doubling time(C)	1.08	0.66	1.11	1.45	1.52	1.60	1.02	1.04	3.84	1.395	1.329	1.389
Hypothetical female in F2 generation	900	484	529	862.75	982.52	1943.06	784	1936	3600	4356.0	4526.25	4423.06

Table 20. Life table parameters of *R. marginatus* on *C. cephalonica* (T_1) *S. litura* (T_5) and *C. cephalonica* with water (T_3)

Parameters	*C. cephalonica*			*S. litura*			*C. cephalonica* with water		
	CC F1	CC F2	CC F3	SLF1	SLF2	SLF3	CW F1	CW F2	CW F3
Net reproductive rate (NRR)	78.00	73.5	66.5	81.50	72.75	70.25	38.00	31.00	68.00
Mean length of generation (Tc)	78.55	95.18	95.44	89.40	91.87	85.6	39.21	32.35	68.52
Innate capacity for natural increase (rc)	0.99	0.44	0.13	0.050	0.045	0.051	0.96	0.96	0.99
Corrected rm	1.00	0.55	0.69	0.56	0.072	0.055	0.98	0.97	1.00
Precise generation time (T)	78.0	88.48	77.95	78.25	81.48	78.21	38.77	31.95	68.0
Finite rate of increase (λ)	2.00	1.51	1.50	1.57	1.061	1.056	1.92	1.92	1.98
Weekly multiplication time	1.98	13.55	14.62	11.41	12.72	11.95	1.96	1.94	2.00
Doubling time(C)	1.00	1.419	1.409	1.477	1.258	1.569	1.04	1.04	3.96
Hypothetical female in F2	1260.84	53658.2	4552.23	6881.56	5025.54	4954.22	1444	961	4624

removed, it induce disease infection on *R. marginatus* nymphs. (Plate 3a) and adults (Plate 3b).

3.7. Gut bacterial profile and their enzyme activities

3.7.1. Total Heterotrophic Bacterial Population (THBP)

The total heterotrophic bacterial population (THBP) varied according to the diet ingested by the reduviid. Among all the categories, the bacterial population was found to be higher in *S. litura* (114.32) category followed by *C. cephalonica* (103.8), *C. cephalonica* with water (98.55), Oligidic diet with *C. cephalonica* (68.5). THBP was drastically decreased in OD diet reared reduviids (40.4) (Figure 7).

3.7.2. Bacterial composition

Fourteen bacterial species such as *Bacillus subtilis, B. megaterium, B. cereus, Carnibacterium xerosis, C. kutcheri, Escherichia coli, Klepciella pneumoniae, Pseudomonas aeroginosa, Micrococcus varians, M. luteus, Proteus vulgaris, Staphylococcus aureus, S. epidermidis and S. saprophyticus* belongs to eight genera were found to be common in the gut of *R. marginatus* reared in three different diets regime. All bacterial species (14) were recorded in oligidic diet reared *R. marginatus,* followed by the *C. cephalonica* (9) and *S. litura* (9). *Micrococcus* sp., *Bacillus subtilis, Pseudomonas fluorescens, Streptococcus faecalis* and *Aeromonas* sp. were also isolated from the predators. *Micrococcus varians* was a dominant (52.70 %) species diet. In natural prey reared reduviid, all the bacteria were maximum than oligidic diet. In contrast, the number of bacteria within oligidic diet reared insect decreased (Table 21).

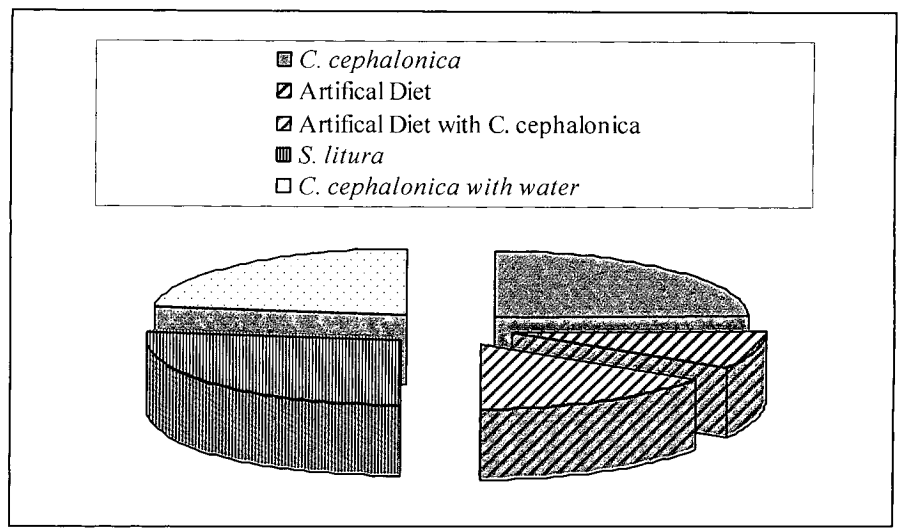


Figure 7: Mean viable bacterial count (CFU x 10^3 ml/g) of *R. marginatus* reared on Oligidic Diets and natural prey

Table 21. Viable bacterial count of *R. marginatus* reared on oligidic diet, natural and factitious host

Proportion of bacterial population					
Bacteria	**T1**	**T2**	**T3**	**T4**	**T5**
Bacillus subtilis	18.96	16.56	8.9	10.83	10.1
B. megaterium	-	3.35	10.81	-	10.10
B. cereus	8.9	8.9	7.20	-	6.1
Carnibacterium xerosis	0.92	3.55	0.71	2.18	2.40
C. kutcheri	-	-	1.01	-	-
Eachericia coli	-	-	10.47	4.25	1.6
Klepciella pneumoniae	1.10	2.25	2.81	-	-
Pseudomonas aeroginosa	1.14	2.25	0.5	0.55	1.4
Micrococcus varians	42.33	36.45	27.20	52.70	40.6
M. luteus	4.3	8.5	3.6	1.74	10.4
Proteus vulgaris	2.40	2.40	3.25	-	-
Staphylococcus aureus	20.33	21.35	16.11	28.0	16.56
S. epidermidis	-	-	6.08	-	-
S. saprophyticus	-	-	5.60	-	-

3.7.3. Hydrolytic extra cellular enzymatic activity

The total of 650 bacterial isolates were produce amylase, protease and lipase (P + A) (Plate 4). Irrespective of the prey categories from T_1 to T_5, the amylolytic activity was observed. In AD category amylase, protease and invertase activities were observed in the foregut and the activities were reduced in hind gut (except protease) and salivary gland (except amylase). Hydrolytic activity was lower in factitious host reared predators. Lipase and invertase activities were very minimum in the control category. Amylase, invertase, protease and lipase activities were higher in foregut and hindgut (except amylase) (see table 22) salivary gland of the control categories.

3.8. Enzymes

3.8.1. Qualitative enzyme profiles

Food quality regulates and influences the production of alimentary canal digestive enzymes. Henceforth, an attempt was made to study the quantitative profile of digestive enzymes in relation to different preys and OD. From the table 21, it was very clear that *R. marginatus* foregut and hindgut contain amylase, protease, invertase, and lipase. But the activity profile is depends on the type of diet consumed by the predators. For instance, amylase, invertase and lipase activities of both the foregut and hindgut were maximum while *R. marginatus* was fed with *C. cephalonica* followed by OD and *S. litura*. The hindgut enzymes activities were decreased (++) and similar kind of observations were recorded for OD and *S. litura*. The results shows that generally prey type and Oligidic diet does not have any influence on the enzyme qualitative of this reduviid. In salivary gland, all the enzyme activities were moderate (++) except lipase (+)

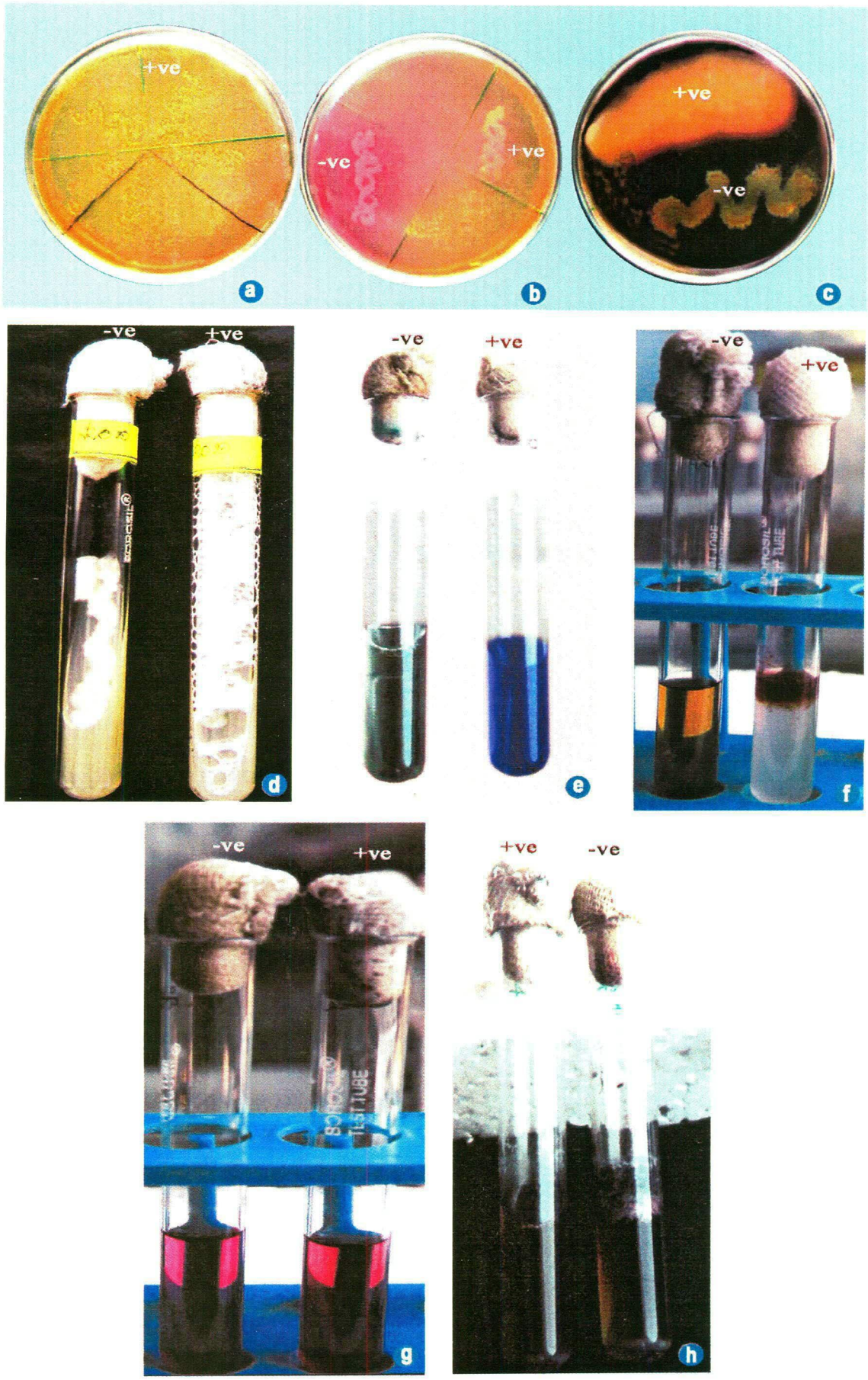


Plate-4. Lactose (a,b) and carbohydrate (e) fermentation, Amylase (c), Catalase (d), Methyle red (f), Voges proskar (g) and Nitrate reduction tests (h) of *R. marginatus* gut microbes

Table 22. Impact of *C. cephalonica* (T_1), *S. litura* (T5), artificial diet (T3), Artificial diets + *C. cephalonica* (T4), *C. cephalonica* with water (T2) on the foregut, hindgut and salivary gland qualitative enzyme profile of *R. marginatus*

Treatments	Protease	Lipase	Amylase	Invertase	Trypsin	Pepsin
Foregut						
T1	+++	+++	+++	+++	++	++
T2	+++	++	++	+	+++	++
T3	+++	++	+++	+++	++	++
T4	+++	+	++	++	++	+++
T5	+++	++	++	++	+++	+++
Hindgut						
T1	+++	+++	++	+++	++	++
T2	++	++	+	+++	++	+++
T3	+++	++	++	++	++	++
T4	++	+++	++	++	+++	++
T5	+++	++	++	++	++	++
Salivary gland						
T1	+++	+	+++	+	++	+
T2	++	+	++	+	++	++
T3	++	+	+++	+	++	++
T4	+	++	++	++	++	++
T5	+++	++	+++	++	++	++

(+) less; (++) moderate and (+++) maximum

when in T1 and T5 categories. The activity was further reduced (+) on *C. cephalonica* and OD for *R. marginatus* (Table 22).

3.8.2. Quantitative enzyme profiles

3.8.2.1. Amylase

Amylase is one of the key enzymes involved in digestion of carbohydrate metabolism in insects. To study the putative hyper production of amylase response of diet, the gut of reduviid adult *R. marginatus* feeding on different preys or on OD reared reduviid gut was analysed for amylase activity. The amylase quantity of reduviid hindgut has the higher in both preys and OD than foregut. Among the five diets, OD reared reduviid hind gut has the maximum activities (0.69 mg protein released/min) (t=17.19, df=2, P=0.003) followed by *C. cephalonica* (0.58 mg protein released/min) (t=1.31, df=2, P=0.320) and *S. litura* (0.44 mg protein released/min) (t=48.32, df=2, P=0.000) same results were also observed in salivary gland also, OD showed highest activity (0.18 mg protein released/min) (t=20.00, df=2, P=0.002) followed by *C. cephalonica* (0.17 mg protein released/min) (t=2.01, df=2, P=0.181), *S. litura* (0.14) (t=19.00, df=2, P=0.003) categories respectively. In the present study foregut has the lowest amylase activity. But further increased level was recorded in the foregut of *C. cephalonica* reared reduviids (Table 23).

3.8.2.2. Invertase

This study showed that irrespective of the categories, invertase activity was higher in hindgut followed by salivary gland and foregut. OD reared predator hindgut showed maximum invertase activity (0.72 mg protein released/min) (t=31.00, df=2, P=0.001), followed by *C. cephalonica* (0.67 mg protein released/min) (t=24.40, df=2, P=0.002). The

invertase activity was significantly increased from 0.6 mg protein released/min to 3.0 mg protein released/min in salivary glands when *R. marginatus* take OD (T_4). In foregut *C. cephalonica* showed the lowest invertase activity (0.10 mg protein released/min) (t=1.176, df=2, P=0.360) but increase in salivary gland (0.2 mg protein released/min) (t=40.06, df=2, P=0.001). Among all the preys tested, *S. litura* reared reduviid salivary gland has the minimum activity (0.68 mg protein released/min) (t=21.71, df=2, P=0.002).

3.8.2.3. Lipase

Lipase activity was minimum in salivary gland of all the categories. In general *S. litura* fed reduviid foregut showed the maximum amount of lipase (2.6 mg/min) (t=61.36, df-2, P=0.000) than those fed with OD (1.24 mg/min) (t=13.62, df-2, P=0.005) and *C. cephalonica* (1.1 mg/min) (t=19.82, df-2, P=0.003) (Table 23). Salivary gland showed meager amount of lipase activity in all the categories. As observed in fore and hind guts *S. litura* (0.2) (t=48.32, df-2, P=0.000) has the higher amount of lipase than reduviids reared on OD (0.1 mg/min) (t=10.78, df-2, P=0.008) and *C. cephalonica* (2.6 mg/min) (t=3.50, df-2, P=0.073).

3.8.2.4. Protease activity

Both in fore and hind guts intense protease activity was observed in OD (13.9 mg protein released/min) (t=42.86, df-2, P=0.001) reared predator followed by *C. cephalonica* (13.4 mg protein released/min) (t=57.89, df-2, P=0.000) reared reduviids. Least protease activity was recorded in *S. litura* (12.0) (t=18.25, df-2, P=0.003) reared predator both in fore and hindguts (5.1 mg protein released/min) (t=6.17, df-2, P=0.025) (Table 23). Similar trend was also observed in salivary gland of *R. marginatus* but *C. cephalonica* has the maximum activity (3.0 mg protein released/min) (t=7.85, df-2,

Table 23. ***C. cephalonica*** **(T_1),** ***S. litura*** **(T_5), Oligidic diet (T_3), Oligidic diet+** ***C. cephalonica*** **(T_4),** ***C. cephalonica*** **with water (T_2) on the foregut, hindgut and salivary gland quantitative enzyme profiles of** ***R. marginatus***

Treatments	Amylase	Invertase	Lipase	Protease
Foregut				
T1	0.24 ± 34	0.10 ± 2.4	1.1 ± 0.2	13.4 ± 1.6
T2	0.23 ± 2.5	0.96 ± 0.2	0.8 ± 0.0	10.2 ± 0.5
T3	0.25 ± 1.3	0.11 ± 1.3	1.2 ± 0.2	13.9 ± 0.3
T4	0.23 ± 1.2	0.95 ± 0.2	0.9 ± 0.0	11.2 ± 0.2
T5	0.19 ± 2.2	0.12 ± 1.2	2.6 ±1.2	12.0 ± 0.2
Hindgut				
T1	0.58 ± 2.5	0.67 ± 1.6	0.7 ± 0.2	5.2 ± 1.0
T2	0.48 ± 0.5	0.55 ± 0.5	0.8 ± 0.2	4.5 ± 0.5
T3	0.69 ± 0.7	0.72 ± 0.5	0.8 ± 0.6	6.9 ± 1.6
T4	0.52 ± 1.2	0.55 ± 0.5	0.6 ± 0.4	4.5 ± 1.2
T5	0.44 ± 0.3	0.24 ± 1.2	1.5 ± 0.5	5.1 ± 0.3
Salivary Gland				
T1	0.17 ± 0.1	0.20 ± 0.4	0.2 ± 0.5	3.0 ± 1.1
T2	0.13 ± 0.5	0.15 ± 0.5	0.3 ± 0.1	2.5 ± 0.4
T3	0.18 ± 0.4	0.30 ± 0.8	0.1 ± 0.1	2.4 ± 0.1
T4	0.12 ± 0.2	0.15 ± 0.05	0.3 ± 0.5	2.5 ± 0.2
T5	0.14 ± 0.3	0.60 ± 0.1	0.2 ± 0.0	1.4 ± 0.2

Amylase - Activity based on mg glucose released/mg. protein/min.

Invertase - Activity based on µl maltose released/ mg. protein/min.

Lipase - Activity based on µl NaOH released/ mg. protein/min.

Protease - Activity based on µl casein released/min.

P=0.016) followed by OD (2.4 mg protein released/min) (t=17.61, df-2, P=0.003) and *S. litura* (0.4 mg protein released/min) (t=15.02, df-2, P=0.004).

3.9. Macromolecular profile

Whole body total macromolecular content of the tested preys and oligidic diet is presented in Figure 8. From the results, it was very clear that total carbohydrates, proteins and lipids contents were higher in *S. litura* followed Oligidic diet and *C. cephalonica.*

3.9.1. Protein profile

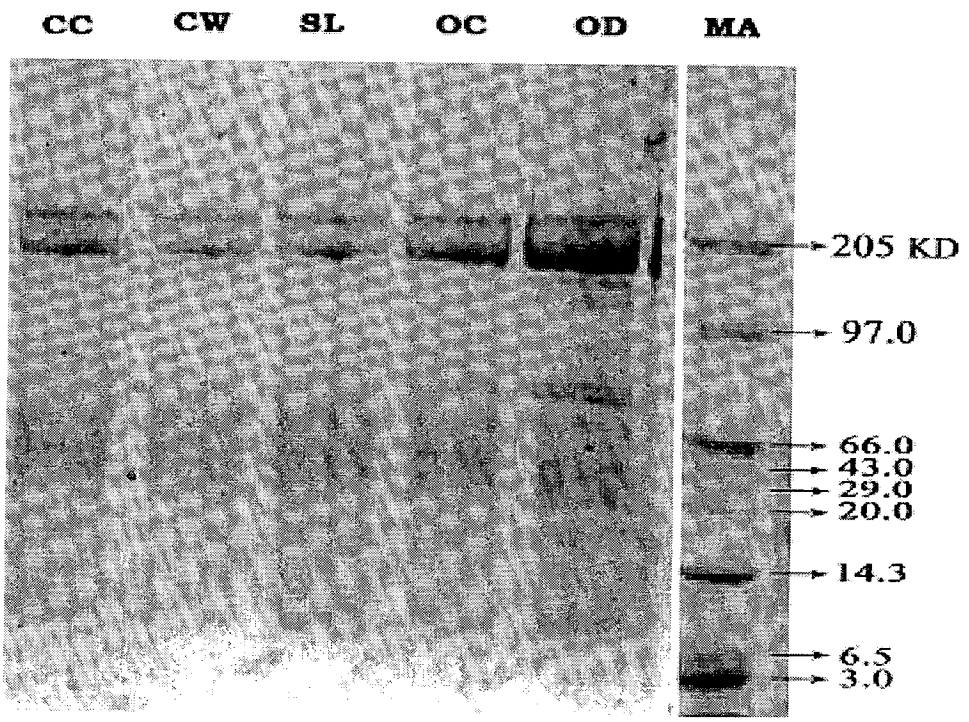


Plate 5. Whole body protein profile SDS profile of *R. marginatus* (Oligidic diet, *Corcyra cephalonica*, *C. cephalonica* with water, S. *litura*, Oligidic diet + *C. cephalonica*, Oligidic diet and Marker protein-MA

SDS-PAGE separation of the gut and whole body homogenate of the *R. marginatus* in a mini gel (10 X 7.5 cm and 5 and 10% acrylamide for stacking and resolving gels respectively, 1.5 mm). The electrophoretic separation of the denatured

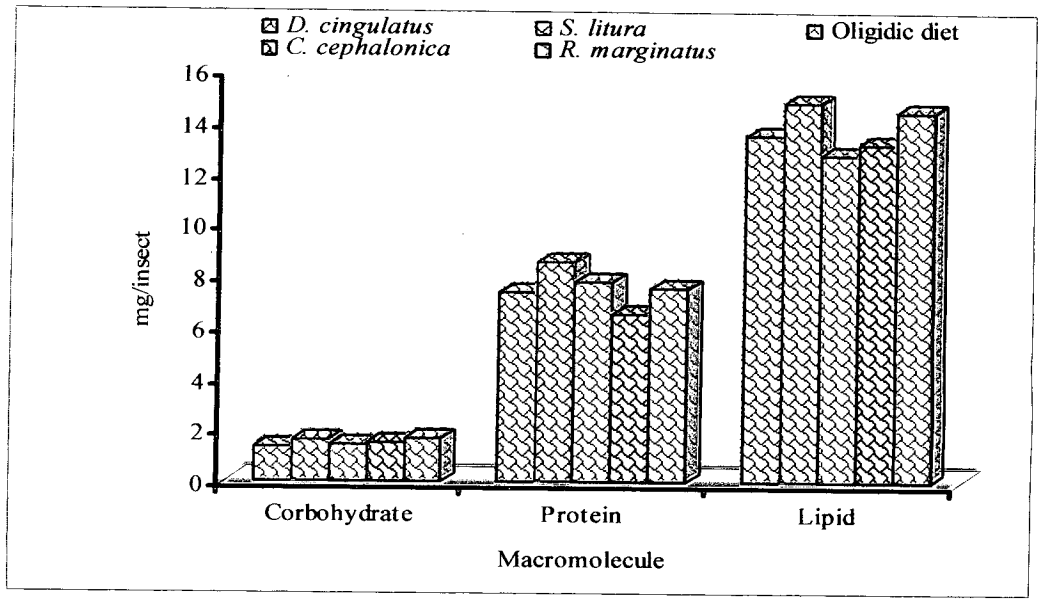


Figure 8: Quantitative macromolecular components of pest, predator and Oligidic Diet in mg / insect

proteins from whole gut and whole body homogenate of *R. marginatus* is shown in Plate 5. The PAGE analysis of proteins of predator revealed that OD influenced the number of bands and intensity of bands. A greater number of protein bands are visible in the separation of the whole body homogenate proteins from insect fed on meat based diet lane 5 and 6 versus when insect were fed control diet *C. cephalonica, C. cephalonica* weekly once with water lanes (1, 2) and natural prey *S. litura* lane 3. There are three distinct molecular weight protein ranged from 205 to 97 kDa in OD

A dendrogram generated by hierarchical clustering techniques is shown in fig 6. Cluster analysis is a standard method to study relatedness and genetic diversity among the different diet reared *R. marginatus.* Five diets reared *R. marginatus* were resolved in to five clusters based on the protein expression. *C. cephalonica* and *S. litura* reared reduviid cluster under one group and their total similarity index was 77.5%. Oligidic diet reared reduviids and *C. cephalonica* +weekly once with water categories protein cluster under the one group. The similarity index was 55%. OD reared category cluster with the above said category with 66% indices. It is clear from the dendrogram that the reduviids reared in *C. cephalonica, S. litura* and oligidic diet are the most divergent in their ability to store amino acids as energy source.

3.10. Immunology

3.10.1. Effect of predator protein content on ELISA sensitivity

Protein content of *Rhynocoris marginatus* in relation to three pest *S. litura, D. cingulatus and C. cephalonica* and pig liver-based Oligidic diet significantly different. The overall mean protein concentration was 400, 330, 400, 475 and 425 µg/insect for *Rhynocoris marginatus, S. litura, D. cingulatus and C. cephalonica* and oligidic diets respectively. After the determination of the total protein content, each predator was

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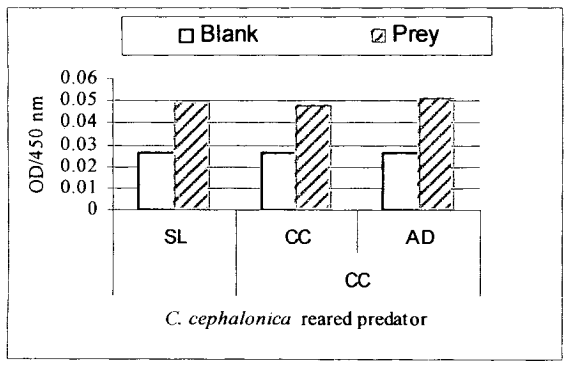
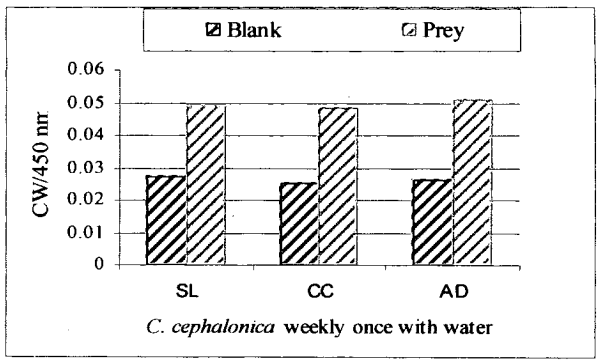
assayed by a standardized indirect ELISA. The standardized ELISA consisted of homogenizing each predator, regardless of its total protein content, in 500 µl of PBS *S. litura, D. cingulatus, C. cephalonica* and pig liver antigen was detected in every *R. marginatus* sample that was spiked with a single pest, yielding a mean ELISA absorbance value. The *R. marginatus* samples valueswere increased with a single pest were not as immuno reactive. To assayed *R. marginatus* for pest antigen, net results of overloading an ELISA plate with predator proteins is an increased probability of obtaining ELISA false negative results. We used a standardized indirect gut content ELISA for all diet reared predator because we would like to find out the qualitative feeding behavior of this predator. We standardized the first step (coating) in the indirect ELISA by grinding all predator species in 500 µl of PBS. However, it appears from the result that a 500-µl dilution yielded too high protein content for *R. marginatus* consume a minimum quantity of a prey/diet. To minimize the high frequency of the ELISA false-negative reactions. First, we added the equivalent of pest from a stock pest antigen to *R. marginatus* samples that were homogenized in with 500 µl (500 µg/well) to 1500 µl PBS. We then analyzed 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.

Indirect ELISA was examined to detect the influence of *S. litura, C. cephalonica,* and oligidic diet numerous in adult *R. marginatus*. The *S. litura* (0.062) and OD reared predator yield the highest frequency of positive immuno assay (fig. 9) followed by *C. cephalonica* reared predator. There is no significant differences were observed in *C. cephalonica.*



a



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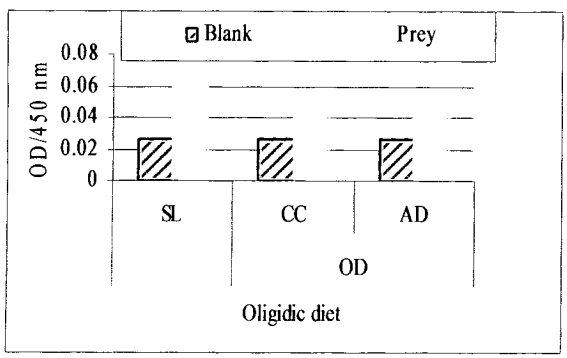
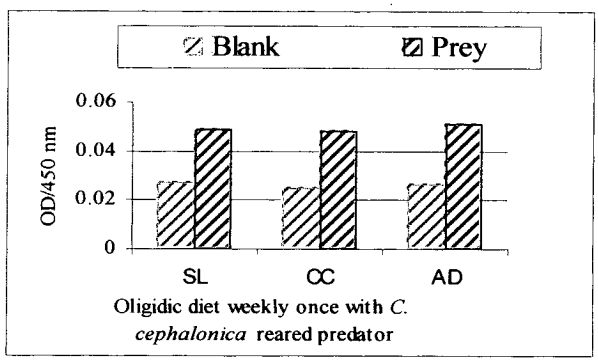
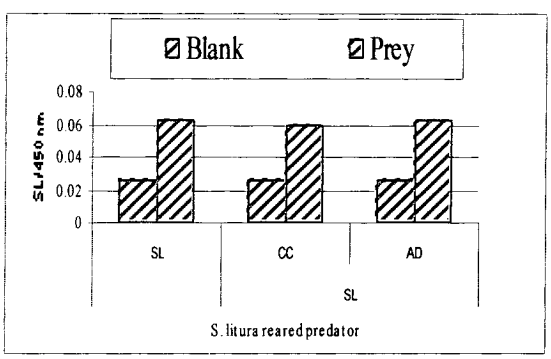


c



d



e

Figure 9: Antibody titers of indirect ELISA with pest antigen from *R. marginatus* gut different diets

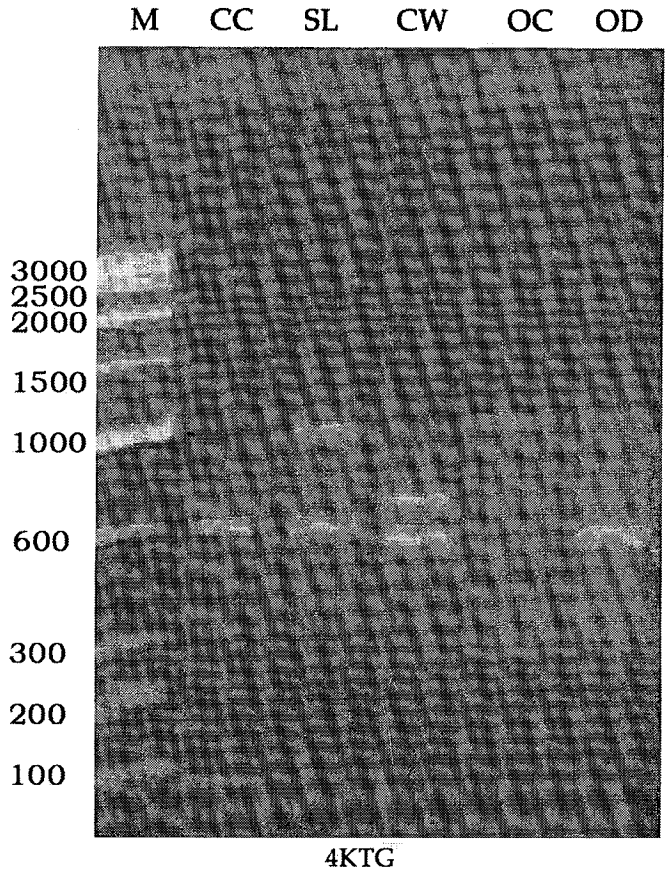
The results from this study showed that the sensitivity of the indirect ELISA depended on the predator species, quantity of prey consumed after feeding. The more preys eaten by a given predator species the greater the probability of obtaining a positive ELISA reaction.

3.11. RAPD (Polymerase chain reaction)

3.11.1. Dendrogram analyses

The dentrogram (Fig 10) demonstrate the ability of RAPD marker to reliable differences between the diets, since phenotyphic differences are not clear know by the diets. The accessions analysed clustered mainly into two groups. Cluster I accession of different diet with range upto 0.80 and cluster two consisting of the remaining 5 to 6 accessions with range 0.12 to 0.85 and accessions lane 1 is more clear than lane 2 to 5. Among the six primers such as KTG-3 and KTG-5 have potentially informative value to distinguishing five diets. The presents of few polymorphic bands with less number of primers and more monomorphic bands with most primers among the five diets were observed. The presence of more number of polymorphic bands between the diets (KTG-3 and KTG-5) have separate status in *R. marginatus* (Plate 6).

The present investigation of DNA profiling in diet clearly demonstrate that it was possible to analyse the RAPD pattern for correlating their similarity between the diets and accessions. On the basis of clustering data for the RAPD profile accessions of the first and second group exhibit greater amongst each other, and genetic variation has been found in accessions of the same populations (Plate 7).



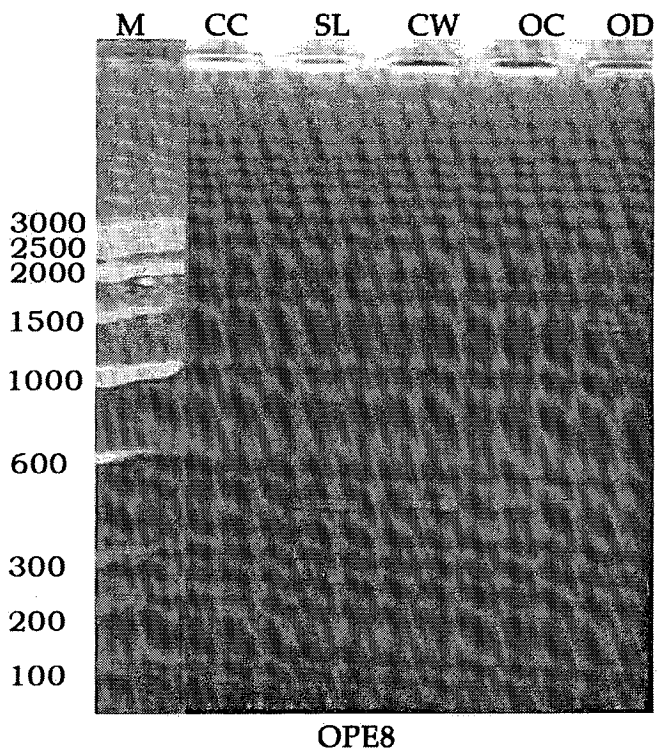
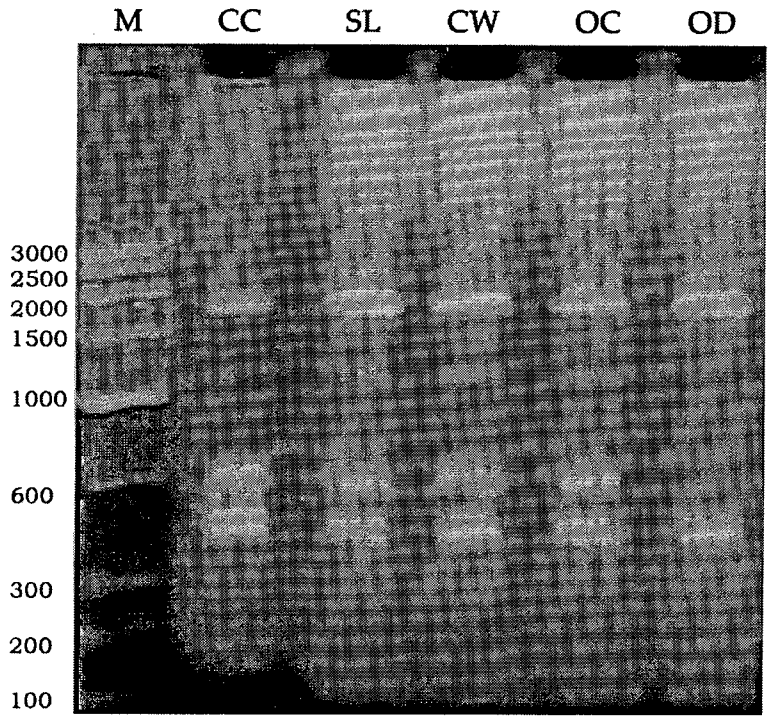
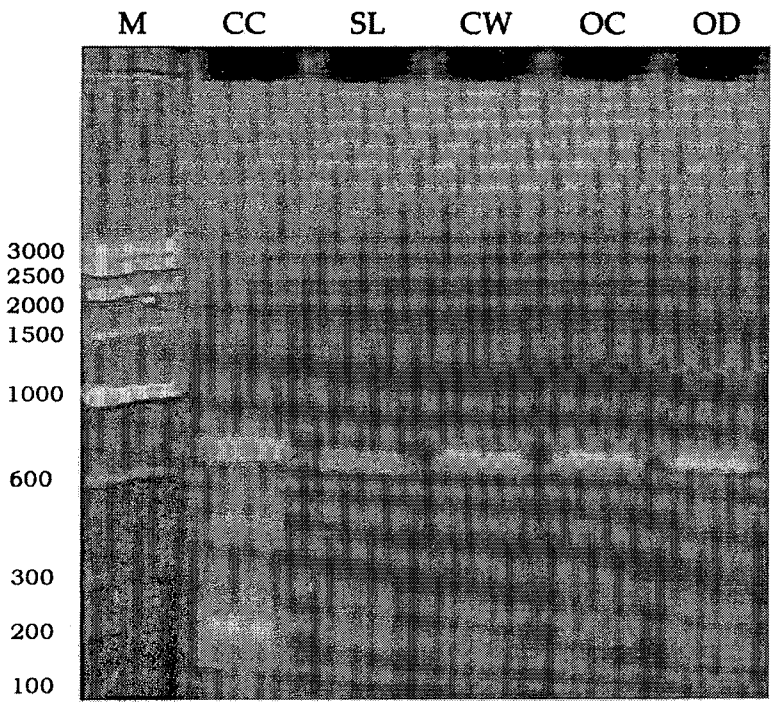


Plate 6. RAPD analyses of *R. marginatus* fed with *C. cephalonica* (CC), *S. litura* (SL), *C. cephalonica* + water (CW), Oligidic diet (OD) and OD + *C. cephalonica* (OC) using primers KTG 4 and OPE 8

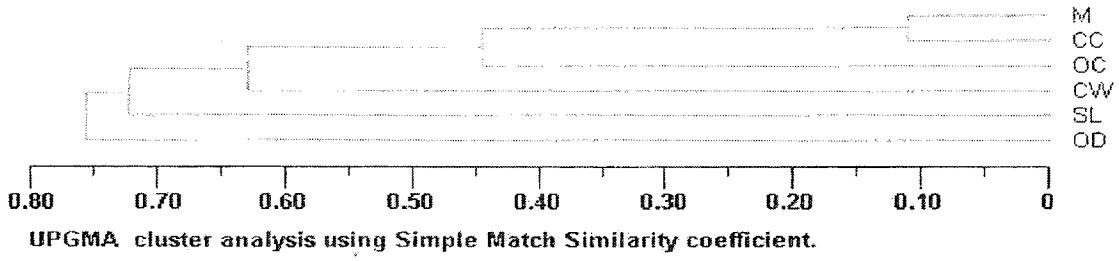


KTG 3



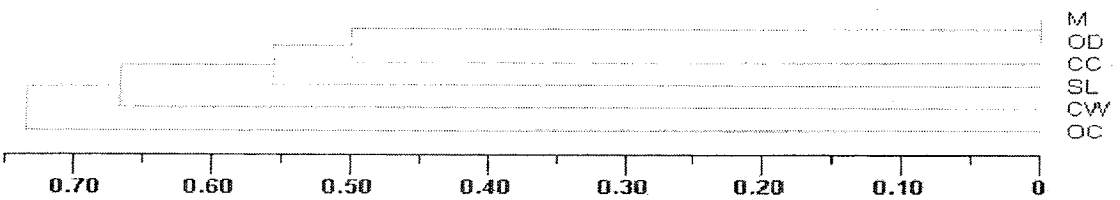
KTG 5

Plate 7. RAPD analyses of *R. marginatus* fed with *C. cephalonica* (CC), *S. litura* (SL), *C. cephalonica* + Water (CW), Oligidic diet (OD), and OD + *C. cephalonica* (OC), using primers KYG 3 and KTG5

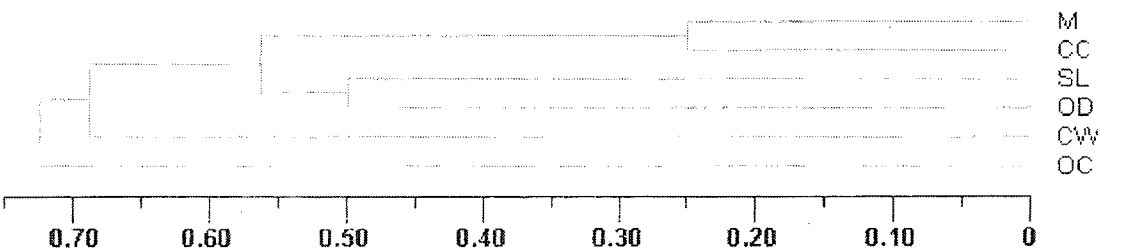


UPGMA cluster analysis using Simple Match Similarity coefficient.

Dendrogram based upon RAPD-PCR (primer OPE-4) data showing genetic relatedness amonst different diets reared Rhynocoris marginatus

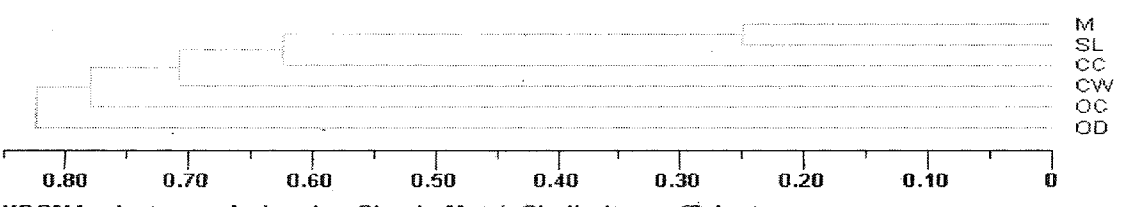


UPGMA cluster analysis using Simple Match Similarity coefficient.



UPGMA cluster analysis using Simple Match Similarity coefficient.

Dendrogram based upon RAPD-PCR (primer KTG 3) data showing genetic relatedness amongst different diets reared Rhynocoris marginatus



UPGMA cluster analysis using Simple Match Similarity coefficient.

Dendrogram based upon RAPD-PCR (Primer KTG-5) data showing genetic relatedness amongst different diets reared Rhynocoris marginatus

Figure 10: Dendrogram analysis of *R. marginatus* reared with different diets using RAPD primers KTG 4, OPE 8, KTG 3 and KTG 5

3.11.2 Primers

R. marginatus reared with *C. cephalonica* (T_1), *C. cephalonica* weekly once with water (T2), oligidic diet (T3), oligidic diet and weekly once *C. cephalonica* (T_4), and *S. litura* (T_5) were subjected to RAPD analyses using six primers. However, universal primers KTG 3, KTG 4, KTG 5 and OPE 8, produced DNA polymorphism. Results reveals that *C. cephalonica* reared *R. marginatus* produced DNA polymorphism having bp rangedfrom 100 to 2000 with absence of 1000, 1250, 1500 and 22500 bp observed in other diets. *C. cephalonica* weekly once with water (T_2), oligidic diet (T_3), oligidic diet and weekly once *C. cephalonica* (T_4), and *S. litura* (T_5) reared *R. marginatus* amplified the DNA of 200 to 2250 bp. The DNA polymorphism with 500 bp and 700 bp (except with *C. cephalonica* with KTG 4) recorded in *R. marginatus* reared with *C. cephalonica* in KTG 5 primer. Irrespective of the prey and diets, and primers DNA with bp of 200, 250, 400, 450, 600 and 2000 were common. However, none of the DNA polymorphism with specific bp wdas recorded in OD. Further studies are necessary to analyse RAPD designing specific primers. While we consider the primers, OPE 8 amplified *R. marginatus* fed with various diets. It showed DNA polymorphism with 450 bp was common among three diets such as *C. cephalonica* weekly once with water (T_2), oligidic diet and weekly once *C. cephalonica* (T_4), and *S. litura* (T_5). Both *C. cephalonica* and OD amplified *R. marginatus* DNA and showed specific bp such as 190 and 1500 respectively. The 190 bp DNA was not amplified by other tested primers like KTG 3, KTG 4 and KTG 5. Similar observation was recorded for bp 1500 with other primers except KTG 3. Among the four primers, maximum and minimum DNA polymorphism was recorded for OPE 8 (5) and KTG 3 respectively.

3. 11.2.1. KTG-3

The resultant dendrogram drawn for *R. marginatus* with KTG-3 primers shown in fig 9. All the five diets divided into two major cluster. Cluster I consisted of high similarity (0.72) obtained for oligidic diet + *C. cephalonica*. Then the cluster II divided into a, b, and c which represented for *C. cephalonica* (0.68), *S. litura* (0.56) and Oligidic diet. Among the five diets. *C. cephalonica* harbour lowest genetic similarity (0.25).

3. 11.2.2. KTG-5

The dendrogram derived with KTG-5 (Fig-10) revealed that genetic similarity was maximum for Oligidic diet (0.82) on cluster I. For cluster II, the maximum similarity was observed at Oligidic diet + weekly once with *C. cephalonica.* Furthermore cluster II subdivided into a, b and c with genetic similarity ranged between 0.25 to 0.78. Genetic similarity specific to KTG 5 oligidic diet shows maximum similarity. Where as the cluster III gives subclusters consisted more or less similar on *C. cephalonica* and *C. cephalonica* weekly once with water. This dendrogram shows very poor similarity value in *S. litura.*

3. 11.2.3. KTG -4

The denrogram primer KTG 4 showed that genetic similarity was maximum for oligidic diet (0.75) on cluster I. For cluster II the maximum similarity index was recorded for S. litura (0.72). Moreover, cluster II subdivided a, b and c and their genetic similarity was ranged from 0.10 to 0.63. Genetic similarity specific to KTG 4 was OD maximum similarity (0.63). As observed for KTG 3, lowest value was observed for *C. cephalonica* (0.10).

3.11.2.4. OPE 8

The OPE 8, (Fig 11), revealed that genetic similarity was maximum at Oligidic diet in Cluster I. In cluster II, high similarity was observed at Oligidic diet + weekly once with *C. cephalonica*. Furthermore, Cluster II subdivided into cluster a, b and c and their genetic similarity was ranged from 0.11 to 0.66. As observed for KTG-3, KTG-5, cluster II subdivided into a, b and c. *S. litura* and *C. cephalonica* + water showed minimum (0.45) and maximum (0.50) similarity index.

The present investigation on DNA profiling *R. marginatus* clearly demonstrated that it is possible to analyze the RAPD pattern for correlating their genetic similarity and dissimilarity distance between the species within the subjected five diets regimes. Further the species specific accompany with individual diets produced the bands can be utilized define the uniqueness. Which will be a helpful in the diet to the diet change it leads to the genetic changes occurred in the predator of *R. marginatus*.

3.12. Field evaluation

3.12.1. Pest population

During the study period five pests such as *Aphis crassivora* Koch, *H. armigera, S. litura, M. indica* and grosshoppers (*Atractomorpha crenulata* and *Chrotgonus trachypterus*) were recorded both in summer (Table 24) and kharif 2006 and 2007. *N. virudula, Millocerous sp., B. tabaci* and Jassids were present only during summer whereas *A. annulipes* and *P. ricini* were present in kharif (Table 25). During this period, the most abundant pest was *A. craccivora* followed by Jassids, grasshoppers. *A. craccivora* population was also prevalent in kharif. ANOVA multiple comparision test showed at 36 DASE, *H. armigera* population was significant ($p=0.083$; $p>0.05$) day khariff. However, *A. annulipes* population was statistically significant at 5% level all the observation periods. In contrast *P. ricini* population was statistically insignificant. Four

Table 24. Impact of *C. cephalonica* and artificial diet (AD) reared *R. marginatus* on the incidence of groundnut pests (No./plant) in summer season

Pests	Treatment	DASE						Mean
		36	43	50	57	64	71	
A. craccivora	Control	88.9	36.1	32	22.7	27.3	21.4	38.08
	OD	96.5	96.0	66.2	44.1	28.00	21.8	55.13
	CC	86.6	54.2	32.4	31.3	28.70	18.5	41.95
H. armigera	Control	1.0	0.4	0.0	0.0	0.7	0.3	0.40
	OD	0.7	4.3	1.3	0.4	0.3	0.0	1.16
	CC	1.9	0.9	0.4	0.7	0.0	0.0	0.65
S. litura	Control	0.1	0.4	3.0	0.0	1.7	0.0	0.86
	OD	3.3	2.6	1.5	1.0	0.9	0.0	1.55
	CC	0.9	0.0	2.4	0.0	1.6	0.0	0.81
N. virudula	Control	0.3	0.0	0.2	0.3	0.0	0.2	0.16
	OD	0.4	0.3	0.2	0.0	0.0	0.0	0.15
	CC	0.0	0.0	0.3	0.1	0.4	0.2	0.11
Millocerous sp.	Control	0.7	1.5	0.0	0.2	1.6	0.0	0.66
	OD	2.2	1.5	0.0	0.0	0.0	0.0	0.61
	CC	0.0	0.0	0.4	0.0	0.2	0.0	0.10
M. indica	Control	3.3	1.3	2.6	2.7	4.2	2.2	2.71
	OD	4.6	2.4	3.7	3.2	3.5	1.7	3.18
	CC	2.2	1.8	1.2	1.3	1.9	1.6	1.51
Grasshoppers	Control	2.8	4.7	2.1	1.6	3.6	3.2	3.00
	OD	6.0	5.8	5.4	5.2	4.2	3.0	4.93
	CC	5.4	4.2	4.2	3.2	2.6	2.2	3.63
B. tabaci	Control	8.6	1.5	12	0.0	0.0	0.0	3.68
	OD	8.3	9.7	7.6	0.0	0.0	0.0	4.26
	CC	6.4	3.8	0.0	0.0	0.0	0.0	1.70
Jassid	Control	22.0	23.5	17.4	18.0	11.1	11.25	17.20
	OD	21.9	17.3	8.7	12.0	8.0	5.0	12.15
	CC	14.7	13.6	10.5	10.2	5.0	5.6	9.93

DASE-Days After Seedling Emergence

Table 25. Impact of factitious (T_1) and artificial diet (T_3) reared *R. marginatus* on the incidence of groundnut pests (No./plant) in Khariff (2007)

Pests	Treatment	DASE						Mean
		36	43	50	57	64	71	
A. craccivora	Control	27.4	27.4	6.0	4.9	9.5	5.0	13.36
	OD	21.6	17.6	8.1	1.2	0.6	0.0	8.18
	CC	2.4	6.0	3.0	1.6	0.0	0.0	2.16
H. armigera	Control	3.2	0.6	0.3	0.7	0.4	0.6	5.2
	OD	1.2	1.4	0.5	0.4	0.0	0.0	0.58
	CC	3.6	1.4	1.5	0.9	0.0	0.0	1.23
S. litura	Control	4.0	6.5	4.2	0.1	0.5	0.0	2.55
	OD	2.6	1.9	0.4	0.2	0.0	0.0	0.85
	CC	2.2	0.4	0.6	0.4	0.0	0.0	0.6
Ants	Control	4.5	4.1	2.8	1.2	1.6	0.5	14.7
	OD	4.0	3.8	4.1	1.0	0.0	0.0	12.9
	CC	4.3	2.6	2.9	1.7	2.0	0.0	13.5
Anisolabis annulipes	Control	0.6	0.4	0.3	0.2	0.0	0.0	0.25
	OD	1.6	0.3	0.2	0.3	0.4	0.5	0.40
	CC	0.8	0.4	0.2	0.3	0.0	0.0	0.28
M. indica	Control	5.6	0.7	3.3	3.8	1.4	1.1	15.9
	OD	2.1	0.2	1.5	0.9	0.2	0.0	0.81
	CC	1.1	0.5	0.4	0.8	0.4	0.0	3.2
Grasshoppers	Control	4.8	3.6	3.1	4.6	2.9	3.8	3.8
	OD	3.3	1.0	0.7	0.5	0.3	0.0	0.96
	CC	2.8	2.0	0.9	0.9	1.0	0.0	1.26
P. ricini	Control	1.1	8.0	10.4	4.2	1.1	0.8	4.26
	OD	9.6	3.6	2.5	1.0	0.0	0.0	2.78
	CC	10.4	4.2	1.5	1.0	0.8	0.0	2.98

DASE-Days After Seedling Emergence

of them were sucking pests and others were defoliators. In the OD reared *R. marginatus* released field, *A. craccivora* was recorded as the predominant pest followed by jassids and grosshopper. The incidence of all the pest decreased significantly when the groundnut grew older. Both 43 and 50 DASE, *C. cephalonica* population was significant at 5% level ($p<0.05$) by multiple comparision test during khariff.

Two grasshoppers (t-0.22, df-9, P>=0.82) were recorded in both the seasons. Among the treatments, grasshoppers population was higher and lower in OD and control categories during summer or and kharif season respectively. In *H. armigera*, except 36 DAS, all other observations were statistically insignificant when ANOVA multiple comparision ws made between control and OD category, 64 DAS comparision between control and OD of *A. craccivora* except at 57 and 64 DAS all other observations were significant 57,64 and 71 DAS of *A. craccivora* observation made insignificant. *P. ricini* population was maximum and minimum in control (4.46/plant) and OD categories (2.78/plant).

3.12.2. Natural enemies

Among all predators recorded, *R. marginatus* was the most abundant and were found to gradually increase when groundnut plant was maturing. Though spiders population was found to be lower densities, its population was maintained throughout the groundnut vegetative cycle. When release rate of *R. marginatus* increased, the ratio of the number of prey per natural enemy decreases. From the results, it was very clear that *R. marginatus* greatly suppressed the population of *S. litura A. craccivora* and *H. armigera* and *P. ricini*. Spiders and *C. septumpunctata* were the most abundant predators in groundnut during summer (Table 26). But *R. marginatus* was dominated in kharif (Table 27). Among the two-diet regime, AD reared *R. marginatus* slightly and highly reduce the pest populations.

Table 26. Impact of *C. cephalonica* and artificial diet (T_3) reared *R. marginatus* on the incidence of groundnut predator (No/plant) in summer season (2006)

Predators	Treatments	DASE						Mean
		36	43	50	57	64	71	
Rhynocoris marginatus	Control	0.0	0.0	0.0	0.1	0.2	0.2	0.08
	OD	0.2	0.3	0.4	0.7	1.1	2.1	0.80
	CC	0.0	0.2	0.3	0.6	0.9	1.5	0.58
Rhynocoris fuscipes	Control	0.1	0	0	0.1	0	0.1	0.05
	OD	0.0	0.1	0.0	0.5	0.5	1.1	0.28
	CC	0.0	0.3	0.6	0.1	0.0	0.5	0.25
Rhynocoris longefrons	Control	0.0	0.0	0.0	0.1	0.0	0.0	0.01
	OD	0.0	0.0	0.0	0.1	0.1	0.1	0.05
	CC	0.0	0.0	0.0	0.0	0.0	0.0	0.00
Spiders	Control	0.3	0.2	0.5	0.1	0.3	0.0	0.23
	OD	0.1	0.2	2.4	4.6	3.3	4.6	2.53
	CC	1.1	1.2	1.4	1.4	2.4	0.7	1.20
Dragon fly	Control	0.2	1.0	0.1	0.2	1.0	0.0	0.08
	OD	0.4	0.0	0	1.1	0.4	0.2	0.35
	CC	0.7	0.4	0.2	0.4	0.6	0.5	0.46
Mantis religiosa	Control	0.1	0.2	0.0	0	0.0	0.1	0.06
	OD	0.3	0.0	0.0	0.4	0.1	0.1	0.15
	CC	0.0	0.5	0.5	0.4	0.0	0.4	0.30
Coccinella septumpunctata	Control	0.3	0.2	0.3	0.1	0.0	0.2	0.18
	OD	0.8	2.1	1.4	1.7	1.7	0.7	1.16
	CC	1.0	1.0	0.5	0.9	1.1	0.5	0.83

DASE-Days After Seedling Emergence

Table 27. Impact of factitious host (T_1) and artificial diet (T_3) reared *R. marginatus* on the incidence of groundnut predator (No./plant) in Khariff (2007)

Predators	Treatments	DASE						Mean
		36	43	50	57	64	71	
R.marginatus	Control	1.0	0	1.4	0.9	0.3	0.3	0.65
	OD	0.2	1.9	2.4	3.6	4.1	5.3	2.91
	CC	1.1	2.1	2.7	3.0	2.8	4.0	2.61
R. fuscipes	Control	0.4	0.0	0.2	0.3	0.3	0.3	0.25
	OD	0.0	0.4	0.0	0.0	0.5	1.1	0.33
	CC	00	0.0	0.1	0.1	0.3	0.5	0.16
R. longefrons	Control	0.9	0.0	0.7	0.4	0.3	0.1	0.40
	OD	0.0	1.2	0.2	0.1	0.1	0.1	0.28
	CC	0.0	0.0	0.2	0.0	0.4	0.6	0.20
Spider	Control	0.5	0.5	0.4	0.7	1.5	1.4	0.66
	OD	1.0	0.4	0.7	0.5	2.2	1.8	0.93
	CC	0.3	0.4	0.8	0.7	0.9	0.5	0.93
Dragon fly	Control	0.7	0.2	0.6	0.2	1.7	0.0	0.56
	OD	0.4	0.3	0.2	0.2	0.4	0.2	0.28
	CC	0.3	0.3	0.7	0.3	0.5	0.6	0.45
Mantis religiosa	Control	0.2	0.1	0.1	0.1	0.0	0.3	0.13
	OD	0.0	0.3	0.1	0.0	0.1	0.1	0.10
	CC	0.1	0.0	0.4	0.1	0.4	0.5	0.25
Coccinella septempunctata	Control	0.6	0.0	0.5	0.5	0.3	0.5	0.40
	OD	0.9	0.7	0.7	0.5	1.7	0.7	0.86
	CC	0.7	0.2	0.9	0.7	1.0	0.6	0.68

DASE-Days After Seedling Emergence

3.12.3. Yield, cost benefit ratio and Percent avoidable loss

Among all the treatments, numbers of two pods were more than three pods in a groundnut plant. For instance in T_1 and T_4 categories, the number of pod per plant were 24.17 and 71.79 respectively. Three pods were not observed during summer 2007. Number of two pods/plant was higher in the oligidic diet reared predator plots (60.39, 57.39 and 15.17 pods/plant in the T_3, T_1 and control plots, respectively) (Table 28).

During summer 2006, groundnut production was maximum in the oligidic diet reared predator plot (1224 kg/h) and followed by T_1 (936.00 kg/h) minimum in the control plot (728.00 kg/h) (Figure 11). Same trend was also observed in kharif 2007. Similarly the cost benefit ratio was high in the T3 (OD) predator released field (1:2) followed by T_1 (CC) reared predator (1:1.8). Similar results were also observed in kharif 2007 too. During 2006, the percent avoidable loss (PAL) was maximum (23.33%) in OD reared predator released field, followed by T1 reared predator released (14.81%). So *R. marginatus* could be used as a biological control agent against groundnut pests.

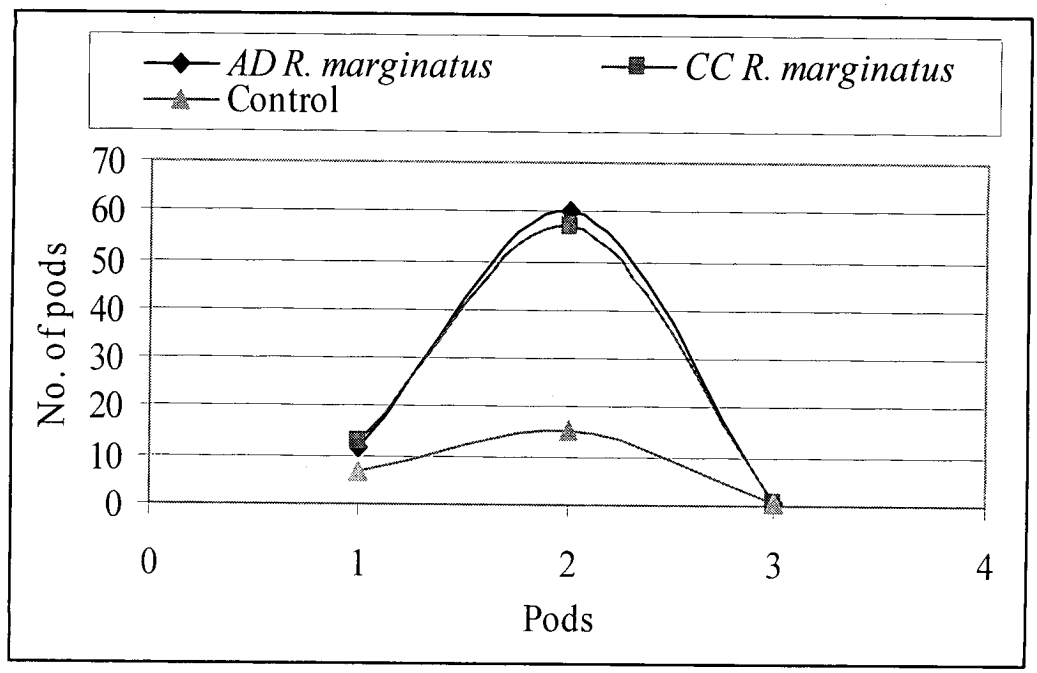


Figure 11. Groundnut production in natural diet reared and artificial diet reared on *R. marginatus* and control (no of pods per 3 plants)

Table: 28. *C. cephalonica* (T_1), artificial diet (T3) reared *R. marginatus* and control on the groundnut production Khariff

Treatements	Total No. of pods/plant	1 Pod/ plant	2 Pod / plant	Weight of total pods/ plant
T_3	58.29	4.13	54.16	78.43
T_1	45.66	8.43	37.23	66.26
Control	47.7	6.1	41.6	69.83

Discussion

CHAPTER - 4 DISCUSSION

4.1. Reduviid predator

Rhynocoris marginatus has been characterised as a polyphagous predator feeding on wide range of insect preys (Ambrose, 1999; Sahayaraj *et al.,* 2006). However, generalised predators usually show some degree of nutritional stringency and may display distinct food preference in choice situations (De Clercq, 2004). The polyphagous predator *R. marginatus* demonstrate a high level of nutritional plasticity and has the capability to utilise artificial foods, although this may results some negative effect on biological parameters as compared with feeding on optimal insects foods, like larvae of *C. cephalonica* and *S. litura*. Previously no artificial diet has been reported to support complete development of this predator.

There are survival factors that determine prey suitability for insect predators, which can be divided into nutritional and non-nutritional factors (Sahayaraj *et al.*, 2004). Under laboratory conditions, *R. marginatus* was noted to have a preference for slow moving soft bodied insect prey and highly nutrient prey , particularly foliage feeding lepidopteran larvae (Ambrose, 2006; Sahayaraj *et al.,* 2004; Sahayaraj and Balasubramanian, 2008). The suitability of a certain prey type may not only be related to its nutritional quality or acceptability for the predator but also to the prey – predator size ratio (Hagler and Naranjo, 2005). In relatively large predator like the Asopinae, the energy cost benefit ratio may be more favorable when large prey used. In order to obtain certain amount of biomass reduviids may need to spent more time and energy for capturing and handling several small prey than they would when feeding on a large prey.

Between the two tested lepidopteran preys, *C. cephalonica* size was smaller than the *S. litura*. Moreover, *D. cingulatus* are characterised by a heavily sclerotized integument may this be less preferred by predator *R. marginatus*. Moreover, it moves very faster than lepidopteran caterpillars. The superior fecundity rates of predators fed with *C. cephalonica* suggest that it is a more suitable food for the laboratory production of *R. marginatus*. Furthermore, it was also suggested by Sahayaraj *et al.* (2004) that *H. armigera* could be used for the laboratory rearing of this bug. However the cost of mass production of *R. marginatus* either with *C. cephalonica* or *H. armigera* was higher. Furthermore, rearing of either natural or factitious host is laborious, tedious and time consuming one.

4.2. Oligidic diet development

The effect of variation in individual dietary factors on growth of nymphs of *R. marginatus* nymph was largely consistent with findings of previous studies by Sahayaraj *et al.* (2005, 2007). The OD can be prepared at low cost using meat pig liver as the main food, along with several chemical components were incorporated in the rearing medium. In this study, we formulated an oligidic diet that contain meat-based diet. The meat liver, blood and or its serum were used as source ingredients. The meat-based diet tested in this study was able to sustain the *R. marginatus* for several generations without supplying any insect prey, showed a good nymphal survival rate. For the first time, initially Sahayaraj *et al.* (2006) developed oligidic diet for rearing this bug based on the comparison of its natural and factitious preys. Factors which influencing the consumption and growth rates of insects were protein, sucrose and water (Karowe and Martin, 1989; Martin and Van't Hof, 1988; Slansky, 1993; Timmins *et al.,* 1988). Shifting of nutritional requirements and capabilities are a general features of heteropteran

nymphal development as most reduviids bugs substantial changes in body size, ecology and physiology across nymphal instars (Avila *et al.,* 2003). Functional goals should change in early instars acquiring nutrients necessary for additional growth to the late instars for metamorphosis, reproduction and adult hood.

4.2.1. Oligidic diet ingredients

De Clercq (2004) classified the artificial diets into three general types: holidic diets, in which all ingredients are known in chemical structures, (2) meridic diets, which have a holidic base supplemented with one or more unrefined or chemically unknown substance (eg: liver extracts, yeast products etc...), and (3) Oligidic diets, which are mainly made up of crude organic materials (like meat diets). We recorded, diet contaminations was common when the diet was devoid of formaldehyde (diet-1-4). After the addition of formaldehyde, nearly 70% of diet contaminations has been prevented. Indirectly, it was also prevent the *Aspergillus flavus* infection to *R. marginatus* nymphs and adults (see plate 2 a and b). Furthermore, devoid of yeast in the diet 5 and 6 may also prevent the contamination and infection. Our results also showed that yeast extract provides a substantial quantity of this factors, since addition of yeast extract to the base diet promote predators development and also the reproduction (diet 5). The yeast extract was much more effective for increasing the number of adult emergence of *R. marginatus* than was the liver diet (Xie *et al.,* 1997). Meat diet produced smaller, lighter weight insect with longer embryonic and nymphal developmental period than the control categories. This is common feature reported, and discussed by Castane and Zapata (2005) and Grenier and De Clercq (2003).

As reported by Hill (1989), sugar is a very important component which promote the egg production. Similarly McEwen and Kidd (1995) had recommended yeast and

sugar for maximum egg production. Honey is also a very important component regarding fecundity. McEven and Kidd (1995) and Kubota and Shiga (1995) analysed that a mixture of honey and yeast autolysate is a suitable adult diet for production of fertile eggs. *R. marginatus* reproduced from the diets 4 to 6 and hence the non-reproduction in other diets must not be due to the honey. Sahayaraj *et al.* (2006) reared *R. marginatus* adult with OD consisting of milk, eggs, sugars and yeast which found to be in favour for fecundity. Higher fecundity was observed in diet containing higher egg yolk (amino acids 15.5%) as observed by Norioka *et al.* (1984). Moreover, *R. marginatus* reproduced after the addition of vitamin E and C, blood serum, casein, cholestrerol and acetic acid. Hence we hypothesised that all these constituents are essential for this reduviid reproduction and development.

4.3. Biology

4.3.1. Development

The total nymphal development period of *R. marginatus* with *S. litura* ($\bar{x}$=41.3 days) and *C. cephalonica* (x=42) were statistically significant. The most direct way in which nutrients can influence predator biology is not only nymphal development but also nymphal mortality. The results of this study indicate that when *R. marginatus* fed on either *C. cephalonica* or *S. litura*, the predator attain into adults and also reproduce. Previously, it was reported that *R. marginatus* completed its nymphal development with in 46.71 days (Sahayaraj and Paulraj, 2001) on *S. litura*; 84.70 days on *Odentotermes obesus* (Ambrose *et al.,* 1994), 45.0 days on *H. armigera* (Sahayaraj *et al.,* 2004). In OD show *R. marginatus* nymphal developmental period was 65 days. However, when we compare to the results of Ambrose *et al.* (1999), OD reduce *R. marginatus* development by 20 days. Moreover, in milk powder-based artificial diet, the total nymphal period of

R. marginatus was 147.8 days (Sahayaraj *et al.*, 2007). In *Podisus maculiventris* developed on meat diet was either slower than on wax moth larvae (De Clercq *et al.*, 1998b). Although cannibalism occurred more frequently in reduviid reared with alive preys, the high yield of adults and eggs in consecutive generations of *R. marginatus* does not support the conclusion that cannibalism may account for the success in development and reproduction. Furthermore, *R. marginatus* was known to be highly cannibalistic behavior when they were reared on their natural host.

4.3.2. Nymphal survival rate

In *R. marginatus* adult female survival rate was low (75/76 days) when OD was offered without insect material and no reproduction in the earlier of the diets. All of the diets had nutritional qualities that allowed complete development of the predators, to a greater or a lesser extent, indicating that there is a potential for rearing this insect on artificial media and that the OD diet used here was adequate, allowing the nymph to acquire food and develop to the adult stage (De Clercq et al., 1998). Moreover, adult weight was also very low. Our results are closer to those presented by Cohen (1995) and Cohen and Urias (1986). The oviposition was not increased when *C.cephalonica* larvae were provided along with the OD. However, predatory rate was higher when compared *R. marginatus* reared on with *C. cephalonica* alone. It is possible that some minor imbalance in the nutrition of meat fed predators could generate a higher predation rate on *C. cephalonica.* It should also be noted that the data analysed in the present work related to first generation meat reared predators and that in successive generation life history traits could be improved due to adaptation to the diet as reflected by the increase in nymphal survivorship from the first (80.55%) to the second (92.76%) and third (98.26%) generations (Wittmeyer and Coudron, 2001).

4.3.3. Reproduction

When *C. cephalonica* and *S. litura* were provided, *R. marginatus* females mate within 27 days of adult life and preoviposition period was slightly (2 to 3 days) affected by oligidic diet taking 28-30 days. Meat-reared females of *Dicyphus tamanini* Waganer (Miridae) did not increase their preovipositional time. In contrast, De Clercq and Degheele (1992) found an increase in preoviposition period when *Podisus* spp. were continuously reared on the meat diet as observed in this study. Also oviposition period was shorter (30-44 days) when *R. marginatus* was reared on *S. litura*. It was prolonged from 51 to 97 days on *C. cephalonica*. Further prolongation was recorded in diet-5 (upto 135 days). But reduced in diet 6. The rate of oviposition of *R. marginatus* species can vary with the species of prey (Sahayaraj *et al.*, 2004). In an average both *S. litura* and *C. cephalonica* provided *R. marginatus* laid 120 eggs/female. It was 2.65 time reduced (56.1 egg/female) when the reduviid was reared with meat-based oligidic diet. Generally the reproductive rate of predators reared on artificial diets was minimum (Carpenter and Greany, 1998; Cohen, 1985a, 1985b, 1992, 2000a; Cohen and Staten, 1994; Coudron *et al.*, 2002; De Clercq *et al.*, 1998; Wittmeyer and Coudron, 2001; Rojas *et al.*, 2000). Cohen and Smith (1998) reported significant cost savings in producing high-quality populations of the predator *Chrysoperla filabris*. When the predator *P. bioculatus* was maintained on an artificial diet, reduced egg production was observed and was attributed to the failure to form mature follicles (Adams, 2000). Our study demonstrated that addition of vitamin E, blood serum and egg yolk enhanced the reproduction of *R. marginatus*. Vitamin A, niacin, riboflavin B12, pantothenic acid, thiamin, pyridoxine, folic acid, Vitamin E and D are present in greater quantity in egg yolk. Similarly folic acid, which is particularly more important for egg productions is much higher (117. g) in egg yolk. Egg yolk also has

higher amount of saturated, mono unsaturated, polyunsaturated oils and lipids. Furthermore, the egg yolk has greater calorific value (303 calories per 100 g). The cholesterol level is particularly very high (1075 mg) in egg yolk (says Rolfes *et al.*, 1978). Diet containing egg yolk is quite rich in proteins, minerals, vitamins and lipids as compared to the diets containing egg white and mixed egg (says Rolfes *et al.*, 1978 and Norioka *et al.*, 1984), which promoted quick growth and completion of the larval period. It was also reported that after pre-oviposition, feeding during adult phase play a more important role than feeding during the nymphal phase in terms of egg production (Cangussu and Zucoloto, 1992, 1995 and Fernandes-da-Silva and Zucoloto, 1993). The result obtained here did not support the above said view. Because we have not either provided any special food and/or not prey or took any special care during the preoviposition period.

Lower fecundity observed in predators reared on artificial diet may there fore be partially attributed to lower adult weights. Female with lower body weights did not however adjust the weight of individual eggs. They decreased reproductive output rather by reducing oviposition frequently total number of egg deposited and size of egg batches. These findings are consistent with those of O'Neil and Wiedenmann (1990) who investigated the effects of feeding regimens on reproduction. A quantitative examination of eggs laid thought the oviposition period was done by Ferkovich, and Shapiro (2004) who suggested that the increase on egg deposition on multiple mated females may be related to hormonal effects in egg production. Though multiple mating was possible also recorded in this study, the total number of eggs laid by and also number of egg batches laid *R. marginatus* was so poor. This might be due to the lower nutritional value, alternation of gut bacterial population and their enzyme and protein profiles recorded in *R. marginatus*..

It also indicates that some nutrient may inhibit the conversion of *C. cephalonica* in to body mass and also the prevent the oogenesis of *R. marginatus*. We hypotheticed that if we alter the ingredients of OD and also rearing medium of *C. cephalonica* the reproductive ability can be increased. Previoulsy (Sahayaraj and Sathyamoorthy 2002) recorded that change of *C. cephalonica* rearing media, could alter the reproductive ability of *R. marginatus*. If the chemical composition of OD was changed, dependly the insect behaviour, biology, reproduction and morphology. Cohen (1992) pointed out that besides the evaluation of behaviour performance, quality control of predators reared on artificial diet should also include immunological procedures and metabolic tests. Such tests may result in a better understanding of the trophic biology of the predators, and may consequently allow for making appropriate dietary adjustments for improve the nutritional value of the artificial diet.

Development of *R. marginatus* in the meat - based was slower and female weight, survival, fecundities and oviposition periods were also lower. However, this indicates that the insects were smaller, they were able to reproduce as successful as conventionally reared insects when offered this same conditions. Normal values of female longevity, and oviposition rate as well as the total number of eggs produced by female with these diets show the potential of in-vitro rearing of this predator. Also, the failure of some individuals reared on artificial diets to produce normal adult did not affect the performance of the first generation adults (De Clercq, and Degheele, 1997).

Lower reproduction in OD category might also be due to lower rates of assimilation and conversion efficiency as observed by Cohen (1989, 1992); Ferkovich and Shapiro (2003). It may also due to the failure of follicle maturity (Adams, 2000a) or reduced juvenile hormone titre or lack of vitellogenin precursors such as amino acids,

lipids, or carbohydrates (Ferkovich and Shapiro, 2004). The author attributed the reduction in egg production not only to a protein deficiency but also to a lack of chemical or behavioral cues that confirmed the presence of live prey. A nutritional deficiency in the artificial diet is likely to suppress *R. marginatus* in succeeding generations. This suggests that larger nymphs and adults of *R. marginatus* have difficulties handling this type of inanimate food, probably because of the low amount of nutrients, and can not extract nutrient at an appropriate rate to fully support growth and reproduction. We recorded abnormalities both nymph and adults of *R. marginatus* while they reared in diet 1 to diet 4. Similarly, abnormal may indicate a nutritional problem (Craig, 1997). This result closely correspond to those presented for *Macrolophus caliginosus* reared on a similar meat based diet (Castane and Zapata, 2005; Iriarte and Castane, 2001).

Females of many insect species may obtain extra nutrients and water through mating (Marshall and McNeil, 1989, Pivnick and Mc Neil 1987). During mating, the male transfer spermatophore which contains sperm, male accessory gland fluids insects in general but for some lepidopterons (Drummond, 1984, Greenfield, 1982) were transferred during copulations. Our results showed that provision of water along with either *C. cephalonica* or artificial diets shows no direct effects on reduviid ovipositions. This study clearly shows that although *R. marginatus* can be reared on an oligidic diet that is economically produce, the fecundity of the female is significantly less than those reared on either *C. cephalonica* or *S. litura.* In the current study, the oligidic diet originally developed for this predatory reduviids, proved suitable food for sustainable development and reproduction of *R. marginatus* reared either in individual or group. Further experiments will investigate whether this diet will sustain < 10 generations of *R. marginatus*, given that nutritional deficiencies. May be expressed only after several generations (Arijs and De Clercq, 2004).

4.3.4. Weight gain

Growth rates are dependent on development time, the only major difference in growth rates between diet groups occurred on the lowest nutrient content of the diet. Weight gains of T_3 and T_4 categories were lower than other categories. Similar trend was a common features in artificial diet reared insects (De Clercq and Degheele, 1992, 2004; Castane and Iriarta, 2005; Cohen, 1985, 1990, 2000). This finding reinforces that compensatory feeding was effective for making up small differences in diet nutrient content, but the lowest nutrient content diet was outside the range of compensatory ability (Lepis and Travis, 1994; Bradshaw and Johnson, 1995; Nylin and Gotthard, 1998; Flanagin *et. al.,* 2000). Though a direct correlationship was observed between the adult weight and its fecundity, this finding was not support the statement. From the result it was very clear that the meat- based artificial diet produced smaller, lighter weight *R. marginatus* with longer embroyonic and nymphal development times than the predator reared on *C. cephalonica* and *S. litura*; this is a commonly reported feature amongst artificially reared insects, as discussed in Grenier and De Clercq (2003).

4.3.5. Sex ratio

The sex ratio as female biased in all the tested categories. It was ranged from 0.62 to 0.85. Similar female biased sex ratio was observed fro *R. marginatus* (Ambrose, 1999; Claver *et al.,* 1996). Moreover, Long and Zaher (1958) reported that insect reared at different diets produced maximum adults with greater effects on female. Field survey also revealed that population of females were higher than males.

4.3.6. Life table

Variation in the quantity of nutrients of prey species appears to have considerable effect on the feeding efficiency and reproductive potential of the predators (Beddington 1975). It reveals that *S. litura* and *C. cephalonica* might be due to the minimum stress developed during feeding on less number of prey due to the comparatively larger size with richer body tissue. Awadallah *et al.* (1986) and George *et al.* (1998) reported same results in reduviids with five different prey . In all diets (From T_1 to T_5) life table statistics varied with the diet. Net reproductive rate, weekly multiplication time, doubling time and hypothetical female in F2 generation were higher in *R. marginatus* on *S. litura* than on other categories. The mean length of generation was shorter in OD (T_3) reared *R. marginatus* followed by T_4 and T_3 (CW). The decrease in corrected rm and extension of developmental period, the population of doubling time increased in *S. litura* categories followed by T_1 and T_3 categories. Similarly finite rate of increase and doubling time were observed for *R.marginatus* of different prey species (George, 2000b; Ravi, 2004) and for *Sphedanolestes minusculus* (Bergroth).

Among the five diets, *S. litura* and *C. cephalonica* for the following reasons faster development higher survival, higher fecundity, higher net reproductive rate, and higher doubling time of the predator *R. marginatus*. The above mentioned parameters were low in OD, OC and CW reared predators. High net reproductive rate and intrinsic rate of population increase have been reported for temperate bugs such as *Oncopeltus guildnii,* and *Clavigrella tomentosicollis* (Iheagwam, 1982) when reared on their preferred hosts. The net reproduction and intrinsic rate of increase were greater for conventionally reared than for oligidic diet reared *M. caliginosus* (Castane and Zapata, 2005) as observed in this study.

4.4. Microbiology

Microbes or active biochemical factors such as enzymes that are usually present in natural and or fac titious prey and OD will be determined. While the oligidic diet optimise growth, development and reproduction, it did not interfere the bioefficacy of the predators. The bioefficacy can be determined and compared between laboratory and field experiments.

4.4.1. Autochthonus gut bacterial content

Trypticase soy agar, Macconkey agar, pseudogel, polymixin private egg yolk, mannitol, bromothymol blue agar were used for isolating total bacteria, enterics, Pseudomonas and Bacillus species (Hunt. and Charnley, 1981). Total 650 isolates of bacteria were grouped based on their morphological characters, their ability to assimilate different types of carbohydrates, urea. Further characterization with fermentation tests suggested that they belongs to eight genera like *Bacillus*, *Carnibacterium, Eachericia, Klepsiella*, *Micrococcus, Proteus, Staphylococcus* and *Streptococcus.*

Sahayaraj (2007) first isolated and identified the gut bacteria of three reduviids such as *Acanthaspis pedestris* Stall *Haematorrhophus nigroriolacous* (Router) and *Catamearus brevepennis* Distant. The gut microflora represent all the aspect of microbial relationship from pathogenic to obligate mutualism (Dillon and Dillon, 2004; Dillon and Charnley, 1996). In natural prey reared reduviid, the number of bacterial cells remained almost constant during the experiment, while the number of bacteria other than artificial diet increased. In contrast, the number of bacterial species within artificial diet reared insect decreased. In natural prey, *S. litura*, 9 bacteria were observed with varing number of bacterial cells. In T1 categories, 10 bacteria species were isolated followed by in T3

categories. 14 bacterial species were isolated from T4 category. The following bacteria were found in *R. marginatus* adult; *Micrococcus spp.*, *Bacillus sp.*, *P. aerogenosa*, *C. xerosis* and *S. aureus* are found in all the three categories suggesting that they were not transient but true intestinal bacteria of this reduviid. Brooks (1963); Tanada and Kaya (1993); Takatsuka and Kunimi (200) reported that *St aphylococcus* spp. are the typical of the intestinal bacteria found in insects. It was also reported that *Klepsiella* spp are the autochthonous bacteria of insects. In addition, we recorded *Bacillus spp.,* and *Carnibacterium spp.*, were the common bacteria particularly in Hemipteran bug. Dillon and Charnley (1995) identified *Bacillus* spp., *E. coli, Micrococcus spp.*, from the fecal extracts of desert locust *Schistocerca gregaria.*

Among the observed bacteria, *M. varians* was the dominant species, its proportion was ranged from 27.20 to 52.70%. Natural host *S. litura* reared *R. marginatus* has only a bacterial species, which increased to OD diet reared predator. In OD, common bacteria *E. coli* dominated followed by *S. epidemidis* and *S. saprophyticus*. This leaves three option, to overcome presence of various nutrients and chemicals impacts predator produced diversified bacteria species, bacterial diversity probably in directly correlated with reduviid feeding which suggests that the reduviid do not have control over bacterial diversity.

4.4.2. Hydrolytic extra cellular enzymatic activity

Protease activity was rarely reported in gut bacterial isolates (Coolbear *et al.,* 1992 and Balasubramanian *et al.*, 1975). Protease and amylase (P + A) activities were only observed in bacterial isolates belonging to the factitious host reared *R. marginatus.* Chemically defined diets have been developed to rear predatory insects are highly suitable for the development of microorganisms. Various antimicrobial agents were used

in insect diet to prevent microbial contamination and propagation (Yazgan and House, 1970; House 1967). But a very limited amount of work has been done on the effect of antimicrobial agents like formaldehyde and streptomycin on predatory heteropterous species (Xie *et al.,* 1986). In adult insects, on the other hand, the symbiont shows a specific localization to the posterior section of the midgut. Therefore, the symbiont must colonise the specific part of the midgut in the developmental course of *R. marginatus*. In adult insects, notably, the alimentary tract is segmented and differentiated into a series of peculiarly compartmentalised structures. It will be of great interest to investigate the development of the alimentary canal of *R. marginatus* in connection with the localisation of the symbiont such as *Bacillus* spp., *Carnibacterium* spp., and *Micrococcus* spp., bacteria these are the bacteria common in all the diets.

The bacteria found in *R. marginatus* were *Streptococcus* spp. and *Staphylococcus* spp., are typical of the intestinal bacteria found in insects (Brooks, 1963; Tanada and Kaya, 1993). In adult insects the bacterium showed a specific localization to the posterior section of the midgut. Disrupted transmission of the bacterium to newborn nymphs by diet or eggs or saliva deposited by other reduviids and that these had to grow inside the egg of the insect resulted in retarded development, arrested growth, abnormal body coloration, and other symptoms. From these results we concluded 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.

The diversity of symbiotic bacteria as well as that of endosymbiotic mechanisms in the Heteroptera suggests that the endosymbiotic associations have evolved multiple times independently in a dynamic manner, although the number of species so far examined is quite limited. Examination of more species from diverse taxa of the

Heteroptera is needed. Notably, the insects with a trace amount of the symbiont also exhibited the deficient symptoms, suggesting the possibility that a threshold titer of the symbiont must be ingested by newborn nymphs to establish normal endosymbiotic association. The symbionts probably provide the host insect with nutritional supplements, such as essential amino acids and vitamins, as has been reported for other plant-sucking insects (Baumann and Maran, 1997; Douglas, 1992).

The artificial diet may have biochemical and chemical effects on metabolic systems of the each developmental stages of the insect. The present work is an important attempt dealing with only the dietary implication on insect with microbial population. Therefore it would be reasonable to suggest that the effect of the diet on survival and population may be related to their effect of the nutritional value of the artificial diet, natural and factitious prey. These data mostly indicate that artificial diet affect microbial population may be of practical importance in artificial mass rearing of reduviids predator. If we decrease the antimicrobial agents and other chemical agents a wider variety of antimicrobial agents and chemical should be screened for use in the nymphal diet of the insect. Predatory insects are known to be more sensitive than their host, which free living insects (Grenier, 1977). If antimicrobial agents added into artificial diet of *R. marginatus* nymphs were weaken the performance of adult individuals. This may become a more important problem in biological control program . So although the low levels of microbial population in artificial diet reared insects with variety of microbial population, it did not affect the survival and development of the insect in natural and factitious prey. To investigate the physiological aspects of endosymbiosis development of a nutritionally defined artificial diet for *R. marginatus* is needed.

4.5. Enzymology

4.5.1. Qualitative enzyme profiles

Digestive enzymes play a major role by converting complex food in to the micro molecules which are necessary to provide energy and metabolites for growth, development and other vital functions to the insects (Wigglesworth, 1972). Food quality regulates and influences the production of alimentary canal digestive enzymes. Henceforth, an attempt was made to study the qualitative and quantitative profile of digestive enzymes in relation to different preys and OD. Results reveals that the activity profile was depends on the type of diet/prey consumed by the insects. For instance, amylase, invertase and lipase activities of both the foregut and hindgut were maximum while *R. marginatus* was fed with *C. cephalonica* followed by OD and *S. litura*. Conversely the true bugs have piercing and sucking mouthparts. The stylets are used for piercing the prey to disrupt the cell wall and cellular contents, and to deliver saliva containing potent digestive enzyme. Together the mechanical damage and the enzyme break down the tissues into a slurry of small particles which are ingested along with the saliva (Cohen, 1998). In salivary gland, all the enzymes were moderate (++) except lipase (+) when *R. marginatus* was reared on *C. cephalonica* and *S. litura*. The results shows that generally prey type and Oligidic diet does not have any influence on the enzyme qualitative of this reduviids.

4.5.2. Quantitative enzyme profiles

Zoophagous insects, particularly generalists, face dietary nutritional variation in hosts, in terms of species, developmental stage and tissues and responses to the environment. *R. marginatus* usually dietary protein (P) and digestable carbohyrates (C)

and in particular, the ratio of these two nutrients (P:C) are critical for proper insect growth and development (Simpson and Raubenheimer, 1993). No previous study was available about the enzyme profile of *R. marginatus* gut and salivary gland except the work of Sahayaraj and Balasubrmanian, (2008).

4.5.2.1. Amylase

High amylase activity found in the foregut alimentary canal of *R. marginatus* suggested that amylase mainly concentrated on the foregut of the insects. Results revealed that small quantity of amylase activity was found in salivary glands. This data also shows the functional role of *R. marginatus* alimentary canal as the main zone for food digestion as observed by Cohen (1996, 1998); Agusti and Cohen (2000). They reported that *L. hesperus* saliva contains amylase activity. In the present study amylase activity of salivary gland was greater in predators reared with OD than those fed with *S. litura, C. cephalonica*. The digestion of glycogen, obtained from meat and arthropod prey, might also explain the presence of amylase in the gut. In vertebrates like pig, carbohydrate is stored as glycogen in liver. To digest the vertebrate glycogen, *R. marginatus* secrete more amount of amylase than to digest insect based carbohydrate. *R. marginatus* ingest intact starch granules, with starch digestion occurring completely in the midgut. Also, the ingestion of soluble glycogen from prey may be an alternative or additional explanation for the presence of amylase in the gut and its absence in the salivary glands. The distribution of amylase in either the salivary glands or guts of heteropterans in various ecological niches remains to be clarified by Boyd *et al.* (2002). Controversially, plant-feeding mirids usually have high levels of amylase in the salivary glands (Agusti and Cohen, 2000). These amylase secretions are thought to be ingested by the mirid along with partially digested starch to be used in the midgut to continue the breakdown of starch (Hori, 1973; Takanova and Hori, 1974; Wheeler, 2001). The lack of detectable

amylase in the salivary glands of *R. marginatus* indicates that plant material is not a part of its diet; this fundamental enzyme is found in the salivary glands of phytophagous heteropterans (Hori, 2000).

Predacious mirids, *Deraeocoris pulchellus* (Reuter), *D. punctulatus* (Fallen) has very low amylase activity in its salivary glands (Hori, 1972). Because they ingest both plant sap and arthropods as their food. But *Deraeocoris nigritulus*a predator has no detectable amylase activity in its salivary glands. A geocorid, *Geocoris punctipe* (Say), showed amylase activity in its salivary glands, indicating its ability to digest starches of plants before ingestion (Zeng and Cohen, 2000a). Similarly *Orius insidiosus* (Say), a predacious anthocorid has amylase activity, detected by whole-organism homogenization, demonstrating its ability to digest starch (Zeng and Cohen, 2000b). Cohen (1996) showed that the predator, *Nabis alternatus* Parshley (Nabidae) and *Sinea confuse* Caudell (Reduviidae), recorded lack of amylase activity in their salivary glands. In another reduviid, *Zelus renardii,* shows amylase activity in its salivary glands. Moreover potent salivary secretions amplify predaceous hemipterans food selectivity and their efficient utilization of prey materials. The presence of this enzyme in abundance in three diets imply that this enzyme has some important physiological role in insect digestion of food materials (Boyd, 2001, 2002, 2003; Azeveira, *et al.,* 2007). Low levels of amylase n salivary gland and high level in hindgut of AC category, indicating that *R. marginatus* might ingest disaccharides and digest them hindgut as proposed by Boyd (2003) in *D. nigritulus.*

4.5.2.2. Invertase

Invertase appears to be on important enzyme for both plants and animals (Heil *et al.,* 2004). Invertase commonly found in the secretion of saliva and digestive tract of various insects has been examined by many authors and was comprehensively reviewed by House (1965). Investigation on alimentary canal carbohydrate of nymphal predators have demonstrated that invertase was concentrated mainly in the foregut. Small quantity of invertase has been identified in the salivary gland. Twelve to eighteen percentage of carbohydrate has been found in foreguts (Yazlovets, 1992). The main activity volume of invertase was found to concentrate in foreguts. This data also confirm the functional role of *R. marginatus* foreguts as the main zone for food digestion (Moontyan and Yazlovetsky, 1988). Invertase also termed as B-fructosidase, saccharadase or sucrase that catalase the cleavage of sucrose into the two monosaccharide, glucose and fructose (Naunoff, 2001).

When *S. litura* was provided as prey, salivary gland secrete more amount invertase. It was double the times and trible the times when we compare to OD and *C. cephalonica* respectively. Since very less quantity of invertase secreted in OC and CW at salivary gland, foregut showed maximum activity (0.95/0.96). The presence of invertase in the salivary glands suggests that *D. nebulosus*can break down the sugary water from plants before ingestion, unless this enzyme is a non-secretable cellular enzyme of the salivary glands as suggested by some heteropteran studies (Hori, 2000). Twelve other mirids were found to have low levels of glucosidase in the salivary glands (Agusti and Cohen, 2000; Hori, 2000). Because the substrates of glucosidase are water soluble and commonly found might explain why only low levels of this enzyme are found

in the salivary glands of many heteropterans. One notable exception is the coreids, which have strong glucosidase activity (Taylor and Miles, 1994; Hori, 2000; Boyd, 2002).

4.5.2.3. Lipase

Proteins are digested externally, but lipid is not hydrolyzed until the ingested material reaches the midgut where lipase activity rises after feeding the prey. The decrease in enzyme activity could be related with some anatomical and physiological modification of predator guts (Edwards, 1961). *R. marginatus* foregut has the maximum activity than other two parts of the insect tested. The prey paralysis may result form damage by certain digestive enzymes in neurons, muscles and storage tissues of the prey. Structural proteins are hydrolysed and liquefied inside the prey by endopeptidases such as trypsin like injected by the predator, while cell membranes, storage tissues and reproductive systems are affected by phospholipase, triacylglicerol lipases and α amylas. Latter, the hydrolysed material is ingested with other nutrients form the prey and additional processing occurs in the gut allowing its absorption by the predator (Cohen, 1993, 1995). So in heteropteran lipase enzymes is one among the key enzymes for digestion and also predation. Predacious arthropods that lack extra intestinal digestive glands, the source of the entire digestive enzymes are the gut, and therefore the extra oral digestion mechanism is a refluxing type (Cohen, 1993, 1998).

Amylase, protease, lipase and trypsin activities were detected in entire salivary gland of *R. marginatus.* Amylase invertase, lipase and protease activities ranged from 0.12 to 0.17, 0.15 to 0.6, 0.1 to 0.3 and 1.4 to 2.5 respectively. Enzymes of the salivary glands showed that *R. marginatus* could be characterised as a zoophagous animal. *C. cephalonica* was reared with starch rice cereals, *R. marginatus* use amylase of their

salivary glands to obtain energy from the starch of cereals ingested by this pest. It as also involved in the glycogen mobilization (Azevedo *et al.*, 2007).

4.5.2.4. Protease activity

Studies on distribution pattern for lipolytic enzymes yield conclusions similar to studies of invertase and amylase. The foregut and hindgut of *R. marginatus* have been found to lower amount than other enzymes. Ishaaya *et al.* (1971) while working on protease activities in the larvae of *Spodoptera litura* recorded that certain protein factors resent in food stimulate digestive enzymes. This decline may result from a greater degradation or lower synthesis of the digestive protease produced by a quantitative decrease of the food intake.

Protease activity of the foregut, hindgut and salivary gland of *R. marginatus* have been found to higher than other enzymes indicates that this reduviid is exclusively predacious. Among the four enzymes studied, protease content was maximum in the salivary gland. Therefore, the protease of the salivary gland of this predator may be involved in the aminoacid mobilization of their prey. Maximum mobilization was took place form *C. cephalonica.* The toxicity of the saliva of heteroptera predators is due to digestive enzymes (Baptist, 1941). The prey paralysis may results for damage by certain digestive enzymes in neurons, muscles and storage tissues of the prey. The presence of protease activity in the alimentary canal and salivary gland complex of this predator was expected. Maximum protease activity was detected in the foregut but the hindgut and salivary gland had low protease activity. The presence of protease enzymes demonstrates the insect was ability to access structural or other insoluble proteins (Cohen 1993, 1998a, 2000). The endoprotease that attack proteins residues. These enzymes are already identified and reported by Zeng and Cohen (2001); Boyd (2002) in the salivary gland of a

reduviid, *Zelus renardii* Kolenati (Cohen, 1998). The strictly phytophagous heteroptera *Poecilocapsus lineatus* (F.) lacks detectable digestive proteases in the gut and salivary gland complex (Cohen and Wheeler, 1998) indicating and inability to use animal protein. However, the phytophagous mirids, *Lygus lineslaris* and *L. Hesperus* have trypsine like protease and elastase like enzymes but not chymotrypsin like enzymes in their salivary gland complexes (Agusti and Cohen, 2000), demonstrating their ability to use animal protein as their nutrients. *Lygus hespersus* produces more proteases when given an artificial diet (Zeng and Cohen, 2001). Same observations were also observed both in fore and hind guts intense protease activity in OD (13.9 mg protein released/min) provided *R. marginatus*. The green mired, *Creontiades dilutus* (Stal) was the first mirid shown to have protease activity in the salivary glands (Colebatch *et al.,* 2001). The protease enzyme secreted on the food from the salivary gland through the salivary canal is thought to be injected by the predator along with food and employed in the gut to continue breakdown of proteins (Cohen, 1998b). Low levels of activity of protease in the hindgut and salivary gland indicate either the ability of *R. marginatus* to synthesize protease in the gut or the presence of symbiotic microorganism in the gut. This proteases help break down protease once they are inside the gut, making the proteins absorbable after further hydrolysis of exopeptidases such as aminopeptidase and caroxypeptidase (Boyd, 2002, 2003).

The presence of protease activity in the salivary gland complex of *R. marginatus* indicates that this reduviid is primarily predacious. The presence of protease activity in alimentary canal and salivary gland enzymes demonstrate an insects ability to access structural or other insoluble protein (Cohen 1993; 1998a; 2000). Structural proteins are hydrolysed and liquefied inside the prey by endopeptidases such as trypsin like injected by the predator, while cell membranes, storage tissues and reproductive systems are

affected by phospholipases, triacylglicerol lipases and α amylase. Irrespective of the prey and meat based oligidic diets, trypsin activity was moderate in salivary gland of *R. marginatus*. Later, the hydrolysed material is injected with other nutrients form the prey and additional processing in the gut allowing its absorption by the predator (Cohen, 1993, 1995). Since the insect has the capacity to alter mid-gut composition within the same generation for neutralising the effect of diet, understanding of the nature of gut protease activity together with the activity induced upon feeding of diet will be important for selecting diet for insect growth development. This process of altering the secretion of proteases may be more complicated in generalised (polyphagous) feeders than the specialised feeders. The generalised feeders are shown to be more adapted to several classes of inhibitors as compared to the specialised feeders (Broadway and Villani, 1995). Previous accounts of proteases from salivary glands of predaceous hemipterans are limited to a few reports on aquatic species (Rastogi 1962; Khan; 1964, Rees and Offord, 1969) and also in a venom spitting reduviids, *Platymeris rhadamanthus* Gaerstaceker (Edwards, 1961). All species studied here showed definite predigest activity with regard to proteases and phospholipases and rather weak triacylglycerol lipases.

All the hunter reduviids are insect-feeders and this ability requires them to have a good complement of digestive enzymes so as to bring about hydrolysis of the complex nutrients that characterise insects. Reduviids inject toxic salivary secretion into the host during paralysing and consumed the body content of the victim (Sahayaraj 2004). Cohen (1998) reported that predatory reduviids consume partially digested food. Before consuming the host, predators predigest the host with the help of the salivary enzyme. Then ingest the partially digest proteins, carbohydrates and lipids and other nutrients, which can be further digested with alimentary canal enzymes. The protease activity of the gut wall is relatively low and does not appear to fluctuate with feeding. Since only tripsin

digestion seems essential for protein absorption, it is possible that the protein breakdown affected by the saliva during external digestion and subsequent storage in the capacious midgut crop of the assassin bug is sufficient to yield assimilable products.

The biochemical and physical environments of the digestive system of *R. marginatus* may be changed appreciably by the presence, absence or quantity of a compound or mixture of compounds. With ingestion, induce or introduced changes in the gut environment my result in changes in p^H, reducing potential, conductivity of specific ions etc.. or in change in activities of many enzymes such as protease or mixed functiones oxidases.

4.6. Protein

The appearance of increased number of Polypeptides and change in their mobility as well as intensity after OD reared indicated that ingredients of the OD stimulating and also initiates the synthesis of some new proteins in *R. marginatus*. These findings get corroborated by the results of Mjeni and Morrison (1976); Kajiura and Yamashita (1989) and Bradfield *et al.* (1990) as they also recorded an increase in the number of protein bands in the OD reared adults.

4.7. ELISA

At least a dozen different immunoassay formats have been used over the past half centuray to analyse arthropod guts for prey remains (Greenstone, 1996), no studies were available for reduviid predators except the studies of Cohen (1993) in *Zelus renardii*. In this study, we followed indirect ELISA format for detecting factitious and natural hosts and OD remaining in adults *R. marginatus*. The overall mean protein concentration (± SE) was maximum in *S. litura* followed by *R. marginatus*, *D. cingulatus* and pig liver

and *C. cephalonica*. This shows a strong linear relationship between insect weight and total protein. This suggests that weight alone can be used to estimate the total protein content of a predator. Therefore, the optimal quantity of PBS that a given predator species showed be homogenized in can be determined simply by weighing the predators (says Hagler *et al.,* 1997). They further reported that using the weight of a animal to predict its protein content was eliminate labor intense protein analyses and chemical waste. *S. litura* have the highest proportion followed by artificial diet reared predator has highest proportion of yield of immunoreactions, suggesting that they are effective at detecting the presence of a small quantity of prey in whole body homogenized predators in examined in the feeding trial were very effective at detecting an *D. cingulatus, S. litura, C. cephalonia* and artificial diet reared predators. *S. litura*, *C. cephalonica* are under order lepidoptera if feed up on leaves chewing type , while *D. cingulatus* are feed plant sap by sucking so body physiological conditions also may entirely different. So this may the reason be for the variation among the diets too.

Variable predator digestive rates (Symondson and Liddell, 1993), predator prey size (Sopp and Sundarland, 1989: temperature (Mclver, 1981), predator metabolic status (Lovei *et al.,* 1990) and the developmental stage of the prey (Hagler *et al.,* 1992) can affect the quantitative outcome of gut content immunoassays (Sundarland, 1996). The data presented have suggest that there is a huge discrepancy in the sensitivity of a gut content immunoassay developed to detect *S. litura* and *D. cingulatus C. cephalonica* remains in the whole homogenized predators. Despite these complications gut content immunoassays after unique opportunities to examine the natural feeding behavior of predators from unmanipulated systems, and after advantage of permitting thousands of predators to be assayed rapidly and inexpensively (Hagler and Naranjo, 1994a, 1994b, 2005; Harwood and Sheppard, 2005; Winder *et al.,* 2005).

4.8. Polymerase chain reaction (RAPD)

On the basis of clustering data for the RAPD profile, accessions of the first and second group exhibit greater similarity amongst each other and genetic variations has been found in accessions of the same populations because samples have been collected from the natural prey reared predator as well as Oligidic diet maintained predators in the laboratory. Molecular profiling provides a rapid means of quantifying prey diversity within predators but when there are specific prey DNA targets, PCR with group specific primers is the principal method of choice (Symondson, 2002). Among the six primers, KTG-3 and KTG-5 have amplified better than other primers which event by amplifying, it may same sequence between the samples. The suitable primer is fine for simple laboratory studies, when there are multiple potential target prey species (Sheppard *et al.*, 2004) or fragments (Hoogendoorn and Heimpel, 2001), the time required to assay each predator for each potential target becomes limiting. The first step in most of this research was the development of the prey specific primer sets that were then used to amplify prey DNA from predator samples. Rapid analysis of samples is possible using this approach to study the diversity of a specific target prey range in predator gut. Generalist predators eat multiple prey species and it is not always practical to analyse predator responses to prey diversity using the species-specific primer approach. In this study KTG-3 and KTG-5 is most preferable primers for *R. marginatus.*

The level of genetic diversity is far greater in the Oligidic diet reared *R. marginatus*. Although there are no available data for *R. marginatus* or any other predatory reduviids, the logical inference is that the diversity existed in *R. marginatus* populations. Rapid PCR-based screening systems for the study of the prey diversity of generalist predators have been developed to expand the potential of molecular detection

into various areas of food-web research. The techniques described by Harper *et al.* (2005) use a single multiplex-PCR to simultaneously amplify DNA from a range of prey species.

It is extremely difficult to evaluate the biocontrol possibilities and their outcomes, when important variation in the biological control agent cannot be identified. Although the different diets (T_1, T_2, T_3, T_4 and T_5) are tested for their impact on *R. marginatus* critical genetic studies between populations of potential biocontrol agent. The retention time for DNA within the gut of a predator during digestion is influenced by factors including the size of the target DNA molecule. Predation by the coccinellid beetle *Coleomegilla maculate* De Geer (Coleoptera: Coccinellidae) upon the eggs of the European Corn Borer *Ostrinia nubilalis* (Hubner) (Lepidoptera: Crambidae) has been characterized by single-plex PCR amplification of four fragments of prey genomic DNA of different sizes from predator guts (Hoogendoorn and Heimpel 2001, 2002). Predator weight, size, developmental stage and meal size had no effect on detection period but in all cases the shortest fragment (150 bp) was detected for the longest time after feeding. A similar study of a coccinellid–Lepidoptera, predator-prey system in Hawaii, produced analogous results with short (151 and 140 bp) prey DNA fragments being detectable in a greater proportion of beetle guts than larger (170 bp) ones (Sheppard *et al.*, 2004).

4.9. Bioefficacy of reduviids

After three generations, reduviids reared on the meat-based diet were as active at preying on the target preys, *C. cephalonica, D. cingulatus and S. litura,* than those tested, in oligidic diet (tables 7-9). This indicated that feeding *R. marginatus* with oligidic diet did not alter their capacity to recognise and feed on their natural and factitious preys. Other artificially reared predators recognised and accepted their target prey, as shown by Hagler and Cohen (1991) and Cohen (2000) for *Geocoris punctipes*; by Chocorosqui and

De Clercq (1999) and De Clercq and Degheele (1993) for *P. maculiventris* and *P. sagitta* by Castane *et al.*, (2002) for *D. tamaninii*; by *Macroloplus caliginosus* (Castane and Zapata, 2005).

4.9.1. Field release

The arthropod predator complex recorded in groundnut fields was highly diverse. We found that total pest populations (Table 24) suppress strengthened significantly when predator communities compared to the average predators species in groundnut field, and this effect was independent of prey diversity. Our experimental design included both artificial diet and factitious prey reared predators, and maintained constant relative abundance of different species, in both experimental plots. A separate issue is whether any single predator species could achieve control equal to that observed by the diverse predators. For example, in our experiment *Coccinella septumpunctata* not support the aphids population and hence its decreased when *C. septumpunctata* increased, it was proved by statistical analyses significance were observed. When such circumstances occurs, conservation of the single most effective species could improve biological control as effectively as conserving the entire predators assemblage (Straub and Snyder, 2006). As observed in *C. septumpunctata, R. marginatus* both artificial diet and natural diet reared insects suppressed *S. litura* and *H. armigera* populations. Similar observations were also observed by Sahayaraj (1999b, 2002c). Claver and Ambrose (2001a) also reported that *R. kumarii* suppressed the lepidopteron pest such as *S. litura* and *H. armigera* in the cotton field. The field cage experiment was also observed by Ambrose and Claver (1999b) was also similar reported that *R. kumarii* suppressed the lepidopteran pest population such as *S. litura* and *H. armigera*. However, whereas *R. marginatus* provide strong suppression in experiment, diverse predatory effect on different pest

communities exerted strong control on both experiments, suggesting that predator *R. marginatus* biodiversity may provide the best result that effective lepidopteran and also other pest control results (Sahayaraj and Ravi, 2007).

Sahayaraj (2002) reported that *R. marginatus* mainly controlled *S. litura* than *Aproaerma modicella*. Present results revealed that *R. marginatus* mainly control jassids, *B. tabaci*, (CC), *M. indica*, *Millocerous* spp. (CC), *N. virudula* (CC), *S. litura* (CC) in summer and *P. ricini,* grasshoppers, *M. indica*, *S. litura, H. armigera* and *A. craccivora* in Khariff. This study indicated that kharif was infavourable for the establishment (mean population was 2.61 and 2.91 for CC and AD categories respectively) and also control of many pests than summer. The composition of the natural enemy complex differed between maize fields, with certain predators favored over others, depending on the location of these fields (Kris *et al.,* 2007). Despite this high variability in abundance and diversity of the predator complex. This finding suggested the role of *R. marginatus*, *R. fuscipes, R. longifrons, C. septumpunctata* in groundnut pest management.

The field release of *P. plagipennis* (Grundy and Maelzer, 2000) and *P. laevicollis* (Antony *et al.*, 1979) were successful in reducing various pests in their released fields. Sahayaraj (2002c) and Sahayaraj and Ravi (2007) integrated *R. marginatus* along with some botanicals in groundnut field and he observed the drastic reduction of pests such as *S. litura* and *H. armigera* and achieved a high yield of groundnut. *R. kumarii* suppressed various pests in the groundnut field as it was reported in the laboratory studies (Ambrose, 1996 and Claver, 1998). But it did not reduce the population of *M. pustulata*. But Ambrose (1999) reported that it feeds on *M. pustulata* in the laboratory. Present study shows that *R. marginatus* during kharif reduced *M. indica* more than 75%.

In the present study, *R. marginatus* greatly suppressed the population of *S. litura* *A. craccivora* and *H. armigera* and *P. ricini.* Biological control potential of *R. marginatus* was not similar in both seasons. Moreover, *C. septumpunctata* and spiders were also established during this season. However, in summer *R. fuscipes,* spiders, dragon fly, *M. religiosa* and *C. septumpunctata* were established. Among the two-diet regime, AD reared *R. marginatus* slightly and highly reduce the pest populations. The studies discussed here suggest that for most augmentative biological control agents, there is rearing of *R. marginatus* on OD that produces effective control of a pest species. This was especially true when predators were used in biological control. It is clear that artificial diet reared predators should be carefully considered before implementing augmentative biological control efforts with natural diet reared insects. The ultimate success of augmentative biological control may depend on releases of biological control agents that maximise establishment, are released in synchrony with the host, and can be integrated into integrated pest management program. Thus, determining OD reared *R. marginatus* that maximize the effectiveness of natural enemies can increase the effectiveness of augmentation biological control and increase its potential economic benefits.

4.9.2. Yield, cost benefit ratio and Percent avoidable loss

Cost benefit ratio in predator released field enhanced the production of groundnut and cost benefit ratio and it is in agreement with the findings of Sahayaraj (1999b and 2002c) and Sahayaraj and Martin (2003) (Sahayaraj and Ravi, 2007) on *R. marginatus* released groundnut field. So *R. marginatus* (reared with OD and natural diet) could be used as a biological control agent against groundnut pests. This work provides the basis for an exploration of opportunities to conserve natural enemies and increase biological

control potential (Barbosa, 1998, Landis *et al.,* 2000). As for many of the natural enemies identified within this study the potential for pest biological control has not been recognized, conservation practices targeting those await formulation for use in subsistence groundnut production systems.

Conclusion

5.0 CONCLUSION

5.1. Oligidic diet

Total nymphal development period was 20 days prolonged in Oligidic diet than factitious host *Corcyra cephalonica*. However, not much variation was observed in sex ratio and adult longevities. The nutritional value of the tested meat-based oligidic diet was sufficient enough to continuously rear *Rhynocoris marginatus*. Moreover production of this diet is simple and inexpensive (@ U$ 3.2 and 1.30 for *C. cephalonica* and OD respectively) hence this OD can be considered as an alternative food source for the mass raring of *R. marginatus*. This diet is a valuable alternative food source for the raring of *R. marginatus*. This reduviid is a voracious predator of many species of arthropods and might be able to sustain itself on pest and oligidic material for long periods of time.

5.2. Microbiology

Among the five diets, *Rhynocoris marginatus* reared with OD has more number of bacterial species with poor proportions. This may be due to the antimicrobial agents. A comparison of extra cellular enzymes produced by the isolated strains reveals that OD and insects did not showed by differences in enzyme activity. This suggested that OD affect the microbial population and also their enzyme secretion.

5.3. Enzymology

The higher of amylase, invertase, and protease activities in the foregut indicates the ability of *R. marginatus* to digest carbohydrates and protein of the prey and oligidic diet. Long term maintenance of the predator on *C. cephalonica* and *S. litura* and OD as well as transmission of adult and nymph form one type of food to another were found to not significantly influence amylase, lipase, protease, and invertase activities in the alimentary canal. The ability to use prey macromolecules might enable to survive in the absence of prey for short periods of time, which would enhance the potential of this bug as a biological control agent.

5.4. ELISA

The quantity of prey consumed, and postmeal time all affected the qualitative and quantitative outcome of the indirect ELISA. These variables make the accurate quantification of predation very difficult using immunoassay procedures. While gut content immunoassays offer a good method of qualitatively estimating predation.

5.5 RAPD analyses

These results also provide a molecular basis for identifying *R. marginatus* nutritional studies of inter specific competition especially feeding strategies. Monitoring inter specific genetic variation of potential classical biological control agents will improve the success of biological control. Further, the data suggest that the opportunity for detecting DNA differs with diets. Its further evaluation as a biological control agent is warranted.

5.6. Biological control potential

Both the laboratory and field studies shows the biological control potential of this reduviid predator. The both oligidic diet and insect prey reread *R. marginatus* greatly suppressed the pest population and their infestation and turned to increase the number of pods, production and cost benefit ratio. Groundnut cultivars can use both OD and insect reared reduviids as biological control agent. However, OD an insect reared reduviids. is better than natural prey reared reduviids.

Summary

6. SUMMARY

Laboratory rearing of a reduviid preadator *Rhynocoris marginatus* (Fab.) was performed with five different diets such as *Corcyra cephalonica* (T_1), *C. cephalonica* +weekly once with water (T_2), Oligidic diets (OD) (4, 5 and 6) (T_3), OD + weekly once with *C. cephalonica* (T_4) and *Spodoptera litura* alone (T_5). Various biological, biochemical and molecular parameters have been considered in this study. The laboratory maintained *R. marginatus* was released in to the groundnut field and recorded the bioefficacy as wells its impact on groundnut production. From the study we arrived the following summary:

1. Six oligidic diet and two preys were offered and recorded their impact on development and reproductive capacity of *R. marginatus*. Among the six diets, diet six was suitable for the reduviid development and reproduction than other five diets. However, low nymphal weight and fecundity were observed in oligidic diet than insect preys tested. Oligidic diet increased the nymphal development period and decrease the adult weight, adult longevity and diet 4 or 5 caused a lot of deformities in the nymphs and adults of *R. marginatus*.

2. *C. cephalonica* + water (T_2), was more or less similar effect like natural preys *Spodoptera litura*. Moreover, egg laying capacity was as more than other tested diets but decreased the adult longevity. Oligidic diet + weekly once with of *C. cephalonica,* increased the total nymphal developmental period and decrease the fecundity and adult longevity.

3. *S. litura*, larvae further reduced the total nymphal developmental period and increased the fecundity, adult longevity, and survival rate.

4. Oligidic diet reared *R. marginatus* predatory efficiency was higher than natural prey reared *R. marginatus*.

5. Gut salivary enzymes such as proteas, lipase, invertase, and amylase activity were observed in all the diet reared *R. marginatus*. However, their distribution and activity was different in different diets.

6. Among the five diets, *S. litura* alone (T_5) reared R. marginatus has the maximum number as well as proportion of bacteria was observed in OD categories followed by *C. cephalonica* (T_1), *C. cephalonica* + water (T_2), Oligidic diets with weekly once with *C. cephalonica* (T_4) categories.

7. Six primers like KTG-3, KTG-4, KTG-5, OPE-8, OPE-13 and OPE-16 were used for amplifing the DNA of different diets reared *R. marginatus*. Among the six primers, four primers only amplified the DNA. Among, the four primers, KTG-5 amplified maximum and DNA polymorphism. Oligidic diet also alter the polypeptides of the whole body protein.

8. Both Oligidic diet and *C. cephalonica* reared *R. marginatus* was released in to the groundnut field and evaluated its impact in terms of pest and groundnut production. It reduced the incidence and infestation of the groundnut pests such as *Spodoptera litura, Helicoverpa armigera*, grasshoppers, and *Aphis craccivora* and significantly increased the yield of groundnut and cost benefit ratio.

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Annexure

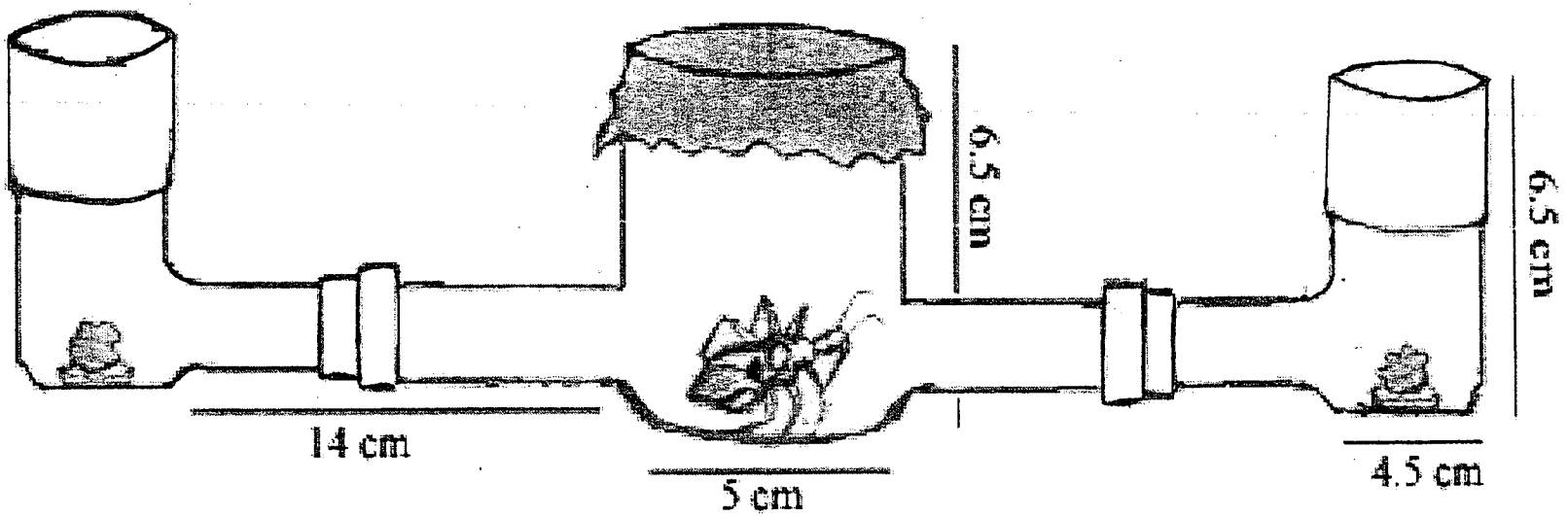

Figure 0. Olfactometer used in feeding behaviour of *Rhynocoris marginatus*.

Details of papers Published

1. **Balasubramanian R.** and Sahayaraj K. 2007. *Pteridium aquilinum* (L) Kuhn and *Metarhizium anisopliae* (Metsch.) Sorokin on *Pericallia ricini* Fab. and *Aphis craccivora* (Koch), *In*; Technology and Management of Bio resources (eds) M. Narayanan, T.A. Sethuramalingam and K. Sahayaraj. PP-22-27.

2. Sahayaraj K., Selvaraj P. and **R. Balasubramanian,** 2007. Cell mediated immune response of *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fab) to fern phytoecdysone. *Journal of entomology*, **4(4)**:289-298.

3. Sahayaraj K. Kumara Sankaralinkam S. and **Balasubramanian R**, 2007. Prey influence on the salivary gland and gut enzymes qualitative profile of *Rhynocoris marginatus* (Fab.) and *Catamiarus brevipennis* (Serville) (Heteroptera: Reduviidae). *Journal of entomology.* **4(4)**:331-336. (Attached).

4. K. Sahayaraj P.Venkatesh and **Balasubramanian, R.** 2007. Feeding Behaviour and Biology of a Reduviid Predator *Rhynocoris marginatus* (Fabricius) (Heteroptera: Reduviidae) on Oligidic Diet. *Hexapoda,* **14(1)** 24-30. (Attached).

5. Sahayaraj K, Lalitha A and **Balasubramanian R**. 2008. Biosafty of *Metarhizium anisopliae* (Metschinikoff) Sorokin on a reduviid predator *Acanthaspis pedestris* (Hemiptera : Reduviidae) *Hexapoda*, **15(1)**:47-50.

6. Sahayaraj, K. and Balasubramanian R. 2007. Biological control potential evaluation of artificial and factitious diets reared *Rhynocoris marginates* (Fab.) on three pest. *Archives of phytopathology.* **(In press).**

7. **Balasubramanian, R.**, Selvaraj, P. and Sahayaraj, K. 2008. Extraction and Characterization of Phytoecdysone from *Cristella parasitica* and Screening for the Pesticidal properties *Spodoptera litura* and *Helicoverpa armigera. Journal of Biopesticides* **(In press).**

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Prey Influence on the Salivary Gland and Gut Enzymes Qualitative Profile of *Rhynocoris marginatus* (Fab.) and *Catamiarus brevipennis* (Serville) (Heteropetera: Reduviidae)

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Abstract: *Rhynocoris marginatus* (Fab.) was reared on two natural lepidopteron pests [*Spodoptera litura* (Fabricius) and *Pericallia ricini* (Fab.)] and a factitious host (*Corcyra cephalonica* Stainton). Whereas *Catamiarus brevipennis* (Serville) reared on *C. cephalonica*, *S. litura* and Thunberg. Impact of these preys on the total body, midgut, hindgut and entire salivary gland weight, their enzymes qualitative profile was recorded. Total carbohydrates and lipids and more amount of proteins were observed in *S. litura* fed *R. marginatus* showed maximum total body, midgut, hindgut and salivary gland weights. Presence of more amount of total lipids and carbohydrates in *M. pustulata* favours the body, gut and salivary gland weight of *C. brevipennis*. Amylase, invertase, lipase, protease, trypsin and pepsin activities were well pronounced in the midgut than the hindgut of both reduviids. *R. marginatus* and *C. brevipennis* salivary gland expressed more amylase, invertase, protease and lipase activities.

Key words: *Rhynocoris marginatus*, *Spodoptera litura*, *Pericallia ricini*, *Mylabris pustulata*, enzyme profile, salivary gland

INTRODUCTION

Rhynocoris marginatus (Fab.) native to India is found to feed on more than 24 economically important pests including *Earias fraterna* (Pawar *et al*., 1986; Ambrose, 1988), *Dysdercus cingulatus* (Fab.) (Imms, 1965), *Papilio demoleus* (L), *Earias vittella* (Fab.) (Nayer *et al*., 1976); *Corcyra cephalonica* (Stainton) (Bhatnagar *et al*., 1983); *Helicoverpa armigera* (Hubner) (Ambrose, 1987); *Spodoptera litura* (Fab.), *Amsacta albistrigia* (Walker) (Sahayaraj, 2000); *Mylabris pustulata* (Faust), *Mylabris indica* (Thunberg), *Achaea janata* (Linn.), *Oxycarenus hyalinipennis* (Costa) and *Aproarema modicella* (Deventer) (Sahayaraj, 1995; Sahayaraj and Kathikraja, 2003). Pest suppression efficacy of this predator was studied both under laboratory (Imms, 1965; Pawar *et al*., 1986) and field conditions (Sahayaraj, 1999; Sahayaraj and Martin, 2003) in India. Scope for the utilisation of *R. marginatus* in groundnut pest management was emphasised by Sahayaraj (1999, 2004) and Sahayaraj and Martin (2003).

Catamiarus brevipennis (Serville) is one of the larger predator of the family Reduviidae and sub-family Peiratinae present in scrub jungles, semi-arid zone, tropical rain forest and agroecosystems of south India (Sahayaraj, 1994). It has been reported as a biological control agent of many agriculture and forest pest like *H. armigera* (Bhatnagar *et al*., 1983), *Pantanga succincta* (Linn.) (Pawar *et al*., 1986), *Earias insulana* (Fab.) and *Mylabris pustulata* Thunberg (Ambrose, 1987), *D. cingulatus* and *Achaea janata* (Linn.) (Sahayaraj, 1991). Sahayaraj (1991) and Sahayaraj and Ambrose (1994) studied the host

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preference of this reduviid on various pests. However, works on biology, ecology and biological control potential of this reduviid has not been undertaken either under laboratory or field situations.

The digestive physiology of reduviid predators solicits greater attention in view of its economic importance. The nutritional need and the knowledge of the functional organisation of digestive system of reduviid predators may be useful in designing oligidic diet for mass production. Moreover this information can be useful to understand how reduviids adopt to its natural and factitious food. Enzymes are proteins, which catalyse a variety of reactions in the biological systems. Digestive enzymes are produced and distributed in different regions of the gut and differ in proportion and quantity. Wide ranges of digestive enzymes were recorded in the alimentary canal of insects and the level varies in relation to moulting and short-term changes in food intake. Food stimulates the secretion of protease in the midgut, the intake of insect substances like water and cellulose. It is a well-known fact that the digestive enzymes play a major role in insect physiology by converting complex food materials into micromolecules necessary to provide energy and metabolites for growth, development and other vital functions. Among the digestive enzymes, protease, amylase, invertase and lipase activity are of great importance in the digestion of food. Utilization of macronutrients from the available prey food depends on the digestive enzymes. Digestive enzymes of alimentary canal and the salivary gland of *Sophrorhinus insperatus* Faust (Hori, 1969; Ravikumar *et al*., 2002) were investigated. Influence of host plants on the activity of digestive enzymes of *Helicoverpa armigera* (Hubner) was also studied. No concrete work has been undertaken so far about digestive enzymes in predaceous reduviids. Digestive enzymes of Heteroptera include proteinase, lipase, phospholipase A1, amylase, pectinase, invertase, hyaluronidase and nuclease (Nuorteva, 1958; Miles, 1972; Cohen, 1998). Digestive enzymes are specific for zoophagic insects include protease, hyaluronidase and phospholipase (Cohen, 1998). However studies on enzyme profile of Indian reduviids were not available in the literature. Based upon the available literature we have undertaken this research to record the qualitative enzyme profile of salivary gland, mid and hindgut of *R. marginatus* and *C. brevipennis*.

MATERIALS AND METHODS

Collection and Maintenance of Reduviids

Life stages of *R. marginatus* and *C. brevipennis* were collected from Sivanthipatti agricultural ecosystem (cotton and bhendi) and also their border ecosystems of scrub jungles, Palayamkottai, Tirunelveli District, Tamil Nadu, India. They were maintained in the laboratory at 28±1°C temperature, 70±10% relative humidity and 11 L: 13 D on *C. cephalonica*, *M. pustulata*, *P. ricini* and *S. litura*.

Rearing of Preys

Larval stages of *S. litura*, *M. pustulata* and *P. ricini* were collected from cotton and ladyfinger fields in Pavoorchatram, Tirunelveli District, Tamil Nadu, India and were maintained in the same laboratory conditions as for reduviids on castor and black gram leaves. Laboratory emerged fifth instars *S. litura*, *P. ricini* and field collected *M. pustulata* adults were used for the experiments. Newly emerged adult of *R. marginatus* were reared on *S. litura*, *P. ricini* and *C. cephalonica* whereas *C. brevipennis* reared on *C. cephalonica*, *S. litura* and *M. pustulata* continuously for three weeks separately. Then the predators were removed from the host and allowed to starve for 6 hours. Twenty predators from each category were used for the enzyme profile analyses studies. Ten uniform sized preys were selected for the macromolecules profile. Total carbohydrates (Nigam and Omkar, 2003), total proteins (Lowry *et al*., 1951) and total lipids (Bragdon, 1951) were estimated with standard procedures.

Enzyme Extraction

Both the reduviid adults (>24 h) were maintained on *S. litura* and *C. cephalonica* continuously for a period of three weeks separately. Anesthetized predators were dissected out aseptically by using pins, fine forceps and razors and a dissection microscope in a petri dish filled with sterile phosphate buffered saline (pH 7.8). Entire gut and salivary gland were dissected out from the predators. From the isolated digestive tract, mid and hind guts were separated individually, washed several times with fresh phosphate buffered saline to minimize possible microbial contamination and used for enzyme bioassay. Similarly entire salivary gland from 10 predators were removed carefully and used for the enzyme study. Transfer the salivary glands and alimentary canal separately in a small test tube containing 2 mL of distilled water and grind them as completely as possible with tissue Homogenizer (Remi 8000 RPM, Mumbai). Transfer the solution to the centrifuge tube and centrifuge at 5000 rpm for 15 min. The supernatant was used as enzyme source for this experiment. Invertase, amylase, lipase (Nigam and Omkar, 2003), pepsin, polypeptidase (Tonapi, 1996), protease, trypsin (Balagun and Fisher, 1970), qualitative profiles were performed from the enzyme samples using sugar, starch, olive oil emulsion, acid casein, peptone, casein, alkaline casein, respectively as substrates. Based upon the colour intensity, the enzyme activities were expressed as less (+), moderate (++) and maximum (+++) activities.

Statistical Analysis

Both for macromolecules and weight of different parts of the reduviids, student t-test was performed. Results of *C. cephalonica* were compared with other pests and their significance was expressed at 5% level.

RESULTS

Digestive enzymes play a major role by converting complex food in to the micromolecules which are necessary to provide energy and metabolites (Wigglesworth, 1972) to the insects. Macromolecules weight of tested preys is presented in Table 1. From the results, it was very clear that total carbohydrates, total proteins and total lipid contents were higher in *C. cephalonica*, *S. litura* and *M. pustulata*, respectively.

Gut Enzyme Profiles

The food quality regulates and influences the production of digestive enzymes. Henceforth, an attempt was made to study the qualitative profile of digestive enzymes in relation to different preys. From the Table 2, it is very clear that both the reduviids midgut and hindgut contain amylase, protease, invertase, lipase, trypsin and pepsin. But their activity profile is depends on the type of host encountered. For instance, protease and lipase activities of both the mid and hindgut were maximum while *R. marginatus* was provided on *C. cephalonica*. But both the trypsin and pepsin levels of midgut were higher (+++) when the predator consumed *S. litura*. *C. brevipennis* midgut amylase, protease, invertase, lipase, trypsin and pepsin activities were maximum same on the other preys except on *M. pustulata* (Table 2). However, the hindgut enzyme activities were decreased (++) and similar kind of observations were recorded for all the three preys. The results further showed that prey type does not have any influence on the enzyme activity of this reduviid.

Table 1: Macromolecule composition (mg/mL) of four pests (mg/100 mg)

Macromolecule	*C. cephalonica*	*S. litura*	*P. ricini*	*M. pustulata*
Pests				
Protein	0.60±0.12	0.77±0.20*	0.63±0.14*	0.56±0.02*
Carbohydrate	0.20±0.07	0.13±0.08*	0.23±0.06*	0.20±0.03*
Lipid	0.12±0.05	0.07±0.01*	0.10±0.03*	0.18±0.05*

*: Significant at 5% level

Table 2: *C. cephalonica* (CC), *S. litura* (SL), *M pustulata* (MP) and *P. ricini* (PR) influence on the midgut and hindgut qualitative enzyme profile of *R. marginatus*

Prey	Protease	Lipase	Amylase	Invertase	Trypsin	Pepsin
R. marginatus midgut						
CC	+++	+++	++	++	++	++
SL	++	++	++	++	+++	+++
PR	++	++	++	++	++	++
R. marginatus hindgut						
CC	+++	+++	++	++	++	++
SL	++	++	++	++	++	++
PR	++	++	++	++	++	++
C. brevipennis midgut						
CC	+++	+++	+++	+++	+++	+++
SL	+++	+++	+++	+++	+++	+++
MP	+++	+++	+++	+++	++	++
C. brevipennis hindgut						
CC	++	++	++	++	++	++
SL	++	++	++	++	++	++
MP	++	++	++	++	++	++

(++) Moderate; (+++) Maximum

Table 3: Influence of *C. cephalonica* (CC), *S. litura* (SL) and *M pustulata* (MP) on salivary gland enzyme profile of *R. marginatus* and *C. brevipennis*

Prey	Protease	Lipase	Amylase	Invertase	Trypsin	Pepsin
R. marginatus						
CC	+++	+++	+++	+++	++	++
SL	+++	+++	+++	+++	++	++
PR	++	++	+++	+++	+	+
C. brevipennis						
CC	+++	+++	+++	+++	++	++
SS	+++	+++	+++	+++	+	+
MP	++	++	+++	++	+	+

(+) Less, (++) Moderate, (+++) Maximum

Table 4: Influence of preys on body, gut and salivary gland weight (mg) of *C. brevipennis* and *R. marginatus*

Preys	Body weight	Midgut	Hind gut	Salivary gland
C.brevipennis				
C. cephalonica	520.84±9.01	11.72±1.02	10.04±1.6	64.40±3.10
S. litura	674.33±11.9*	18.50±2.8*	10.93±1.2^{NS}	65.45±2.2^{NS}
M. pustulata	779.33±12.1*	18.62±1.9*	11.93±2.4^{NS}	65.78±1.6^{NS}
R. marginatus				
C. cephalonica	132.42±4.11	8.78±1.30	8.34±1.12	30.72±2.13
S. litura	134.7±5.81*	9.01±1.41^{NS}	8.68±1.21^{NS}	31.6±2.4^{NS}
P. ricini	129.94±2.41*	7.54±1.21*	6.98±1.10*	31.50±2.8^{NS}

* Shows significant at 5% level by t-test; ^{NS}- Stars for not significant

Salivary Gland Enzyme Profile

Polypeptidase activity was not observed in the salivary gland of both *R. marginatus* and *C. brevipennis* (Table 3). Both the trypsin and pepsin activities were moderate (++) when *R. marginatus* was provided with *C. cephalonica* and *S. litura* and *C. brevipennis* on *C. cephalonica*. The activity was further reduced (+) on *P. ricini* for *R. marginatus*, *S. litura* and *M. pustulata* for *R. marginatus* and *C. brevipennis*, respectively. In *R. marginatus* and *C. brevipennis*, the amylase, invertase, lipase and protease activities were higher with *C. cephalonica* and *S. litura*.

Body and Body Parts Weight

Table 4 showed the total body, alimentary canal and salivary glands weight of *R. marginatus* and *C. brevipennis*. The weight was varied while *R. marginatus* provided with different types of prey. Statistically significant low body (129.94±2.14 mg), midgut (7.54±1.21 mg), hindgut (6.98±1.10 mg) and salivary glands were recorded while *R. marginatus* was fed with *P. ricini*. From the Table 4, it was very clear that *S. litura* was the suitable prey for the rearing of *R. marginatus*. But the statistical

comparison between *S. litura* and *C. cephalonica* were insignificant. Body weight was higher while *R. marginatus* was reared on *S. litura* (134.7 mg). But it was not statistically insignificant when compared to *P. ricini* and *C. cephalonica*. Similar statistical insignificance was also recorded for salivary gland weight (Table 4). *M. pustulata* slightly influence *C. brevipennis* weight (779.33 mg). It was statistically significant at 5% level. However, prey has no influence on salivary gland (64.40, 65.45 and 65.78 mg for *C. cephalonica*, *S. litura* and 65.78 mg, respectively) as well as alimentary canal weight (Table 4). Body weight was statistically increased when *C. brevipennis* provided with both *S. litura* and *M. pustulata*. Similar trend was also observed for midgut.

DISCUSSION

All the hunter reduviid bugs are insect-feeders and this ability requires them to have a good complement of digestive enzymes so as to bring about hydrolysis of the complex nutrients that characterize insects. Reduviids inject toxic salivary secretion in to the host during paralysing act and consumed the body content of the victim (Sahayaraj, 2004). Cohen (1998) reported that reduviid consume partially digested food. Before consuming the host, predators predigest the host with the help of the salivary enzyme. Then ingest the partially digest proteins, carbohydrates and lipids and other nutrients, which can be further digested with alimentary canal enzymes. As observed in other insects, the production of midgut enzymes is not a continuous process and the level varies in relation to moulting and food intake (House 1905; Isaiarasu *et al*., 2003). Furthermore Khan (1964) in *Locusta migratoria* and Ishaaya *et al*. (1971) in *S. litura*, the secretion of digestive enzyme were stimulated by the intake of food. Digestive enzymes are vital determinants for growth and survival of predatory insects.

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FEEDING BEHAVIOUR AND BIOLOGY OF A REDUVIID PREDATOR *RHYNOCORIS MARGINATUS* (FABRICIUS) (HETEROPTERA: REDUVIIDAE) ON OLIGIDIC DIET

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ABSTRACT: Feeding behaviour, nymphal development and survival rate of *Rhynocoris marginatus* (Fab.) on insect [*Spodoptera litura* (Fab.)] based oligidic diet (OD) was tested under laboratory conditions. All the life stages of *R. marginatus* prefer OD than water. The act of feeding behaviour was similar when they are fed with OD and also a factitious host (*Corcyra cephalonica* Stainton). *R. marginatus* preferred 5 % OD than 10 % OD. Fortification of protein in OD enhanced the handling time (HT) of the predator. Handling time gradually increased from the first instar to the third instar and decreased in the fourth instar. Further increase was observed in the fifth instar and also the adult. Among the nymphal instars, third instar took more time for handling the OD than other instars. Predators spend more time for resting than handling the OD. *R. marginatus* life stages preferred the cotton as a feeding object followed by paraffin capsule, foam and liquid. *R. marginatus* took 147.8 days for completing its life cycle on OD. The survival rate was minimum and maximum in the second and first instars, respectively. None of the laboratory emerged adults laid eggs. Rearing of reduviids in OD enhanced the predatory rate of this predator on *Helicoverpa armigera* (Hub.) and *Spodoptera litura*. Further, improvement in the nutritional quality of AD is needed in order to minimize the developmental period, and nymphal mortality and enhance the fecundity.

Key words: Reduviid predator, Oligidic diet, Object preference

INTRODUCTION

The reduviid, *Rhynocoris marginatus* (Fab.), is found in diverse agricultural and natural habitats, where it is reported to feed on more than 23 prey species including several economically important pests (Ambrose, 1999). Biological control potential of *R. marginatus* either alone (Sahayaraj, 1999, 2000; Sahayaraj and Martin, 2003) or in combination with intercrops and plant products (Sahayaraj, 2002) has been reported The development of effective and economically profitable technologies for mass rearing of entomophagous insects is important for the successful biological control programme. Successful development of such technologies based on artificial media will require a thorough knowledge of feeding behaviour and metabolism (Thompson, 1986). Inspite of some promising results obtained in the development of artificial diets for entomophages, the use of artificial diets in mass propagation programmes is currently limited to only a few species of predators (Cohen and Urias, 1986; De Clercq and Degheele, 1992; Chocorosqui and De Clercq, 2001). Sahayaraj *et al.* (2004) took an initiative for utilizing artificial diet in the mass production of reduviid. Recently, Sahayaraj *et. al.* (2006) evaluated the impact of both insect based and meat based artificial diets on feeding behaviour of *R. marginatus*. It was reported that in general, artificial diet reared *R. marginatus* consumed more number of preys than those reared on natural diet and/or factitious hosts (Sahayaraj and Balasubramanian, 2006). In this paper the effect of artificial diet i.e., *Spodoptera litura* based OD on the biology of this reduviid peredator has been presented.

MATERIALS AND METHODS

1. Test materaials

1.1. Reduviid predator

Rhynocoris marginatus life stages collected from agroecosystems were maintained in the laboratory at 30 ± 2°C temperature, 65±10% relative humidity and 11L and 13D on *Corcyra cephalonica* larvae. The egg batches laid by the adults were incubated in small plastic vials (30 ml volume) with moist cotton swabs to provide humidity. The newly hatched first instar nymphs were used for the experiment.

1.2. Preparation of oligidic Diet (OD)

Spodoptera litura larvae were reared on groundnut leaves. Third and fourth instar larvae were selected and kept in hot air oven at 60°C for 12 hours. The dried larvae were powdered by using domestic mixer, which formed the main source of the OD. Hundred ml of distilled water was taken in 500 ml capacity beaker and boiled at 100°C. From this 10 ml of boiled water was taken, 2.0 g of milk powder dissolved in it and allowed to cool. Beef extract (3.0 g), honey (15 ml), egg yolk (2.0 g), sucrose (200 mg), 10 % acetic acid (3.7 ml), NaCl (5 mg) and KCl (5 ml) were added to the remaining 90 ml of water, and once again boiled at 100°C. After 10 minutes, temperature was reduced to 40°C. Then to the source ingredient *viz.*, 5.0 and 10 g for 5 and 10 %, respectively, streptomycin (Sarabhaipiraman, Vadodara) (25 mg) and multivitamin (Glaxo, Gujarat) (40 mg) were added and boiled at 40°C for another 10 minutes. The prepared diet was cooled down to reach the normal room temperature. The milk powder solution was then added, mixed well and filtered through Whatman filter paper No.1 and the filtrate used for the study.

2. Test methods

2.1. Diet and object preference test

Covered petridish was used for all the experiments (10 cm diameter). Three small pieces of cotton (50 mg) and impregnated with 1 ml each of 5 and 10 % OD and water alone were placed inside the petridish. One 24 hours starved first instar *R. marginatus* nymph was introduced into the central part of the petridish and closed. After 30 minutes number of predators feeding on the OD and also the preferred concentration was recorded. Thirty different nymphs were maintained individually in each life stage. The preferred concentration was used for the subsequent experiments. The preferred diet was fortified with protein (Protein-X) at four concentrations (5, 10, 15 and 20 %). All the four concentrations along with the control (water) were tested to find out the suitable concentration. One 24 hour starved first instar *R. marginatus* was introduced and the concentration preferred by the predator was observed. Time spent by the predator in the preferred diet was recorded. Once the feeding was over the weight gained was recorded. Similar observations were recorded for the third instar and adult separately. Fifteen replications were kept for each nymphal instar and also adults. The observations were defined in terms of an Access Proportion Index (API). API work calculated according to Sahayaraj and Paulraj (2001). API= NS-NC/ NS+NC, where NS -Number of animals choosing the sample side and NC-number of animals choosing the control side. The preferred concentration was used for the subsequent experiments.

In the third experiment, four objects like cotton, paraffin capsule, foam and cavity slide were tested to find out their suitability for providing the artificial diet. The objects were placed inside the petridish. One ml of AD was poured into each object separately.

Twenty-four hour starved first instar (3 individuals) *R. marginatus* nymph were introduced in the central part and closed with the lid. Number of predators feeding the OD present in the object was observed for 30 minutes visually. Furthermore, feeding time of *R. marginatus* was noted on different objects separately. Similar procedure was followed for fifth instar nymphs and adults. Six replications were maintained for each life stage separately. Number of predators showing preference to each object was recorded and the percentage of predator preferring the objects was calculated using the following formula. The preferred object was used for further studies.

Preference = Number of predators feeding on particular object/ no. of predators introduced x 100

2.2. Feeding behaviour and strain selection

Two small pieces of cotton (50 mg) were taken and placed inside the petridish. One was impregnated with one ml of preferred OD that was selected from the previous experiment and another one was impregnated with water. Twenty-four hours starved first instar nymphs (2 individuals) of *R. marginatus* were introduced in the central part, and then the petridish was closed with lid. Feeding behaviour was observed for 30 minutes continuously. The approaching, feeding, and resting times were recorded and the data were pooled as handling time as described by Cohen (2000). Similar observation was observed for second, third, fourth, fifth nymphal instars and adults separately. Fifteen replications were maintained for each nymphal stage and adults. Further, number of predators which approached and consumed the OD was recorded. From the observation, the food preference was calculated.

Food preference = Number of nymphs feeding OD/ Total No. of nymphs introduced.

Similar procedure was followed for the remaining life stages. Karl Pearson's coefficient correlation was worked out for API, and handling time.

3. Biology

Plastic boxes (650 ml volume) were used for rearing the predators. Before introducing the first nymphal predators in to the plastic boxes, blotting paper was placed at the bottom. Reference card strip of 14 x 9 cm size was selected and cut them into zigzag pattern and placed in the container for resting of the predator. The Konica film vial lid was taken and 50 mg cotton was placed at the centre. One ml of OD (selected from the previous study) was poured on the cotton, and then kept inside the plastic container. Five newly hatched first instar nymphs were introduced into each container. Totally 210 newly hatched nymphs were taken for this experiment. After every 24 hours the cotton was replaced and the OD was provided once in two days. The number of immature predators surviving, and moulting changes were recorded. Based on this, developmental period and survival rate were calculated. Laboratory emerged adults provided with artificial diet. After 30 days, they were divided into two groups (each group having five adults). One predator was supplied with six fourth instar larvae in each of *H. armigera* and *S. litura* separately. After 24 hours number of prey consumed by a predator was recorded and the results were expressed as predatory rate (no. of prey / predator / day). Adults were maintained till their death. Student 't' test was used to find out the significance between concentrations of the diets. Same test was also performed for the predatory rate between *H. armigera* and *S. litura*. One-way ANOVA was used to determine the differences between the objects tested, different concentrations of protein fortified diet and handling time and resting time. Karl Pearson's correlation coefficient was used to know the significance between OD and water in feeding acts (Zar, 1984).

RESULTS AND DISCUSSION

1. Diet and object preference

The greatest barrier to mass production of predatory insects as a major force in pest control is the lack of suitable artificial diets. It has been developed for many predatory insects like chrysopids, coccinellids, anthecorids, geocorids, pentatomids etc. Information on artificial diet for reduviids is lacking. *R. marginatus* when provided with artificial diet it oriented towards the OD and fed on it. When the predator was provided with 5 and 10% OD, it preferred 5% diet (82.5%) over 10% diet (13.6%).

2. Protein fortified AD and Object preference tests

The results indicated that the first and third nymphal instars highly preferred 5 % protein fortified diet whereas the adults preferred 10 % diet (Table.1). Among the four objects tested, *R. marginatus* preferred OD soaked in cotton and the maximum feeding time was noticed (Table 2). Feeding time increased with the growth of the predator. For instance first instar spent 3.13 min whereas the fifth instar spent 5.15 minutes in cotton. Adults spent 7.09 min in the same object. Statistical analyses showed all the comparison were significant at 5% level except between capsules and liquid for fifth instar.

Sahayaraj *et al.* (2004) took an initiative for utilizing artificial diet in the mass production of this reduviid. Further Sahayaraj *et. al.* (2006) evaluated the impact of both insect based and meat based artificial diet on feeding behaviour of *R. marginatus*. The result of the present study has revealed that AD portified with protein-x has been accepted by this predator, which suggests that the predator can be reared in artificial diet. Object preference result suggested that cotton is suitable and inexpensive. Once the predator approached the cotton impregnated with synthetic diet or water, it protruded and inserted its rostrum and sucked the content (water and artificial diet). This observation suggested that synthetic artificial diet attracted the bugs and the act is similar to the prey location behaviour of *R. marginatus* (Sahayaraj and Paulraj, 2001). During feeding *R. marginatus* tries to insert its rostrum into the cotton and such kind of activity is well pronounced in the latter instars and adults than the early nymphal instars. Furthermore, *R. marginatus* pierced and sucked at various places, a habit of harpactorine reduviids (Ambrose, 1999).

3. Access Proportion Index (API) and superior strains selection

Access proportion Index (API) showed that OD had the attractant activity and it was ranging from 0 to 1.0. Results indicated that, the API was maximum in third instar (0.5) and similar in both first and fifth (0.3) instars and also in second, fourth and adults (0.4). It is evident that 54.6 % of the first nymphal instars preferred artificial diet and it increased to second (57.7 %), third (60.6 %), fourth (64.3 %) and fifth nymphal instars (65 %). The preference reached the peak (72 %) when adults were provided with the artificial diet. The above datas were statistically significant except the second nymphal instar at 5% level.

4. Feeding behaviour

In general, the handling time increased when the predator grew older. It was higher in the third nymphal instar (11.44 min) and decreased in the fourth instar. Afterwards it increased in the fifth instar (8.23) and further increased in adult. However, handling time in water gradually increased from the first instar to the adults. Predator spent more time for testing than handling either the AD or water (Table 3.). Karl Pearson's Correlation coefficient showed that the handling time of *R. marginatus* life stages in artificial diet with water showed high coefficient correlation (0.78).

5. Developmental Period, survival rate and Predatory potential

First, second, third, fourth and fifth instar took 23.3, 16.2, 20.7, 35.3 and 52.5 days, respectively for completing their nymphal instars. Shortest and longest nymphal developmental periods were observed in the second instar and fifth instar respectively. The total nymphal period lasted for 147.8 days. Maximum survival rate was observed in first nymphal instar (74.76 %) and was almost equal in the fourth (47.5 %) and total (45.5 %) nymphal instars. Minimum survival rate was observed in the second nymphal instar (40.1 %). Though 210 *R. marginatus* were reared on artificial diet only ten adults emerged. After feeding on artificial diet for about 30 days, adults of *R. marginatus* readily attacked active prey. It shows feeding on artificial diet did not affect the predation capacity of the reduviid and this increased predatory activity might be due to the continuous stress excerted by malnutrition. For instance adults reared on artificial diet attacked 2.82 fourth instar *H. armigera* and 5.60 fourth instar *S. litura* larvae. This was highly significant when compared to the reduviid reared on *C. cephalonica* (3.21), and *H. armigera* and *S. litura* (4.85) larvae respectively. Males and females adult longevity recorded an average of 34.5 and 39.2 days respectively. But females did not lay any eggs during their lifetime. The total nymphal period of *R. marginatus* was 46.71 days on *S. litura* (Sahayaraj and Paulraj, 2001) and it was higher when this reduviid was reared on *Odontotermes obesus* in solitary condition (Ambrose *et al.*, 1990). Generally in harpectorine reduviid the second and fifth nymphal instars are having shortest and longest developmental period respectively. There was no significant difference was observed on the development time of *Geocoris punctipes* (Say) reared on artificial diet and insect prey (Cohen and Urias, 1986). However, it is essential to analyse the chemical composition of its natural prey to improve the dietary quality of artificial diet which may helps to reduce the developmental time and increase the survival rate, adult emergence and fertility.

The predatory efficiency of the adult reduviids reared on artificial diet did not affected when provided with natural host. These results substantiate the findings of Chocorosqui and De Clercq (2001) and De Clercq and Degheele (1993). They also reported that prolonged rearing on artificial diet did not affect the predation capacity of nymphs and adults of *P. maculiventris* and *P. nigrispinus*. Similarly after prolonged maintenance of *G. punctipes* bugs on artificial diet, their predation capacity was identical to that of their field collected predators (Hagler and Cohen, 1991). None of the laboratory emerged adults laid eggs, indicating that it is essential to increase the nutritional quality of the OD. It is concluded from the present results that reduviid orient towards the artificial diet and feed them even if the nutrients in the diet (both qualitative and quantitative) cannot support normal growth as evident from the prolongation of developmental time and lower nymphal survival rate. However, the increased predatory activity of the reduviids reared on OD is a good sign to incorporate them in IPM programme.

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Table 1. Influence of protein x fortification on the diet preference (in %) of *R. marginatus* life stages

Life Stages	Concentration (%)			
	5	10	15	20
I	55.30^{a}	36.56^{b}	0	0
III	74.13^{a}	39.36^{b}	6.93^{c}	2.33^{d}
Adult	5.41^{a}	39.46^{b}	3.33^{c}	0.21^{d}

Same alphabets in the row are not significant at 5% level by DMRT.

Table 2. Objective preference (in %) and feeding time (in min) of *R. marginatus* life stages

Parameter	Life stage	Objects			
		Cotton	Paraffin capsule	Foam	Liquid
Object Preference	I	48.3^{a}	33.3^{b}	0.0^{e}	11.1^{d}
	V	51.7^{a}	33.3^{b}	22.2^{c}	22.2^{c}
	A	56.5^{a}	55.5^{ab}	33.3^{c}	22.2^{d}
Feeding time	I	3.13^{a}	2.89^{b}	0^{e}	0.85^{d}
	V	4.86^{a}	1.73^{b}	0.17^{c}	1.22^{b}
	A	7.09^{a}	2.44^{b}	0.06^{c}	1.51^{d}

Same alphabets in the row are not significant at 5% level by DMRT.

Table 3. Handling (HT) and resting (RT) times (in min) of *R. marginatus* life stages on artificial diet and water

Life Stages	Artificial diet (AD)		Water	
	HT	RT	HT	RT
I	6.81	23.13^{a}	2.13	27.82^{a}
II	10.42	19.60^{a}	2.25	27.66^{a}
III	11.44	18.41^{a}	2.43	27.58^{a}
IV	6.46	23.28^{a}	3.51	26.49^{a}
V	8.23	21.68^{a}	11.09	18.21^{a}
Adult	8.91	21.08^{a}	11.34	18.69^{a}

Same alphabets between the parameters of the same aspect (AD and water separately) in the row are not significant at 5% level by DMRT.