AUGMENTATIVE BIOLOGICAL CONTROL POTENTIAL EVALUATION OF COTTON PESTS USING REDUVIID

Thesis submitted to Manonmaniam Sundaranar University in partial fulfillment of the requirement for the award of the degree of

Doctor of Philosophy in Zoology

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CERTIFICATE

I certify that the thesis entitled **"Augmentative biological control potential evaluation of cotton pests using reduviid"** submitted by **Mr. S. KALIDAS (Reg. No. 4342)** for the award of the Degree of **Doctor of Philosophy in Zoology** at Manonmaniam Sundaranar University is a bonafide record of research work done by him independently in the Crop Protection Research Centre, Department of Zoology, St. Xavier's College (Autonomous), Palayamkottai under my guidance and supervision. The details furnished in the thesis are the original work of the candidate and have not been submitted elsewhere in part or full for the award of any other degree, diploma, associateship or other similar titles. It is not plagiarism of any other work either published or unpublished without acknowledgement.

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DECLARATION

I do hereby declare that the thesis entitled **"Augmentative biological control potential evaluation of cotton pests using reduviid"** is the result of the original study carried out by me under the guidance of **Dr. K. Sahayaraj,** Associate Professor, Department of Zoology, St. Xavier's College (Autonomous), Palayamkottai for the award of the degree of **Doctor of Philosophy in Zoology**. This work has not been submitted elsewhere in part or full for the award of any other degree, diploma, associateship or other similar titles. It is not plagiarism of any other work either published or unpublished without acknowledgement.

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PREFACE

Cotton is an important cash crop which grows in many parts of the world. This economical crop is damaged by over more than 160 different species of insect pests. Particularly, cotton has been regularly damaged by sucking pests like *Dysdercus cingulatus* Fabricius (Hemiptera: Pyrrhocoridae), *Aphis gossypii* Glover (Hemiptera: Aphididae) and *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) and defoliator, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae). Almost all the pests have developed resistance against insecticides which are routinely used by the farmers. Furthermore, *D. cingulatus* is a migratory insect, which very easily moves from place to place, whereas *P. solenopsis* has a waxy white external coating which acts as a defensive mechanism to prevent easy penetration of insecticides. Concerning environmental pollution and health hazards of insecticides, worldwide biointensive integrated pest management (BIPM) has been practised where natural enemies have considered as an important BIPM components.

Reduviids belong to the family Reduviidae (Insecta: Hemiptera). It comprises true bugs which contain more than 6300 species; most of them are zoophagous predators. The Reduviidae is the largest family of predaceous land hemipterans, abundant worldwide. Most of them are ploy phagous, however, prey specific, and are voracious predators. They are abundant in semi-arid zones, scrub jungles, forests of all kinds and their bordering agroecosystems like cotton, chillies, bhendi, brinjal, coconut form, mango orchard, greens, beans, wheat, etc. They are one of the important natural enemies of the cotton hemipteran and lepidopteran pests too. Under natural conditions, they hunt other insects. Hence, enormous hunter predators have been identified as biological control

agents against many economically important pests in India and also in other parts of the world. So far enormous literatures are available about the distribution and diversity, biology, mass rearing, stage preference, host preference and biological control potential of hunter reduviids worldwide particularly in India.

Rhynocoris longifrons Stål is more obscurely coloured beneath; lateral areas of head, behind eyes black; head a little longer than pronotum, membrane fuscous, abdominal margin palely spotted. It has a prey record of more than six insect species such as *D. cingulatus, A. gossypii, P. solenopsis, H. armigera, S. litura* and *Mylabris pustulata.* It is mainly living in the agroecosystems, scrub jungles, semi-arid zones and tropical forests and is considered as a good biocontrol agent in India. However, no one has studied the detailed biology and life table of this predator on cotton pests like *A. gossypii*, *D. cingulatus*, and *P. solenopsis.* Very minimum literature is available about the prey stage and prey preference, biological control potential under laboratory; But bioefficacy under pot condition and field condition (irrigated and rain fed) are not available in the literature. Moreover, these studies are very crucial to consider this predator in biological control worldwide. We considered these entire lacunas in our mind and initiated to fill the existing lacuna with the following objectives:

1. To make a survey of hunter reduviids in different varieties of cotton cultivated in all conditions from Tirunelveli, Thoothukudi, Kanyakumari, Theni, Virudhunagar, Sivagangai and Madurai districts of Tamil Nadu. Our survey reveals that one of the predominant reduviid is *Rhynocoris longifrons*; hence it has been selected for further studies.
2. To observe the biological traits and life table parameters of *R. longifrons* against *A. gossypii, D. cingulatus, H. armigera* and *P. solenopsis* (young ones and

adults) for three generation continuously and correlated these parameters with whole body total protein, lipid and carbohydrate of these four cotton pests.

3. To record the prey stage preference and prey preference of *R. longifrons* life stages on four economically important cotton pests using visual methods. The prey preference of the reduviid was confirmed by using preys crude kairomone by 'Y'- shape glass olfactometer. Furthermore, the bioactive chemical of the tested preys was documented.
4. To evaluate the predatory potential of *R. longifrons* on *A. gossypii, D. cingulatus, H. armigera* and *P. solenopsis* under pots and field condition and also to evaluate the hiding behaviour of the *R. longifrons* under screen house.

The thesis findings are represented in four chapters. The parts of each chapter are Title, Abstract, Keywords, Introduction with review of literature, Materials and methods, Results, Discussion and Conclusion.

The first chapter entitled **Survey of Reduviids in Cotton Agroecosystem** deals with the intensive survey of reduviid predators from cotton cultivating villages [Kavalkinaru (8°16' 33.19" 77°31' 50.55" E) and Alankulam (8°51'56.74" N 77° 29' 49.31" E) in Tirunelveli district; Aralvoimozhi (8°15' 07.49" N 77° 31' 50.55" E) in Kanyakumari district; Sathankulam (8°26' 28.73" N 77°54' 49.99" E) and Killikulam (8°42' 22.35" N 77° 51' 22.59" E) in Thoothukudi district; Srivilliputhur (9°30' 38.97" N 77° 38' 17.76" E) and Idayankulam (9° 29' 14.04" N 77° 35' 13.60" E) in Virudhunagar district; Rajagopalanpatti (10° 00' 16.81" N 77°38' 44.66" E) and Vaigai dam (10° 01'22.65" N 77°34' 05.15" E) in Theni district; Thirupachethi (9°46'29.35" N 78°20' 37.20" E) and Vellikurichi (9°45' 23.87" N 78°21' 43.07" E) in Sivagangai district and Chatrapatti (10°02'55.79" N 78°09'55.18" E) and Umachikulam (9°59'42.52" N 78°08' 45.61" E) in

Madurai district] of Tamil Nadu, India from April 2009 to March 2010. More than nine reduviid species [*Acanthaspis pedestris* (25), *Catamiarus brevipennis* (9), *Ectomocoris tibialis* (6), *Lophocephalus guerini* (1), *Rhynocoris fuscipes* (41), *Rhynocoris kumarii* (8), *Rhynocoris longifrons* (29), *Rhynocoris marginatus* (5) and *Sphedanolestes variabilis* (6)] from six genus were recorded. The reasons for their abundance have been discussed with concise conclusion.

The second chapter entitled **Biology and Life table of *Rhynocoris longifrons*** deals with the biology and life table traits of *R. longifrons* for three generations consecutively and correlated with whole body total carbohydrate, total protein and total lipids. The *H. armigera* larvae fed *R. longifrons* quickly developed with higher fecundity and hatchability. The life table traits of the *R. longifrons* reveals that the innate capacity for increase (r_c), intrinsic rate of increase (r_m), finite rate of increase (λ) and weekly multiplication (WM) of the predator were high when *H. armigera* was served as food. The total body protein and lipid was higher in *H. armigera*. The total body protein was correlation with nymphal development time (r^2=0.8 and 0.6 for *H. armigera* and *D. cingulatus,* respectively), nymphal survival (r^2=0.8 for *H. armigera, P. solenopsis* and *A. gossypii*), fecundity (r^2=0.6). The total body carbohydrate level was also correlation with nymphal development time (r^2=0.82) for *P. solenopsis* reared *R. longifrons.* The reasons for the enhancement of reproduction and shortest nymphal developmental time of the predator *R. longifrons* have been discussed briefly.

Stage preference, host preference (visual method and prey kairomone preference method) and functional response have been explained in the third chapter. The predator particular life stages preferred specific stage of *D. cingulatus, H. armigera* and *P.*

solenopsis. The host preference and prey crude kairomone of *R. longifrons* life stages reveal that *H. armigera* was the preferred cotton pest. GC-MS analysis of the preys crude kairomone reveals that predominant bioactive compounds were celidoniol, dodicamethyl, dodecanoicacid and dotriacontane for *H. armigera, D. cingulatus, P. solenopsis* and *A. gossypii,* respectively. Functional response of *R. longifrons* adult showed maximum prey consumption (8 preys) in 16 number prey densities of *D. cingulatus*, *H. armigera* (3.8 preys) in 10 densities and *P. solenopsis* (5 preys) in 10 densities. The third instar predator consumed maximum number of *A. gossypii* at 40 density rather than fourth, fifth and adult.

Chapter four deals with the bioefficacy of *R. longifrons* under pots and field conditions (irrigated and rainfed) and also the hiding behaviour of life stages of this predator. The hiding behaviour reveals that most of the animals hide under the small pebbles excised in the field followed by fallen leaves and other objects. The predatory potential of *R. longifrons* adults shows higher predatory rate against *H. armigera* (2.66±0.42) followed by and *P. solenopsis* (2.5±0.76), *D. cingulatus* (2.16±0.30). Third and fourth instar *R. longifrons* on *A. gossypii* was higher in the morning and in the evening. Field release of *R. longifrons* was evaluated in field condition in cotton field. The results revealed that the predator highly reduced *D. cingulatus* (53.80 %) population and *A. gossypii* (11.8 %) at rain fed condition and *P. solenopsis* (26.0%) under irrigated condition. Among the two field conditions, *R. longifrons* release gave more yield than the control in both conditions and the cost benefit ratio was higher in irrigated field followed by rainfed field.

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S. KALIDAS

CONTENTS

ABBREVIATIONS AND SYMBOLS USED

%	-	Percentage
µl	-	Micro litre
ANOVA	-	Analysis of Variance
BIPM	-	Biointensive Integrated Pest Management
BDV	-	Binomial Distribution Value
BSA	-	Bovine Serum Albumin
CBR	-	Cost Benefit Ratio
CKE	-	Crude Kairomone Extract
cm	-	Centimetres
$CuSo_4$.	-	Copper sulphate
Fig.	-	Figure
g	-	grams
GC – MS	-	Gas Chromatography Mass- Spectrometer
ha	-	hectare
hr	-	hours
IPM	-	Integrated Pest Management
kg/h^{-1}	-	kilogram/hectare
km	-	kilometres
kpa	-	kilo pascals
mg	-	milligrams
min.	-	minutes
ml	-	millilitre
mm	-	millimetres
Na_2Co_3	-	sodium carbonate
NaOH	-	sodium hydroxide

nm	-	nanometre
^{o}C	-	Degree centigrade
OD	-	Optical Density
PST	-	Potassium Sodium Tartrate
RBD	-	Randomized Block Design
Rh	-	Relative humidity
rpm	-	Rotation per minute
Sq. m	-	Square meter

ABSTRACT

Reduviid predators survey was conducted in seven district (Tirunelveli, Thoothukudi, Kanayakumari, Virudhunagar, Theni, Sivagangai and Madurai) from Tamil Nadu, India. Nine species [*Acanthaspis pedestris* (Stål), *Catamiarus brevipennis* (Servile), *Ectomocoris tibialis* (Distant), *Lophocephalus guerini* (Lap.), *Rhynocoris fuscipes* (Fab.), *Rhynocoris kumarii* (Ambrose and Livingston), *Rhynocoris longifrons* (Stål), *Rhynocoris marginatus* (Fab.) and *Sphedanolestes variabilis* Distant] from six genus were recorded from cotton field. *Rhynocoris fuscipes* was the predominant reduviid species in cotton followed *R. longifrons, A. pedestris, R. kumarii, E. tibialis, S. variabilis, R. marginatus* and *L. guerini.* Among the seven districts, Kanyakumari district harboured number reduviids of *Rhynocoris* spp. than *A. pedestris* and *E. tibialis.*

Studies on effect different hosts on biology of *R. longifrons* carried under laboratory condition. The result revealed that *Helicoverpa armigera* Hubner was most suitable for this predator because it was decreased total nymphal development time and mortality. The adult longevity, ovi-position days, fecundity and hatchability were enhanced when the predator was reared with *H. armigera.* Innate capacity for increase (r_c), intrinsic rate of increase (r_m), finite rate of increase and weekly multiplication at the predator were high when reared with *H. armigera.* Presence of more protein, lipid and carbohydrate may be the reason for the predator's activity.

The stage preference of this predator was on four cotton pests. The results revealed that the nymphal instars preferred small size preys and adults preferred large size preys. In host's preference of the *R. longifrons* comes first which is for lepidopteran pests followed by other hemipteran pests. This result was also observed in using kairomone.

The GC-MS analysis of the kairomones indicate that *H. armigera, Dysdercus cingulatus* (Fab.), *Phenacoccus solenopsis* Tinsely and *Aphis gossypii* (Glover) contains celidoniol, dodicamethyl, dodicanoic acid and dotriacontane, respectively. These bioactive compounds are responsible for the preference of reduviid against these pests.

Biocontrol potential of *R. longifrons* on four cotton pests under pot condition was examined. The result revealed that the adult of *R. longifrons* showed higher predatory rate against *H. armigera* than by *D. cingulatus* and *P. solenopsis* in both morning and in evening study. Invariably during day time the predator showed maximum consumption rate when compared with that in the evening. In hiding behaviour of *R. longifrons,* most of the predator life stages preferred pebbles than by fallen leaves and plants. *R. longifrons* was easily acclimatized in the field, even in the plants and fallen leaves. The augmentative release of the reduviid predator *R. longifrons* was evaluated against selected cotton pests of farmer's field at irrigated and rain fed condition. The results revealed that the total pest population reduction was higher in *D. cingulatus* and *A. gossypii* for rain fed condition than in irrigated cotton field condition and higher pest population reduction was observed for *P. solenopsis* under irrigated cotton field condition. Between the two tested field conditions, higher yield was recorded in *R. longifrons* released cotton plots compared with control in irrigated condition. The cost benefit ratio was higher in the predator released field in irrigated field condition than in the rain fed condition.

CHAPTER - 1

SURVEY OF REDUVIIDS IN COTTON AGRO-ECOSYSTEM

1.1. ABSTRACT

Cotton, *Gossypium hirsutum* L. is grown as a key fibre crop in the world. A study was undertaken to explore the diversity of reduviid predators in cotton plantation of seven southern district of Tirunelveli, Thoothukudi, Kanyakumari, Theni, Virudhunagar, Sivagangai and Madurai in Tamil Nadu, India, from April 2009 to March 2010. The study revealed the presence of nine species belonging to six genus namely *Acanthespis, Catamiarus, Ectomocoris, Lophocephala, Rhynocoris* and *Sphedanolestes*. They were observed and nine major pests [*Aphis gossypii, Dysdercus cingulatus, Dysdercus koengii, Helicoverpa armigera, Odontotermes obesus, Mylabris pustulata, Mylabris indica, Phenacoccus solenopsis* and *Spodoptera litura*] in all the districts were also recorded. Among the nine reduviids, *Rhynocoris fuscipes* population was higher than that of *Rhynocoris longifrons*.

Key words: Cotton, India, reduviid predators, Pests, Tamil Nadu.

1.2. INTRODUCTION AND REVIEW OF LITERATURE

Reduviids are distributed in semi-arid zones, scrub jungles, forests which borders the agro-ecosystems and they consume considerable numbers of preys (Schaefer, 1988; Ravichandran, 1998; Ragupathy and Sahayaraj, 2002; Sahayaraj and Raju, 2004; Rakhshani *et al.,* 2010). However, generalist reduviid predators are predominantly found in agro-ecosystems such as cotton (Johnson *et al.,* 2000; Shower and Greenberg, 2003; Majesh *et al.,* 2011; Sahito *et al.,* 2011; Sahayaraj, 2012), groundnut (Sahayaraj and Raju, 2003; Padmavathy and Poyyamoli, 2011), legumes and rice (Heinrichs and Barrion, 2004; Hassan, *et al.,* 2009), tea (Das *et al.,* 2010), coffee (Abasa, 1981), cowpea (Niba, 2011),

pigeonpea (Bhatnagar *et al.*,1983; Ambrose and Claver, 2001; Claver, 2011), sorghum (Balakrishnan *et al.*, 2010a), cabbage (Freddy *et al.*, 2011), millet (Singh *et al.*, 2009), tropical and rain forest (Woodward *et al.*, 1970; Minja *et al.*, 1999), forest (Malipatil, 1985; Hawkeswood, 1990; Revel *et al.*, 2010), sunflower (Grundy *et al.*, 2000), cucumber (Matoka, 2012) and tobacco (Jahnke *et al.*, 2003) worldwide. The following reduviid species were recorded in the agro-ecosystem, *Rhynocoris marginatus*, *Rhynocoris fuscipes, Rhynocoris longifrons, Catamiarus brevipennis, Coranus* sp, *Irantha armipes, Rhynocoris kumarii,* and *Sycanus pyrrhomelas* (Ambrose and Claver, 2001; Sahayaraj and Raju, 2004; Claver, 2011), *Rhynocoris ventralis* and *Peirontis modesia* (Paiero and Marshal, 2003), *Rhynocoris christophi, Rhynocoris ibericus, Rhynocoris iracundus, Rhinocoris punctiventris* and *Rhynocoris rubricoxa* (Rahimi *et al.*, 2010; Dursun, 2011) and *Rhynocoris rubricus* (Limonta *et al.*, 2003) in world wide.

Cotton, *Gossypium hirsutum* L. (Malvaceae), is one of the most commercially important fibre crops in the world. It is a perennial semi-shrub grown as an annual crop in both tropical and warm temperate regions. Cotton production is adversely affected by many insect pests, which have been traditionally controlled by large quantities of insecticides. Although chemical insecticides provide an excellent control, resistance in insect pests of cotton have been reported against many pesticides. This enhances the use of increased amount of insecticides (Ahmed *et al.*, 2011). Many natural enemies are found in the cotton agro-ecosystem. The parasitoids of *Cotesia marginiventris*, *Copidosoma floridanum* (Baur and Boethel, 2003) and *Trichogramma chilonis* (Davies *et al.*, 2009; Godhani *et al.*, 2009), *Bemisia tabaci* (Cetintas and McAuslane, 2009) and predators like, spiders (Ghavami, 2008; Chen *et al.*, 2010; Helsdingen, 2011; Matar and Kandel, 2011; Sahayaraj and Jeyaparvathy, 2011), pentatomidae (McPherson, 1982;

Niba, 2011; Tillman, 2011) and coccinelids (Nemade *et al.,* 2009; Balakrishnan *et al.,* 2010b; Tillman and Cottrell, 2012) are worth recording.

The reduviid predators are found in cotton agro-ecosystem and they are one of the important natural enemies on the cotton hemipteran and lepidopteran pests (Johnson *et al.,* 2000; Shower and Greenberg, 2003; Limonta *et al.,* 2003; Sahayaraj and Raju, 2003; Niba, 2011; Majesh *et al.,* 2011; Sahito *et al.,* 2011; Sahayaraj, 2012). However, to date, no one has recorded the reduviid predator population in cotton agroecosystems at any part of the world. We intended to record the distribution of reduviid predators in cotton agro-ecosystems of selected districts of Tamil Nadu with the following objective:

OBJECTIVE

1. To carry out a survey of reduviid predator and their dominant host insect in the cotton agro-ecosystem of Tamil Nadu, India.

1.3. MATERIALS AND METHODS

1.3.1. Survey of reduviids

Field survey was conducted from April 2009 - March 2010 in seven districts at Tamil Nadu (Plate 1). Two cotton cultivating villages [Kavalkinaru (8°16' 33.19" 77°31' 50.55" E) and Alankulam (8°51'56.74" N 77° 29' 49.31" E) for Tirunelveli, Aralvoimozhi (8°15' 07.49"N 77° 31' 50.55"E) for Kanyakumari, Sathankulam (8°26' 28.73"N 77°54' 49.99" E) and Killikulam (8°42' 22.35" N 77° 51' 22.59" E) for Thoothukudi, Srivilliputhur (9°30' 38.97" N 77° 38' 17.76" E) and Idayankulam (9° 29' 14.04" N 77° 35' 13.60" E) for Virudhunagar, Rajagopalanpatti (10° 00' 16.81" N 77°38' 44.66" E) and Vaigai dam (10° 01'22.65" N 77°34' 05.15" E) for Theni, Thirupachethi (9°46'29.35" N 78°20' 37.20" E) and Vellikurichi (9°45' 23.87"N 78°21' 43.07" E) for Sivagangai and Chatrapatti (10°02'55.79"N 78°09'55.18" E) and Umachikulam (9°59'42.52"N 78°08' 45.61" E) for Madurai districts] are

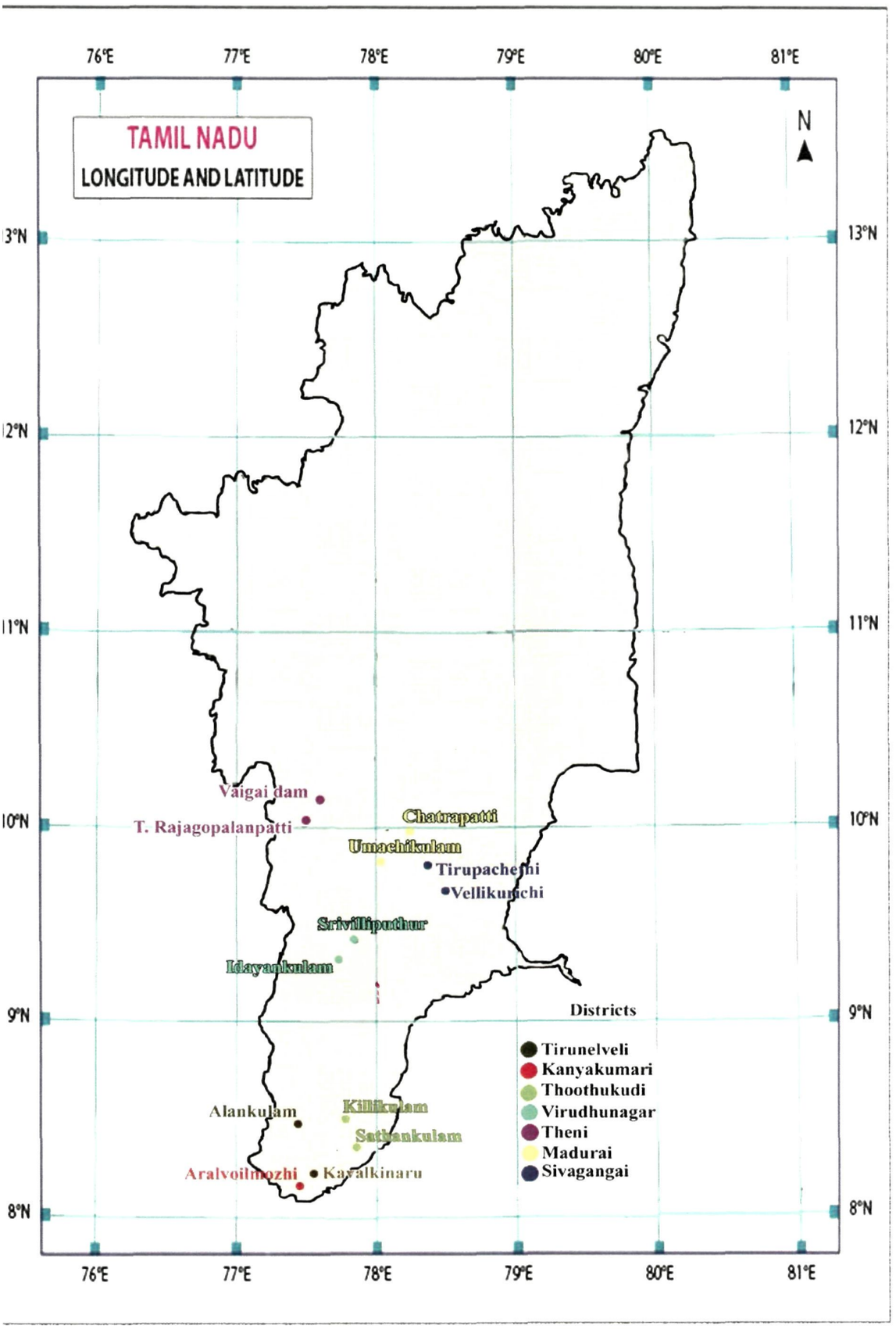

Plate 1. Map showing reduviids collection spots of different districts in Tamil Nadu

randomly selected from each district for this study. In each village, more than two cotton fields of 1 ha were considered for the reduviid survey, variety of cotton at these districts such as MCU, SVPR, Bunny, local variety and Uganda. Sweeping net and hand picking methods were used to collect the reduviids. Immature stages and adult predators were considered for this study. The reduviid and predominant pests prevailed during the study time were recorded carried out from flowering and bolling stage (40 to 90 days old plant) of cotton plant. Three field visits in a month were made in each district. All the observation were made either in the morning (7.00 am to 9.00 am) or in the evening (4.30 pm to 6.30 pm).

1.3.2. Meteorological data

Monthly records of abiotic factors such as maximum and minimum temperature (° C) relative humidity (Rh) (%), wind velocity (km/hr) and total rain fall (mm) which prevailed during the periods of the field experiments were collected from the District Meteorological Stations or District Statistical Department.

1.4. RESULTS

1.4.1. Survey of reduviids

Totally 131 individuals belonging to Harpactorinae (*Rhynocoris* spp., *Sphedanolestes* sp. and *Lophocephalus* sp.), Peiratinae (*Catamiarus* sp. and *Ectomocoris* sp.) and Reduviinae (*Acanthaspis* sp.) were observed in the cotton agro-ecosystems (55%) and their bordering ecosystems (45%) of Tamil Nadu, India. Most of the reduviids were found on the tender part of the cotton (eg: *R. fuscipes,* and *S. variabilis*) or under the leaves (eg: *R. fuscipes, R. marginatus,* and *S. variabilis*). *Acanthaspis pedestris, C. brevipennis, R. longifrons* and *E. tibialis* were found underneath cotton litters or small pebbles either individually or in groups. The number of reduviid predators collected from

the cotton agro-ecosystem and their bordering ecosystem like scrub jungle and semi-arid zones of seven districts of Tamil Nadu, India are presented in Table 1. It shows the reduviid predators collected from different localities with their dominant cotton pests like *A. gossypii, D. cingulatus, M. pustulata, M. indica,* and *P. solenopsis*. Among the seven districts, Kanyakumari district harboured more number of reduviids of *Rhynocoris* spp. than *A. pedestris* and *E. tibialis.*

Rhynocoris fuscipes (41) was observed in more number. For instance, in Kanyakumari district the *R. fuscipes* (18) population was high followed by Thoothukudi district (9) and Tirunelveli district (7) from April 2009 to March 2010. *Rhynocoris longifrons* (18) was also collected in large numbers at Kanyakumari district. *Rhynocoris marginatus* and *S. variabilis* were recorded from three districts (Table 2). The fluctuation of rainfall (Tirunelveli and Thoothukudi) and wind velocity (Kanyakumari) have no effects on the population of these reduviids (Table 3-9). However, the correlation was between minimum temperature and maximum temperature does not showed any influence on the population of reduviid predator in Tirunelveli district. Similar relation was also observed in Kanyakumari district. In the present study, it was observed that in all the study sites, the reduviid predators were high in June 2009 followed by May 2009 and July 2009. Furthermore, results also reveal that tibialorate reduviids were preferred to those that dwelt in scrub jungles and semi-arid zones, whereas non- tibiarolite reduviids dwelled in cotton agro-ecosystem.

Bunny; SVPR 2, 4; MCU 5 and 7; Local variety and Uganda cotton varies have been cultivated in the study areas. More than 67 % reduviids (*E. tibialis, L. guerini, R. fuscipes, R. kumarii, R. marginatus,* and *R. longifrons*) preferred to SVPR 2 than MCU 5 (55%), SVPR 4 (33%), MCU 7 (22%), and Bunny, Local and Uganda cotton varieties (11%).

Table 1. Reduviid predators recorded in cotton agroe-cosystem and their border ecosystem of seven districts of Tamil Nadu from April 2009 – March 2010

Reduviid Predators	Distribution	Number of insects		Dominated pests during the observation
		Cotton ecosystem	Bordering ecosystem	
Acanthespis pedestris (Stål)	Kanyakumari and Tirunelveli districts (MCU 5)	11	15	*Dysdercus cingulatus, Odontotermes obesus*
Catamiarus brevipennis (Servile)	Tirunelveli district (MCU 5 and MCU 7)	3	6	*Dysdercus cingulatus, Spodoptera litura, Mylabris pustulata.*
Ectomocoris tibialis (Distant)	Tirunelveli and Kanyakumari districts (Bunny and SVPR II)	2	4	*Dysdercus cingulatus, Spotoptera litura, Mylabris pustulata.*
Lophocephalus guerini (Lap.)	Kanyakumari district (SVPR II)	-	1	*Dysdercus cingulatus, Spotoptera litura*
Rhynocoris marginatus (Fab.)	Tirunelveli district, Sivagangai, Virudhunagar and Tuticorin districts (SVPR II, SVPR IV and MCU 5)	5	-	*Dysdercus cingulatus, Aphis gossypii, Helicoverpa armigera, Phenacoccus solenopsis*
Rhynocoris fuscipes (Fab.)	Tuticorin, Tirunelveli, kanyakumari, Madurai, Sivagangai, Theni and Virudhunagar districts (SVPR II, SVPR IV and Local variety)	34	7	*Dysdercus koengii, Spodoptera litura, Mylabris pustulata, Mylabris indica, Helicoverpa armigera.*
Rhynocoris longifrons (Stål)	Kanyakumari and Tirunelveli districts (MCU 5, SVPR IV and SVPR II)	11	18	*Dysdercus cingulatus, Spodoptera litura, Phenacoccus solenopsis*
Rhynocoris kumarii (Ambrose and Livingston)	Kanyakumari district (Uganda, SVPR II)	-	8	*Dysdercus cingulatus, Aphis gossypii*
Sphedanolestes variabilis Distant	Tuticorin, Tirunelveli and Virudhunagar (MCU 5 and MCU 7)	6	-	*Dysdercus cingulatus, Aphis gossypii, Phenacoccus solenopsis*
TOTAL		**72**	**59**	

Districts: Tirunelveli, Thoothukudi, Kanyakumari, Virudhunagar, Theni, Sivagangai and Madurai

Table 2. Distribution of reduviid predators in cotton agro-ecosystem of seven districts of Tamil Nadu, India from April 2009 to March 2010

Month	Tirunelveli districts							Kanyakumari districts						Thoothukudi districts			Virudhunagar districts			Theni districts	Sivagangai districts		Madurai districts
	AP	CB	ET	RM	RF	RL	SV	AP	ET	RF	LG	RK	RL	SV	RF	RM	RM	RF	SV	RF	RF	RM	RF
April 2009	4	-	-	-	1	2	-	7	1	5	-	3	5	-	-	-	-	-	-	-	-	-	-
May	-	-	1	-	1	-	-	6	1	3	-	-	-	-	4	1	-	-	-	-	-	-	-
June	4	3	-	1	1	-	1	-	2	-	1	-	-	1	1	-	-	1	1	1	1	1	1
July	2	-	1	-	-	6	-	-	-	-	-	-	3	-	1	-	1	2	1	-	-	-	-
August	-	-	-	-	-	-	-	-	-	3	-	-	-	-	1	-	-	-	-	-	-	-	-
September	-	4	-	1	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-
October	-	2	-	-	2	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-
November	-	-	-	-	-	3	-	-	-	-	-	4	1	-	-	-	-	-	-	-	-	-	-
December	-	-	-	-	-	-	1	-	-	7	-	-	-	-	1	-	-	-	-	-	-	-	-
January 2010	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-
February	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
March	-	-	-	-	1	-	-	3	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Total	10	9	2	2	7	11	2	16	4	18	1	8	18	2	9	1	1	3	2	2	1	1	1

AP- *Acanthaspis pedestris*, **CB** – *Catamiarus brevipennis*, **ET**- *Ectomocoris tibialis*, **LG**- *Lophocephalus guerini*, **RF**- *Rhynocoris fuscipes*, **RL**- *Rhynocoris longifrons*, **RK**- *Rhynocoris kumarii*, **RM**- *Rhynocoris marginatus* and **SV**- *Sphedanolestes variabilis*

- Indicates no reduviids observed during the study period

Table 3. Meteorological data recorded at Tirunelveli district from April 2009 to March 2010.

Month	Rain fall (mm)	Temperature (°C)		Rh (%)	Wind velocity (km/hr)
		Minimum	Maximum		
April 2009	12.0	29.1	31.4	60.8	31.5
May	33.0	30.4	35.0	49.0	13.0
June	8.0	30.0	33.6	51.6	2.6
July	4.30	31.5	34.9	63.3	27.2
August	8.12	31.6	33.4	75.6	2.0
September	0	29.5	31.2	70.2	4.6
October	25.0	28.6	32.6	67.0	1.8
November	157.1	29.3	32.2	75.3	6.4
December	64.5	27.4	31.4	72.1	10.0
January 2010	0	28.2	33.4	73.0	10.0
February	0	27.3	30.2	78.5	12.2
March	0	30.1	33.2	65.1	8.0
Correlation coefficient	-0.12	-0.35	-0.03	0.50	0.36

Table 4. Meteorological data recorded at Kanyakumari district from April 2009 to March 2010.

Month	Rain fall (mm)	Temperature (°C)		Rh (%)	Wind velocity (km/hr)
		Minimum	Maximum		
April 2009	25	28.1	35.2	63.0	12.2
May	66.5	29.0	32.3	70.1	23.6
June	2	28.6	35.3	68.8	34.5
July	2	27.3	34.8	79.5	22.0
August	0	26.6	32.5	69.2	22.6
September	23	28.1	32.3	56.3	12.3
October	3	26.0	32.6	61.0	6.40
November	233	28.6	31.3	78.0	15.4
December	13.6	25.3	29.3	68.8	34.5
January 2010	7	24.2	32.9	48.6	15.0
February	0	26.8	31.7	55.6	27.2
March	34	26.8	30.0	60.5	20.3
Correlation coefficient	0.12	0.43	0.23	0.13	-0.18

Table 5. Meteorological data recorded at Thoothukudi district from April 2009 to March 2010.

Month	Rain fall (mm)	Temperature (°C)		Rh (%)	Wind velocity (km/hr)
		Minimum	Maximum		
April 2009	22.0	29.1	36.2	64.0	3.20
May	34.0	30.6	37.8	59.5	14.1
June	7.0	30.2	36.3	43.3	2.2
July	4	33.3	36.3	70.3	11.7
August	0	28.7	36.6	68.5	3.5
September	0	29.5	36.6	64.0	7.4
October	27	28.4	36.1	67.6	4.3
November	184.1	27.7	31.2	64.0	3.2
December	72.0	29.6	33.8	41.5	14.1
January 2010	0	30.1	35.3	43.3	2.2
February	0	29.1	36.6	41.0	16.3
March	0	31.4	32.6	52.8	14.1
Correlation coefficient	-0.07	-1.00	0.06	0.02	0.30

Table 6. Meteorological data recorded at Virudhunagar district from April 2009 to March 2010.

Month	Rain fall (mm)	Temperature (°C)		Rh (%)	Wind velocity (km/hr)
		Minimum	Maximum		
April 2009	37.5	29.7	35.2	66.0	5.0
May	25.0	30.8	35.0	54.3	48.0
June	16.2	29.8	34.1	59.1	9.3
July	28.5	29.7	34.2	67.6	8.7
August	80.5	29.1	34.6	62.1	9.2
September	87.5	28.6	31.5	65.6	1.53
October	69.2	28.3	32.1	67.6	1.40
November	193.3	26.8	31.6	66.2	7.0
December	27.5	25.8	30.9	73.5	1.50
January 2010	0	24.6	31.1	71.0	1.06
February	0	27.3	30.9	62.9	1.38
March	8.10	28.9	33.3	62.8	0.79
Correlation coefficient	0.40	0.31	0.05	-0.05	0.03

Table 7. Meteorological data recorded at Theni district from April 2009 to March 2010.

Month	Rain fall (mm)	Temperature (°C)		Rh (%)	Wind velocity (km/hr)
		Minimum	Maximum		
April 2009	30.5	30.5	35.2	63.2	5.03
May	44.2	30.1	35.9	640	7.0
June	4.9	28.2	33	59.1	6.65
July	4.4	28.9	36.2	71.2	7.89
August	39.8	26.4	34.9	76.5	6.29
September	10.7	25.9	31.5	72.3	5.73
October	50.7	27.4	34.5	63.9	6.12
November	112.3	23.5	32.7	72.7	3.66
December	35.5	24.5	31.7	73.8	3.26
January 2010	0	23.9	31.5	57.5	2.74
February	0	27.5	34.2	76.9	6.40
March	10.7	28.2	35.8	63.7	5.61
Correlation coefficient	-0.48	-0.16	-0.44	-0.16	-0.24

Table 8. Meteorological data recorded at Sivagangai district from April 2009 to March 2010.

Month	Rain fall (mm)	Temperature (°C)		Rh (%)	Wind velocity (km/hr)
		Minimum	Maximum		
April 2009	53.4	27.1	32.8	66.5	1.28
May	21.6	29.4	34.3	59.7	3.10
June	0	29.0	36.1	49.1	14.0
July	0	28.6	32.1	69.8	12.0
August	0	25.3	33.2	71.3	7.1
September	21.4	26.0	32.3	57.6	1.17
October	63.5	28.2	32.1	67.4	0.91
November	102.4	24.0	28.4	88.3	2.30
December	59.1	23.7	30.2	82.6	1.0
January 2010	0	24.2	26.6	65.3	0.57
February	0	26.8	29.5	63.0	1.12
March	0	28.6	34.4	63.7	0.72
Correlation coefficient	-0.28	0.39	0.48	-0.48	0.48

Table 9. Meteorological data recorded at Madurai district from April 2009 to March 2010.

Month	Rain fall (mm)	Temperature (°C)		Rh (%)	Wind velocity (km/hr)
		Minimum	Maximum		
April 2009	55	28.4	34.4	68.4	3.1
May	89.8	30.3	35.1	90.3	10.2
June	41.8	27.2	32.3	58.6	7.6
July	65.0	28.4	32.0	65.5	5.4
August	108.2	26.8	34.2	72.4	2.8
September	125.6	27.1	32.3	65.9	10.2
October	42.8	26.0	33.1	56.2	8.1
November	304	26.1	33.7	82.1	2.3
December	25.6	24.2	33.3	76.3	1.44
January 2010	0	24.0	30.1	59.3	1.79
February	0	29.3	32.4	69.0	1.10
March	0	30.1	36.6	67.3	2.28
Correlation coefficient	-0.13	0.04	-0.26	-0.39	0.26

1.5. DISCUSSION

Cotton, *Gossypium hirsutum* L., is an important fibre crop is the most economically important natural fibre material in the world. One of the major obstacles hindering cotton cultivation is insect pest infestation. The survey conducted in cotton agro-ecosystem of seven districts of Tamil Nadu revealed that more number of reduviids were recorded in Tirunelveli (7 species) and Kanyakumari (6 species) districts as reported by Ragupathy and Sahayaraj (2002). This might be due to:

(i) multiple crops cultivation recorded in and around the study site, and

(ii) preys of different insects order were abundant in these study areas.

Rhynocoris spp. were recorded from all the seven districts, and *R. fuscipes* were distributed in all districts. Previously these reduviids have been recorded from pigeon pea (Claver, 2011), grams (Claver and Reegan, 2010), chilli (Sahayaraj, 2007), rice (Singh, 1985) and also in cotton (Martin and Brown, 1984; Singh *et al.,* 1987; Ambrose and Livingstone, 1989a; Sahayaraj, 1991; Grundy, 2004; Sahayaraj, 2012) which indicated that *R. fuscipes* is a potential reduviid predatory on more than 45 insect pests belonging to various insect order. Moreover, more than 39 *Rhynocoris longifrons* recorded either from cotton (SVPR II, SVPR IV, and local variety) (38%) or their bordering ecosystems (62%).

The survey of 6 genus (*Acanthespis, Catamiarus, Ectomocoris, Lophocephala, Rhynocoris* and *Sphedanolestes*) of reduviid predators from these districts revealed that the reduviid population may be regulated by preys, because some abiotic factors like rainfall (r^2= -0.12, -0.07, -0.48, -0.28 and -0.13 for Tirunelveli, Thoothukudi, Theni, Sivagangai and Madurai districts, respectively); wind velocity (r^2= -0.18, and -0.24 for Kanyakumari and Theni districts, respectively) and relative humidity (r^2= -0.05, -0.16, -

0.48, and -0.39 for Virudhunagar, Theni, Sivagangai and Madurai districts, respectively) were negatively correlated to the reduviid population. Previously, Ambrose and Livingstone (1978) reported that physical factors like temperature, relative humidity, rainfall and wind velocity have no correlation with reduviid population under semiarid zone condition. Moreover, Vennison and Ambrose (1990 and 1991), Sahayaraj and Raju (2004) and Ragupathy *et al.* (2001) reported the occurrence of four species of reduviid such as *Acanthaspis* sp., *Ectomocoris* sp., *Rhynocoris* spp., and *Onococephalus* sp were influenced by both abiotic and biotic factors.

Plant character is often influenced the distribution of herbivore and their natural enemies distribution. It was also previously reported that cropping condition provides suitable microclimate, continuous supply of food and suitable sites of reproduction for arthropod natural enemies including reduviids (Das *et al.,* 2010). It was also reflected in the present study that more than 67 % reduviids preferred to SVPR 2 than MCU 5 (55%), SVPR 4 (33%), MCU 7 (22%), and Bunny, Local and Uganda cotton varieties (11%) as reported by Atakan *et al.* (1996) and Solangi *et al.* (2011). They reported that cotton varieties altered the pests and their natural enemies pollution.

1.6. CONCLUSION

1. It is concluded from this study that 131 reduviids from Harpectorinae, Peiratinae and Reduviinae were recorded from Tirunelveli, Thoothukudi, Kanyakumari, Virudhunagar, Theni, Sivagangai and Madurai districts of Tamil Nadu.
2. More number of reduviids species were recorded from Kanyakumari (65) than from Tirunelveli (43) district.
3. More than 67 % reduviids preferred to SVPR 2 than MCU 5, SVPR 4, MCU 7, and Bunny, Local and Uganda cotton varieties

4. Reduviids population was influenced by both abiotic and biotic factors.
5. Among the nine reduviids, *R. fuscipes* was recorded in all the districts. Since enormous literature was available about the distribution and diversity of this predator (Singh, 1985; Singh and Singh, 1987; Ragupathy and Sahayaraj, 2002; Claver, 2011), bioecology (Ambrose, 1980), ethology (Ambrose and Kumaraswami, 1993; Ambrose, 1997; Sahayaraj *et al.,* 2002a; Sahayaraj and Selvaraj, 2003) and biological control potential of *R. fuscipes* (Sahayaraj *et al.,* 2002a; Nagarajan *et al.,* 2010; Majesh *et al.*, 2011), we selected *R. longifrons* for this proposed thesis work.

CHAPTER - 2

BIOLOGY AND LIFE TABLE OF *RHYNOCORIS LONGIFRONS*

2.1. ABSTRACT

The biological traits and life table parameters of *Rhynocoris longifrons* was quantified three generations continuously using *Dysdercus cingulatus* (Hemiptera: Pyrrhocoridae) nymphs, *Aphis gossypii* (Hemiptera: Aphididae) nymphs and adults, *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae and *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) adults as preys. *Helicoverpa armigera* the most suitable prey for this predator, because it decreased the total nymphal developmental time and mortality and enhanced the female adult longevity, oviposition days, fecundity and hatchability. The female predator lived longer than the male. Eggs were laid in batches with minimum of 6 and a maximum of 20 eggs in an egg batch. Innate capacity for increase (r_c), intrinsic rate of increase (r_m), finite rate of increase (λ) and weekly multiplication (WM) of the predator were high when *H. armigera* served as food. However, *P. solenopsis* provided the predator to take significantly longer periods to attain adulthood (63 days) and they were dead. Macromolecule profile (Protein, Carbohydrate and Lipid) of pests revealed that lepidopteran pest *H. armigera* has higher quantity of protein and lipids. This too may be responsible for enhancing the reproduction of *R. longifrons.* However, carbohydrate is high in hemipteran pest *P. solenopsis*. The laboratory rearing of *R. longifrons*, *H. armigera* can be used as a prey rather than *D. cingulatus.*

Key words: Biology, cotton pests, life table, macromolecular, *Rhynocoris longifrons*

2.2. INTRODUCTION AND REVIEW OF LITERATURE

In agriculture, predatory insects are the main natural enemies of economically important pests (Waterhouse, 1998; Ignacimuthu, 2002; Butler and O'Neil, 2008). The Reduviidae is the largest family of predaceous land Heteroptera, abundant worldwide and are voracious (James, 1994; Cohen and Tang, 1997; Ehler *et al.,* 1997; Ambrose, 1999; Wignall and Taylor, 2008, 2009; Jackson *et al.,* 2010). Reduviid predators are potential biocontrol agents against many insect pests and have the potential for an even greater role in Integrated Pest Management (IPM) (Ambrose, 2000; Grundy and Maelzer, 2000; Sahayaraj *et al.,* 2002b, 2003b, 2004). Reduviids (Hemiptera: Reduviidae) constitute an important group of predatory insects. For instance, reduviid bugs often constitute a large and diverse group, have many fascinating specialized habits and predators have considerable but un realised potential as biocontrol agents (Ambrose, 1999; Nagarajan *et al.,* 2010). Reduviids suppressed more than 71 lepidopteron dominate the prey followed by hemipteran (41) and coleopteran (27) pests. They reduced most of the pest population both in laboratory and field situation (Ambrose, 1999; Sahayaraj, 2003; 2007).

2.2.1. Biology and life table of *Rhynocoris* spp.

Reduviids are important predatory insects that could be harnessed for biological control of insect pests. The genus *Rhynocoris* counts more than 150 species in the world fauna. Most of them have been distributed in agro-ecosystem (Putschkov, 1994; Paiero and Marshal, 2003; Sahayaraj and Raju, 2004; Rahimi *et al.,* 2010). Studies on the biology and the construction of the life table for a predator species is an important component in the understanding of its population (Carey, 1993; Sahayaraj and Paulraj, 2001b; Sahayaraj and Jeyalakshmi, 2002; Ambrose *et al.,* 2006, 2007b; Sahayaraj and Sujatha, 2011). Adequate literature is available on the biology and the life table of *Rhynocoris* spp.

The biological traits such as incubation and stadial period, adult longevity, sex ratio, oviposition, hatchability and nymphal survival etc. were described for *R. segmentarius* (Ullyett, 1930), *R. iracundus* (Muller, 1937), *R. lapidicola* (Joseph, 1959), *R. albopilosus* (Signoret) (Odhiambo, 1959; Kwadjo *et al.*,2008), *R. carmelita* Stal (Edward, 1962a), *R. bicolor* (F) and *R. tropicus* (Parker, 1969), *R. albopunctatus* (Stal) (Nyirra, 1970), *R. tibialis* (Abasa, 1981), *R. cupsidatus* Ribaut (Gammara, 1981), *R. marginatus* (Ambrose and Livingstone, 1985, 1989b; Vennison and Ambrose, 1988; Ambrose *et al.,* 1990 a and b; Sahayaraj, 1995a; Sahayaraj and Paulraj, 2001a, 2001b; Sahayaraj, 2002a; Sahayaraj and Jeyalaksmi, 2002; Sahayaraj and Sathiamoorthi, 2002; Sahayaraj *et al.,* 2003a; Sahayaraj *et al.,* 2004; Ambrose *et al.,* 2007a), *R. fuscipes* (Vennison and Ambrose, 1986; George and Ambrose, 1998; George *et al.,*2000; Claver and Ambrose, 2001a; Sahayaraj and Paulraj, 2001a; Sahayaraj and Selvaraj, 2003) and *R. kumarii* Ambrose and Livingstone (Ambrose and Livingstone , 1987a, 1987b; Claver *et al.,* 1996; Ravi, 2004), *R. tristis* (Beal, 2006; Beal and Tallamy, 2006), *R. persicus* (Putshkov, 2002) etc.

2.2.2. *Rhynocoris longifrons* Stål

Rhynocoris longifrons (Stål) is a multivolatine, voracious harpactorine reduviid predator inhibiting tropical rain forests, scrub jungles, semi-arid zones bordering agro-ecosystems (Ambrose *et al.,* 2003). *Rhynocoris longifrons* are more obscurely coloured, lateral areas of head behind eyes black, membrane fusious, and abdominal margine palely spotted and head a little longer than pronotum. The length of this predator is 10 – 12 mm and weight is approximately 40 to 57 mg. It feeds on some important insect pests such as *Helicoverpa armigera* Hubner (Ravichandran *et al.,* 2003; Ravi, 2004), *Odontotermes obseus* Rambur *Clavigralla gibbosa* Spinola (Claver *et al.,* 2002; Ambrose *et al.,* 2003), *C. cephalonica* (George *et al.,* 2000; Ambrose *et al.,* 2007b; Shirley and Prasanna Kumar,

2010), *Nezara viridula* L., *Exelastis atomosa* (L.) (Ambrose and Claver, 2001). The predatory potential and biological parameters were studied against on *S. litura* and *H. armigera* larvae (Kumar and Ambrose, 1996; Ambrose *et al.,* 2003; Ravichandran *et al.,* 2003; Ambrose *et al.,* 2007b; Sahayaraj and Ravi, 2007a; Kumar *et al.*, 2009; Ganesh Kumar, 2011).

The relationship between the quantity of food eaten and fecundity for several species of reduviids is well documented (Ambrose and Subburasu, 1988; Ambrose *et al.,* 1990b, 2003; Ambrose and Rani, 1991; Sahayaraj and Ambrose, 1994a; Venkatesan *et al.,* 1997; George *et al.,* 1998a, 2002; Sahayaraj and Sathiamoorthi, 2002; Sahayaraj *et al.,* 2004; Chandral and Sinazer, 2011). However, there are no studies on the effect of different cotton pests species on the biology, life table parameters, fecundity and adult longevity of *R. longifrons,* despite its importance as a predator of many aphid pests in India. For example, requirements for the pre-imaginal development and reproduction of adults of *R. longifrons* have not been studied (Ambrose *et al.,* 2003). The importance of the nutritional quality of the prey for this predator is also unknown. The aim of the present study was to evaluate four cotton pest species (*A. gossypii, D. cingulatus*, *H. armigera* and *P. solenopsis*) and the larvae of *C. cephalonica* as food for *R. longifrons* in terms of survival, development and reproduction under laboratory conditions. Such information would be helpful for optimizing the mass rearing of *R. longifrons* and for understanding its population dynamics in the field in the presence/absence of the various species of prey tested. Ultimately, the results may also help in designing biointensive integrated pest management (BIPM) programs involving the use of *R. longifrons* as a biocontrol agent of pests on cotton crop.

2.3. MATERIALS AND METHODS

2.3.1. Collection and rearing of insects

A mixture of life stages of *Rhynocoris longifrons* was collected from the cotton agro-ecosystem bordering the scrub jungle of Kanyakumari and Tirunelveli districts, Tamil Nadu and maintained individually in plastics containers (15 cm diameter × 7 cm height) under laboratory condition at 31. 2 ± 1.0 °C, 56.0 ± 5.0 % with photoperiod of 11L: 13D hr on a larval *Corcyra cephalonica* Stainton. All the pest species were collected from the same cotton field from which the reduviid was collected. *Aphis gossypii, D. cingulatus*, *H. armigera* and *P. solenopsis* were maintained in groups in plastic trough (7 litre) (32 cm diameter × 15 cm height) and plastic container (50 ml) on young leaves, flowers and bolls.

2.3.2. Biology

The eggs laid by the predator in the laboratory were allowed to hatch in Petri dishes (9 cm diameter × 2.0 cm height) with wet cotton swabs for maintaining humidity. The swabs were changed once in two days in order to prevent fungal attack. Only one egg mass was placed in each petridish so that uniform cohorts of nymphs could be reared and the incubation period was recorded. Laboratory emerged nymphs were maintained in plastic boxes (16 cm diameter × 8 cm height and the lid moulded with nylon mesh for aeration) and divided into five diets: 1) *A. gossypii* (all stages approximately 150 insects per leaf), 2) *D. cingulatus* [40 second (8.76 mg) and 50 third (11 mg) instar nymphs], 3) *H. armigera* [40 second (11 mg) and 50 third (23 mg) instar larvae respectively], 4) *P. solenopsis* [120 to 150 of adults (40 mg)] and 5) *C. cephalonica* [30 and 40 of second and fifth instar larvae respectively] host.

The sex ratio was computed (number of female/total number of adults emerged) on the basis of laboratory emerged adult. *Rhynocoris longifrons* adult (1 male: 1female) were transferred to another plastic container (6 cm height × 4.5 cm diameter) and maintained till their death. The following adult fitness traits were determined: pre oviposition period (the period from adult female eclosion to the age at first oviposition commenced), total number of eggs laid by a female, minimum and maximum number of eggs present in each batch of egg and post-oviposition period (last oviposition upto death), oviposition index (total number of eggs laid /total oviposition days), percentage of nymphs hatched, and adult longevity of male and female. Nymphs emerging from the ten randomly selected egg batches were maintained for each prey species and the predators were reared in the laboratory for three generation continuously (except *A.* gossypii).

2.3.3. Life table

The life table parameters of the predator was calculated by Birch (1948). The equation which was later elaborated by Southwood (1978) and Carey (1993):

$\Sigma e^{rm} l_x m_x = 1$

Where 'e' is the base of natural logarithms,

'x' the age of the individual in days,

l_x the number of individual alive at age 'x' as a proportion of one and

'm_x' the number of female or the net reproductive rate (R_o) was the rate of multiplication of population in each generation.

The approximate value of cohort generation time (T_c) was calculated from the birth of parents of birth of offspring ($Tc= \Sigma x\ l_x m_x/R_o$). The value of innate capacity for increase

(r_c) was calculated using r_c= log e R_o / T_c. The arbitrary value of r_m and the values of negative exponent of e^{rmx} as were certained from this experiment often by outside range. For this reason both sides of the equation were multiplied by a factor of $\Sigma e^{7\text{-}rmx}\ l_x m_x$ = 1096.6 (Birch, 1948). The precise generation time (T) was then calculated from the formula: T= log e R_o/r_m. The finite rate of increase (λ) was determined as (antilog e^{rm} /female/ day). Doubling time (DT), the time required to double in number was calculated as log $2/r_m$ and weekly multiplication (WM = antilog e^{rm}) was also calculated.

2.3.4. Macromolecular profile of preys

The macromolecules like total body carbohydrate, total body protein and total body lipid (Bragdon, 1951; Lowry *et al.,* 1951; Sadasivam and Manikam, 1997) contents of *D. cingulatus, A. gossypii, P. solenopsis* and *H. armigera* were quantified using standard procedure.

2.3.4.1. Total whole body carbohydrate

Preparation of Anthrone reagent: Take 200 mg of anthrone (0.2%) added with 5 ml of ethanol solution and to this solution was added with 95 ml of 75 % Con H_2SO_4.

Standard glucose solution: Take 10 mg of glucose to be added with 100 ml of distilled water and it was considered as a standard glucose solution.

Extraction of carbohydrate from insect prey: 100mg weight of live animal was taken in a homogenizer tube with phosphate buffer and homogenised. Centrifuge it at 3000 rpm for 30 minutes. Collect the supernatant (1 ml) and take particular volume of aliquots for analysis of carbohydrate.

Procedure for carbohydrate estimation

Prepare the 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml of standard glucose in a series of test tubes. '0' serves as blank. Make the volume to 1 ml in all the test tubes including the sample test tube by distilled water. Then add 4 ml of anthrone reagent. Heat them for 10 minutes in a boiling water bath. Cool rapidly and read the green colour at 630 nm. Standard graph was drawn according to the optical density (OD) of the standard. From the graph and also using formula the amount of carbohydrate present in the sample was calculated:

$$\text{Carbohydrate content in sample mg/100 mg} = \frac{\text{Con. of standard} \times \text{OD of the sample}}{\text{OD of the standard}}$$

2.3.4.2. Estimation of prey whole body total protein

Preparation of reagents

Reagent A: 2% sodium carbonate (Na_2CO_3) in 0.1N sodium hydroxide (NaOH) (prepare freshly).

Reagent B: 0.5 % copper sulphate ($CuSO_4$. $5H_2O$) in 1% potassium sodium tartrate (PST) (prepare freshly).

Reagent C: Mix 50 ml of reagent A and 1 ml of B (prepare prior to use).

Reagent D: Folin-ciocaltteau reagent dilutes the commercially available reagent with an equal volume of distilled water on the day of use.

Preparation of standard protein: Take 10 mg Bovine Serum Albumin (BSA). Add 50 ml of distilled water and it is considered as a standard.

Working standard: Dilute 10 ml of protein solution to 5 ml of distilled water in a standard flask.

Extraction of protein from insect prey: Take 100 mg of alive animal and homogenise with the 1 ml of phosphate buffer (pH 7.2) and centrifuge it at 3000 rpm for 30 minutes. Supernatant (1 ml) was used for estimating the protein.

Procedure for protein estimation

Pipette out 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml of the working standard protein solution into a series of test tubes. Pipette out a known volume of sample into a test tube. The volumes of the sample as well as standard were made up to 1 ml by distilled water. A test tube with 1 ml of water was serves as the blank. Add 5 ml of reagent (to each test tube including blank). Mix well and allow it to stand for 10 minutes, then add 0.5 ml of reagent D mixed well and incubate at room temperature for about 30 minutes. Blue colour was developed; the intensity of the colour was measured at 650nm. The optical density (OD) was compared with standard graph to estimate protein in mg/100g.

$$\text{Protein content in sample mg/100 mg} = \frac{\text{Con. of standard} \times \text{OD of the sample}}{\text{OD of the standard}}$$

2.3.4.3. Estimation of whole body total lipid

Preparation of Acetic anhydride solution: About 50 ml of acetic anhydride solution was taken in a beaker which was kept in an ice bucket. To this, 2 ml of concentrated H_2SO_4

acid was added carefully and gently stirred and kept in a cool place for some time. The mixture should be colourless otherwise it should be discarded and prepare freshly.

Preparation of Potassium dichromate: Potassium dichromate ($K_2Cr_2O_7$) (2%) was mixed with con. H_2SO_4. This reagent was prepared freshly.

Standard cholesterol solution: Take 10 mg of cholesterol; make it up to 100 ml of chloroform.

Preparation of standard graph

Standard cholesterol was pipette out into a series of test tubes in different volumes from 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml. All the test tubes were made up to 1 ml with chloroform. A test tube with 1 ml of distilled water was served as blank. Then to each test tube, 5 ml of acetic anhydride was carefully added and mixed well. The test tubes were covered with dark black colour cloth and kept for about 15 minutes without any disturbance. In concentrated solution one can notice the colour becoming rosy red, to blue and greenish blue. The developed colour was measured at 640nm in a spectrometer and optical density (OD) value was recorded. Standard graph was drawn by applying concentrations of cholesterol on X axis and optical density (OD) on Y.

Extraction of lipid from insect prey

Take 100 mg of animal was taken in a homogenizer tube and homoginize it with 2 ml of chloroform. This was centrifuged at 3000 rpm for 30 minutes. The supernatant (1 ml) was transferred to a test tube and evaporated to dryness. This was kept at room temperature for 2 days. Three ml of distilled water and an equal amount of freshly prepared potassium dichromate solution was added. The intensity of the colour developed

was measured at 640nm in spectrophotometer. The optical density (OD) of the sample was compared with standard graph to estimate lipid in mg/100g.

$$\text{Lipid content in sample mg/100 mg} = \frac{\text{Con. of standard} \times \text{OD of the sample}}{\text{OD of the standard}}$$

2.4. STATISTICAL ANALYSIS

The analysis of variance (ANOVA) was used to determine the difference among four cotton preys and also with factitious host *C. cephalonica*. It was applied to all the biology parameters like nymphal total developmental period, adult longevity, fecundity, number of eggs/ batch and hatchability. Deference of significance was expressed at 5 percent level using SPSS 11.5 software.

2.5. RESULTS

2.5.1. Nymphal total developmental period

The incubation periods of *R. longifrons* varies from 7 to 8 days. *Rhynocoris longifrons* lay their eggs either in single, or in small cluster of 4 to 18 eggs. The total nymphal developmental time of the predator fed on *H. armigera* was significantly ($F = 6.906$; df 13,121; $P < 0.05$) shorter than *D. cingulatus* ($F = 1.846$; df 12, 94; $P < 0.05$) and *P. solenopsis* ($F = 4.912$; df 8, 76; $P < 0.05$), when compared to *C. cephalonica* (Fig. 1). However, maximum nymphal survival rate was recorded while the predator was reared with *A. gossypii* (61%) rather than other cotton pests used in this study (Table 10).

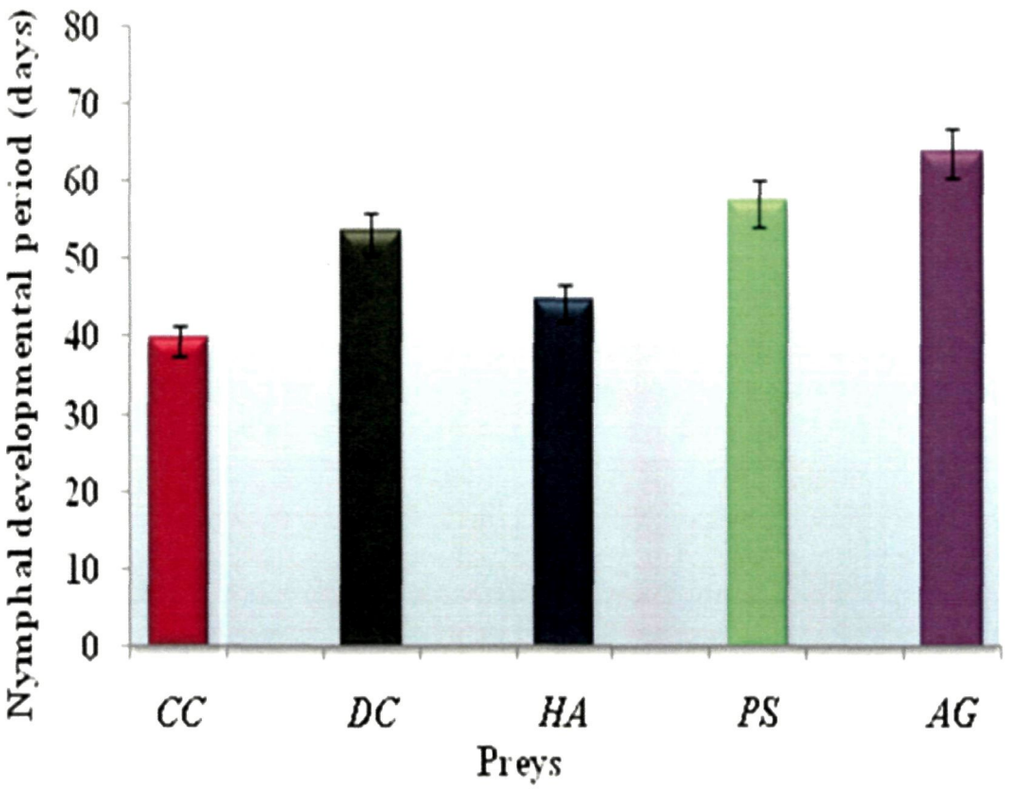

Figure 1. The total nymphal developmental period (day) of *R. longifrons* reared on various types of preys (CC- *Corcyra cephalonica*; DC – *Dysdercus cingulatus*; HA- *Helicoverpa armigera*; AG- *Aphis gossypii,* and PS- *P. solenopsis*

Table 10. Nymphal survival (%) and reproductive parameters of *R. longifrons* on *C. cephalonica* (n=250), *D. cingulatus* (n=236), *H. armigera* (n=246), *P. solenopsis* (n=205) and *A. gossypii* (n=242) (mean value ± SE)

Parameter	*C. cephalonica*	*D. cingulatus*	*H. armigera*	*P. solenopsis**	*A. gossypii**
Nymphal survival (%)	60.4±3.3^{a}	45.3±4.1^{ab}	54.4±4.0^{ac}	27.8±3.9^{abcd}	61.4±2.7^{abcde}
Male longevity (days)	67.6±0.50^{a}	60.4±0.64^{ab}	65.4±0.34^{bc}	57.6±0.98^{bc}	55.1±0.82^{aed}
Female longevity (days)	68.0±0.30^{a}	67.9±0.54^{ab}	71.3±0.68^{abc}	65.4±0.45^{abcd}	63.0±0.42^{abcde}
Sex ratio (Male: Female)	1:48	1:0.59	1:0.54	1:0.56	1:0.37
Pre-oviposition days	10.4±0.16^{a}	10.6±0.20^{ab}	9.5±0.18^{ac}	10.6±0.30^{ab}	10.8±0.43^{abe}
Oviposition days	43.6±0.62^{a}	37.6±0.52^{ab}	47.2±0.80^{abc}	38.2±0.73^{abc}	34.0±0.73^{abcde}
Minimum number of eggs/batch	5.54±0.23^{a}	6.0±0.28^{ab}	6.7±0.34^{ac}	4.8±0.39^{acd}	4.6±0.50^{abcde}
Maximum number of eggs/batch	16.1±0.31^{a}	12.0±0.45^{a}	18.3±0.41^{ac}	10.2±0.46^{acd}	9.39±0.37^{abcde}
Oviposition index	0.050±0.001	0.052±0.001	0.054±0.002	0.047±0.002	0.045±0.002
Post-oviposition days	14.8±0.40^{a}	15.7±0.41^{ab}	14.4±0.37^{abc}	15.9±0.58^{abcd}	18.3±0.43^{abcde}
Fecundity (eggs/ female)	39.3±0.42^{a}	32.6±0.63^{a}	43.5±0.55^{cb}	30.6±0.64^{bcd}	29.7±0.89^{acde}
Hatchability (%)	93.5±0.74^{a}	93.8±0.74^{b}	94.4±0.80^{bc}	90.1±1.56^{ad}	86.1±1.43^{abcde}

*Mean value for two generations
The tables followed by same letters are not significantly different

2.5.2. Sex ratio and adult longevity

In general, male (0.43) and female biased (0.73) (Tables 11 - 15) sex ratio was recorded in *R. longifrons*. The longevity of *R. longifrons* female fed with *H. armigera* was significantly longer ($F = 1.056$; df 7, 44; $P < 0.05$) than *A. gossypii* ($F = 0.892$; df 7, 23; $P < 0.05$) and *P. solenopsis* ($F = 0.554$; df 9, 40; $P < 0.05$). Similar results were also recorded for *R. longifrons* male ($F = 1.941$; df 14, 37; $P < 0.05$ and $F = 4.488$; df 10, 19; $P < 0.05$ for *H. armigera* and *P. solenopsis*, respectively).

2.5.3. Oviposition periods

Table 10 shows that the pre-oviposition period of the predator fed on *H. armigera* ($F = 0.564$; df 6, 53; $P < 0.05$) was shorter than *D. cingulatus* ($F = 0.757$; df 6,50; $P < 0.05$), *P. solenopsis* ($F= 0.302$; df 4, 26; $P> 0.05$) and *A. gossypii* ($F = 0.985$; df 4,23; $P < 0.05$) fed groups. Similar observation was also observed for the oviposition period of the *R. longifrons* fed on *H. armigera* ($F = 0.891$; df 19,40; $P < 0.05$, $F = 1.899$; df 22,37; $P < 0.05$ and $F = 1.221$; df 16,11; $P < 0.05$ for *H. armigera*, *D. cingulatus* and *A. gossypii*, respectively). The post-oviposition of the *R. longifrons* were significantly shorter ($F = 2.757$; df 8, 19; $P < 0.05$ and $F = 2.807$; df 8, 19; $P < 0.05$ for *D. cingulatus* and *P. solenopsis*, respectively).

2.5.4. Fecundity and hatchability

Fecundity and hatchability of *R. longifrons* reared with different preys are presented in table 10. The fecundity of *R. longifrons* was higher when reared with *H. armigera* ($F= 1.698$; df 13, 46; $P<0.05$) but it is not significant when compared with *D. cingulatus* ($F= 0.627$; df 15, 44; $P>0.05$), *P. solenopsis* ($F= 0.871$; df 10, 20; $P>0.05$) and *A. gossypii* ($F= 0.431$; df 10, 17; $P>0.05$). The hatchability was also significantly higher in *R. longifrons*

Table 11. Nymphal survival (%) and reproductive parameters of *R. longifrons* on *C. cephalonica* (n= 100, 75 and 75for three generations)

Parameter	First generation	Second generation	Third generation	Mean
Nymphal survival (%)	54.0±2.1	68.0±4.1	61.3±3.5	60.4±3.3
Male longevity (days)	68.2±0.43	66.8±0.56	64.6±0.51	67.6±0.50
Female longevity (days)	70.3±0.82	68.0±0.48	67.7±0.74	68.0±0.30
Sex ratio (Male: Female)	1:0.48	1:0.53	1:0.43	1:48
Pre-oviposition days	9.8±0.19	10.9±0.22	10.7±0.42	10.4±0.16
Oviposition days	46.5±1.08	42.2±0.66	41.4±1.18	43.6±0.62
Minimum number of eggs/batch	5.40±0.26	5.2±0.35	6.1±0.63	5.54±0.23
Maximum number of eggs/batch	17.0±0.45	15.6±0.50	15.2±0.41	16.1±0.31
Oviposition index	0.056±0.002	0.046±0.003	0.047±0.003	0.050±0.001
Post-oviposition days	14.04 ± 0. 79	15.1±0.56	15.43±0.71	14.8±0.40
Fecundity (eggs/ female)	41.0±0.44	38.8±0.78	36.7±0.62	39.3±0.42
Hatchability (%)	95.0±0.67	94.0±0.60	89.1±2.3	93.5±0.74

Table 12. Nymphal survival (%) and reproductive parameters of *R. longifrons* on *H. armigera* (n= 71, 75 and 100 for three generations)

Parameter	First generation	Second generation	Third generation	Mean
Nymphal survival (%)	71.8±3.41	37.3±4.2	55.0±2.6	54.4±4.0
Male longevity (days)	66.2±0.57	65.2±0.57	65.0±0.82	65.4±0.34
Female longevity (days)	73.8±0.69	73.6±0.82	65.3±0.63	71.3±0.68
Sex ratio (Male: Female)	1:0.53	1:0.57	1: 0.52	1:0.54
Pre-oviposition days	9.6±0.81	9.3±0.33	9.73±0.34	9.5±0.18
Oviposition days	50.61±0.93	49.0±0.85	40.7±1.16	47.2±0.80
Minimum number of eggs/batch	6.8±0.54	6.8±0.62	6.8±0.46	6.7±0.34
Maximum number of eggs/batch	20.4±0.44	18.3±0.42	15.6±0.7	18.3±0.41
Oviposition index	0.057±0.002	0.054±0.003	0.050±0.003	0.054±0.002
Post-oviposition days	13.84±0.42	14.80±0.98	14.9±0.68	14.4±0.37
Fecundity (eggs/ female)	45.5±0.89	45.1±0.82	39.2±0.82	43.5±0.55
Hatchability (%)	97.4±0.83	95.2±0.90	89.5±2.0	94.4±0.80

Table 13. Nymphal survival (%) and reproductive parameters of *R. longifrons* on *D. cingulatus* (n= 71,107 and 58 for three generations)

Parameter	First generation	Second generation	Third generation	Mean
Nymphal survival (%)	57.7±4.2	39.2±3.5	41.3±5.1	45.3±4.1
Male longevity (days)	60.7±1.25	60.4±1.13	60.1±0.64	60.4±0.64
Female longevity (days)	70.1±0.92	66.1±0.58	65.3±1.10	67.9±0.54
Sex ratio (Male: Female)	1:0.73	1:0.52	1:0.45	1:0.59
Pre-oviposition days	9.8±0.66	11.2±1.19	11.6±0.23	10.6±0.20
Oviposition days	38.8±0.72	36.8±0.007	35.7±1.2	37.6±0.52
Minimum number of eggs/batch	6.5±0.61	5.5±0.83	5.3±0.64	6.0±0.28
Maximum number of eggs/batch	14.3±1.00	9.8±0.58	10.2±0.75	12.0±0.45
Oviposition index	0.056±0.002	0.050±0.002	0.047±0.004	0.052±0.001
Post-oviposition days	15.2±0.65	16.0±0.66	16.8±0.63	15.7±0.41
Fecundity (eggs/ female)	34.6±1.60	30.0±1.11	30.3±0.86	32.6±0.63
Hatchability (%)	94.9±1.14	94.1±1.50	92.1±2.46	93.8±0.74

Table 14. Nymphal survival (%) and reproductive parameters of *R. longifrons* on *P. solenopsis* (n= 81, 74 and 50 for three generations)

Parameter	First generation	Second generation	Third generation	Mean for all generations
Nymphal survival (%)	37.0±5.2	29.7±4.1	10.0±4.3	27.8±3.9
Male longevity (days)	55.3±1.93	59.8±1.18	-	57.6±0.98
Female longevity (days)	65.6±0.58	65.2±1.41	-	65.4±0.45
Sex ratio (Male: Female)	1:0.70	1:0.45	-	1:0.56
Pre-oviposition days	10.0±0.46	11.8±0.86	-	10.6±0.30
Oviposition days	38.7±1.18	37.2±1.16	-	38.2±0.73
Minimum number of eggs/batch	5.0±0.55	4.0±0.69	-	4.8±0.39
Maximum number of eggs/batch	10.8±0.79	8.8±0.61	-	10.2±0.46
Oviposition index	0.048±0.003	0.045±0.003	-	0.047±0.002
Post-oviposition days	16.8±0.63	16.2±1.0	-	15.9±0.58
Fecundity (eggs/ female)	32.0±0.69	27.8±1.13	-	30.6±0.64
Hatchability (%)	93.6±1.29	82.8±2.80	-	90.1±1.56

Table 15. Nymphal survival (%) and reproductive parameters of *R. longifrons* on *A. gossypii* (n= 73 and 94 two generations)

Parameter	First generation	Second generation	Mean
Nymphal survival (%)	86.3±3.6	19.1±4.5	61.4±2.7
Male longevity (days)	52.7±1.91.	58.8±1.25	55.1±0.82
Female longevity (days)	63.4±0.31	62.3±0.26	63.0±0.42
Sex ratio (Male: Female)	1:0.38	1:0.33	1:0.37
Pre-oviposition days	10.0±1.41	13.5±0.64	10.8±0.43
Oviposition days	35.1±0.77	30.0±0.63	34.0±0.73
Minimum number of eggs/batch	4.8±0.69	3.6±0.92	4.6±0.50
Maximum number of eggs/batch	9.6±0.87	8.5±0.55	9.39±0.37
Oviposition index	0.045±0.003	0.042±0.005	0.045±0.002
Post-oviposition days	18.1±0.54	18.8±0.47	18.3±0.43
Fecundity (eggs/ female)	30.9±1.60	25.7±1.10	29.7±0.89
Hatchability (%)	86.8±1.80	83.3±2.30	86.1±1.43

when fed with *H. armigera* (F=2.668; df 19, 40; P< 0.05) when compared with other preys like *D. cingulatus* (F=1.738; df 20, 40; P< 0.05), *A. gossypii* (F=0.960; df 9, 18; P< 0.05) and *P. solenopsis* (F=1.811; df 9, 21; P< 0.05).

2.5.5. Life table

The life table statistics of *R. longifrons* on four cotton pests reveal that the net reproductive rate (R_o) was lower than that of the gross reproductive rate for all the pests. For instance, the net reproductive rate (R_o) on *H. armigera* was higher (115.0) than on *P. solenopsis* (76.0), *D. cingulatus* (68.3) and *A. gossypii* (43.8) (Table 16). Similarly, the rate of natural increase on *H. armigera* was higher than *D. cingulatus, A. gossypii* and *P. solenopsis*. The finite rate of increase (λ) and weekly multiplication rate (WM) were higher (1.11, 1.10, and 1.083 days and 2.7, 1.95 and 1.74 with their doubling time of 6.30, 6.93 and 8.7 days for F1,F2 and F3 generation, respectively) in *H. armigera* fed predator. The intrinsic rate of increase (r_m) of the *R. longifrons* has highest value (0.11, 0.10 and 0.080 female progeny/female/ day) when predator was provided with *H. armigera* and then decreases to 0.057 when predatory reduviid provided with *A. gossypii*. The finite rate of increase e^{rm}, means the population multiplication of predatory bug in a unit of time. The present results show that e^{rm} ranges between 1.06 to 1.11, according to the number of preys offered to the *R. longifrons* (Table 16).

2.5.6. Macro molecular profile of cotton pests

Among the four cotton pests analysed, the larvae of *H. armigera* (13.1 mg/100 mg) contain more amount of total body protein followed by nymphs of *D. cingulatus* (10.8 mg/100 mg) and *P. solenopsis* (4.50 mg/100 mg) adult (Fig. 2). The total body carbohydrates content was higher in the adult of *P. solenopsis* (22.9 mg/100 mg) followed

Table 16. Life table parameters of *R. longifrons* provided with *C. cephalonica, H. armigera, D. cingulatus, P. solenopsis* and *A. gossypii* (Mean value for F1, F2 and F3)

Parameters	*C. cephalonica*	*H. armigera*	*D. cingulatus*	*P. solenopsis*	*A. gossypii*
Gross reproductive rate	133.8±2.21	149.4±3.0	115.9±8.6	131.0±2.7	135.1±3.2
Net reproductive rate (Ro)	86.8±24.7	115.0±31.1	68.3±12.9	76.7±19.8	43.8±15.39
Length of generation Tc	46.3±0.87	45.9±1.47	56.7±1.20	62.6±25.6	60.6±1.75
Innate capacity for increase (r_c)	0.094±0.007	0.09±0.008	0.073±0.001	0.068±0.002	0.060±0.004
Intrinsic rate of increases Corrected (r_m)	0.090±0.006	0.09±0.007	0.073±0.002	0.71±0.002	0.062±0.005
Finite rate of increase (λ)	1.09±0.005	1.09±0.001	1.076±0.003	1.07±0.002	1.06±0.006
Weekly multiplication of population	1.86±0.06	1.69±0.31	1.67±0.03	1.63±0.02	1.54±0.55
Doubling time(in days)	7.71±0.53	7.28±0.70	9.42±0.31	9.7±0.30	11.4±1.27
Hypothetical female in next generation	8765.3±48.33	15220.3±75.53	5007±19.29	6276.5±30.43	2162±13.51

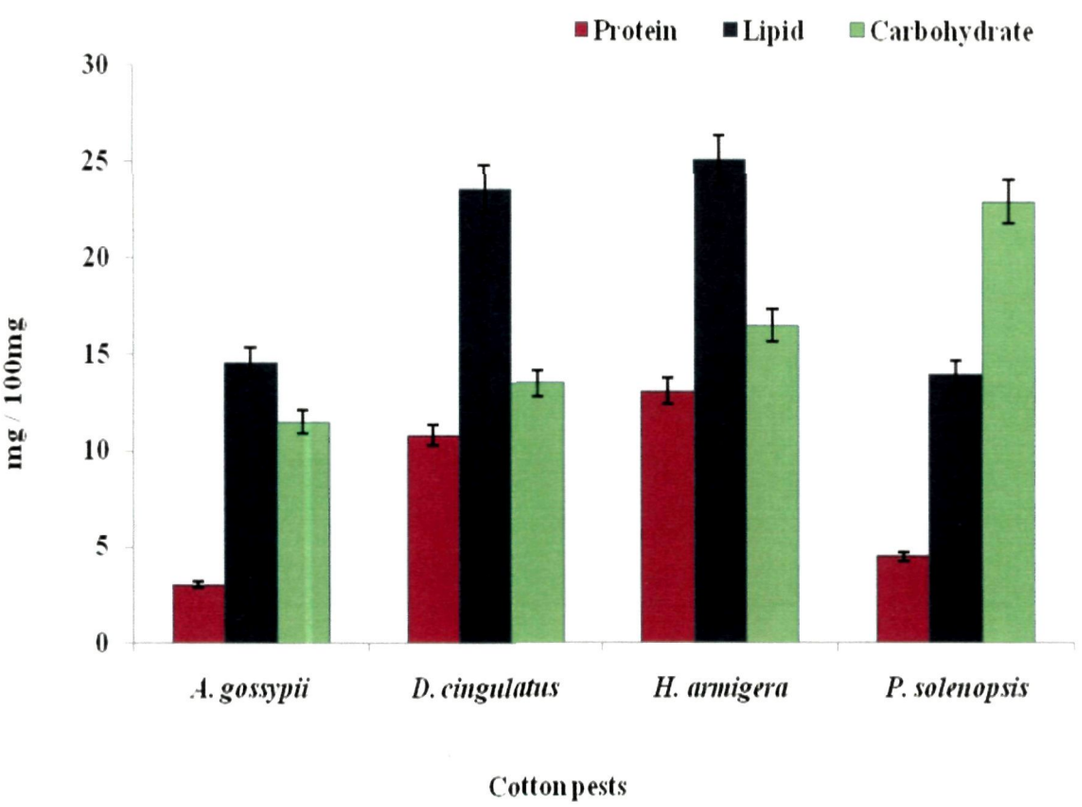

Figure 2. Biochemical analysis of total body protein, lipid and carbohydrate in selected cotton pests (mg/100mg)

by larvae of *H. armigera* (16.5 mg/100 mg) and nymphs of *D. cingulatus* (13.5 mg/100 mg). The lipid content was also higher amount in *H. armigera* (25.1 mg/100 mg).

2.6. DISCUSSION

Reduviids are generalist predators, but some species are known to exhibit preferences for particular prey when they are simultaneously offered a number of different species. For example, in the presence of mixed pests, *R. marginatus* attacks termites first, then grasshoppers and finally ants (says Ambrose *et al.,* 1990b). Similarly *R. longifrons* shows preference against *Spodoptera litura* larvae than its factitious host, *C. cephalonica* (Ravi, 2004). In the present study *R. longifrons* shows higher growth when the predator is reared with *H. armigera* rather than other cotton pests tested and also with a factitious host *C. cephalonica.*

There are several factors that determine the prey suitability for insect predators, which can be divided into (i) nutritional factors (quality and quantity of micro and macro molecules) and (ii) non-nutritional factors (prey texture, movement, agility, type of mouth- parts, cuticular nature, presence and absence of hairs over the body, flying capacity etc.). For a prey species to be suitable, it must provide all nutritionally important factors as proteins, carbohydrates, lipids, vitamins and minerals in a balanced proportion and concentration to meet a predator's metabolic requirements (House, 1966, 1977; Sahayaraj *et al.* 2004). Analysis of macromolecular profile of preys reveals that *H. armigera* has more amounts of total body protein and lipid reflecting higher growth and reproduction of the predator.

The influence of different prey on the biology of reduviids such as *Acanthaspis pedestris* (Ambrose and Subburasu, 1988), *Neohaematorrhophus therasii* (Sahayaraj and Ambrose, 1994a), *Rhynocoris kumarii* (Ambrose and Rani, 1991), *Rhynocoris marginatus* (Ambrose *et al.* 1990b; George *et al.,* 2002; Sahayaraj and Sathiamoorthi, 2002; Sahayaraj *et al.* 2004), *Cydnocoris gilvus* (Venkatesan *et al.,*1997), *Rhynocoris longifrons* (Kumar, 1993; Ambrose *et al.,* 2003; Ravi, 2004) were available in literature. It is still assumed by some authors (DeBach and Rosen, 1991; Hoy, 1994) that an effective biocontrol agent should be highly prey or host specific in order to be considered near as good/viable biological control agent.

Rhynocoris longifrons has been recorded from various verities of cotton (SVPR 2 and 4, MCU 5 and 7, Local and Uganda) agro-ecosystem where there are lot of possibilities to feed wide range of prey which belongs to various insects order. Such information is imperative to utilize this predator in cotton pest management. This study is an initial step to generate such information. To find out the host preference, we offered three hemipteran and one lepidopteran insect which are commonly distributed in the cotton agro-ecosystem. All these preys were provided to *R. longifrons* and its various biology traits were recorded.

The longest nymphal developmental period, lower fecundity and hatchability observed in *R. longifrons* reared on *A. gossypii* and *P. solenopsis* might be due to very smaller size and the presence of a white coating in the latter prey respectively. It is widely reported that unsuitable food can extend the pre-imaginal development of reduviid and decrease the survival, fecundity and longevity of the adults (Ambrose *et al.,* 1990b, Sahayaraj and Sathiamoorthi, 2002; Sahayaraj *et al.* 2004). Ambrose *et al.* (2003) for the first time studied the biology of this predator using a *C. cephalonica, H. armigera* (the stage not mentioned) offered, *Odentotermes obesus,* and *Clavigralla gibbosa* (Spinola). They recorded a shorter nymphal developmental period of 51 days in *H. armigera* (Ravi,

2004) or 53 days in *C. cephalonica* in contrast to our observation (36, 41 and 42 days for first, second and third generation, respectively) for *C. cephalonica*. This indicates that provision of predator stage dependant prey offered is crucial to minimize the predator nymphal stadial period. In this study we evaluated the pre-imaginal developmental period as well as adult longevity and fecundity of *R. longifrons* provided with different species of prey. The prey also increased/ enhanced the survival rate (60%). Maximum nymphal survival was recorded while the predator was reared with *A. gossypii*, as against *H. armigera* recorded by Ambrose *et al.* (2003). The presence study reveals that *H. armigera* was found to be the highly suitable prey for *R. longifrons* due to the following reasons:

i) faster nymphal development,

ii) higher nymphal survival,

iii) higher fecundity and hatchability,

iv) higher net and gross reproductivity and intrinsic rate and

v) shorter population doubling time.

Abnormal hatching caused more nymphal mortality. Earlier, it was reported that sex ratio was female-biased (Ambrose *et al.*, 2003) for *R. longifrons*. However, sex ratio was altering in F2 generation. The male and female adult longevity of *R. longifrons* fed with *H. armigera* was shorter than that with other pests. *Corcyra cephalonica* fed male and female lived 75 to 85 and 75 to 90 days respectively (Ambrose *et al.,* 2007b). Similar observation was recorded by Ambrose *et al.* (2003) while the predator was fed by different hosts. Further, they reported that the longer adult longevity was reported in *H. armigera* reared predator followed by *C. gibbosa* and *O. obesus*. Sahayaraj *et al.* (2004) reported that the higher fecundity of *R. marginatus* was influenced by the provision of *H. armigera* which has higher macromolecular content as observed

here. However, the mean egg production (43 eggs/ female) was lower than that observed by Ambrose *et al.* (2003).

The life table statistics of *R. longifrons* on three pests reveals that the net reproductive rate was lesser than that the gross reproductive rate. This is in accordance with the results of George *et al.* (1998a). They attribute such finding to the sharp decline in the survivorship value of parent females. The net reproductive rate observed in *R. longifrons* is comparatively higher (96.54) than that of other horpactorine reduviids like *Sycanus collaris* Fabricius (R_o = 30.46) (George *et al.* 1998b), *Rhynocoris marginatus* (R_o = 27.90) (George, 2000) and *Sphedanolestes minusculus* Bergroth (R_o = 79.35) (Ambrose *et al.* 2006) and also *Rhynocoris longifrons* (R_o = 46.606 and 115.0) (Ambrose *et al.*, 2007b; Ganesh Kumar, 2011). However, an opposite trend was recorded for *Rhynocoris marginatus* (R_o = 181.979) (Sahayaraj *et al.*, 2004) and *R. fuscipes* (R_o = 55.01) (Sahayaraj and Selvaraj, 2003). The value of true intrinsic rate of increase was slightly higher than the capacity for increase in number, as expected for insects having overlapping generations (Southwood, 1978). The value of true intrinsic rate of increase in the present study was higher (0.11) than those reported for the reduviids such as *R. longifrons* (0.042) (Ganesh Kumar, 2011), *Cydnocoris gilvus* (0.060) (Venkatesan *et al.*, 1997), *Acanthaspis siva* (0.063) (George *et al.*, 1998a), *R. fuscipes* (0.041 and 0.053) (George, 2000; Sahayaraj and Selvaraj, 2003) and *R. marginatus* (0.070) (Sahayaraj *et al.*, 2004). The daily finite rate of increase (λ) ranges from 1.06/ female/ day (*A.* gossypii) to 1.09/ female/day (*C. cephalonica* and *H. armigera*). At this rate the population of *R. longifrons* is expected to double every 7.71 (for *C. cephalonica*) to 11.4 days (for *A. gossypii*). These statistical values are high when compared to the data of Ambrose *et al.* (2007b) for the same reduviid. This might be due to differences in the rate of fecundity and the proportion of survival of female insects. Doubling time for populations of *R.*

longifrons was lower for *D. cingulatus* than for *P. solenopsis* and *A. gossypii* as observed in another reduviid, *Rhynocoris fuscipes* (George, 2000).

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). The reproductive potential such as fecundity and percent hatchability of insect predators are determined by the nutrient composition of the prey species (Fuller, 1988). The egg laying potential, hatching success and longevity of adults were maximum on *H. armigera* than on other pests due to higher content of primary nutrients. Reduced level of fecundity and longevity of *Cydnocoris gilvus* Burm was observed on *Odontotermes obesus* than on *Spodoptera litura* (Venkatesan *et al.,* 1997). Shortest nymphal developmental period, maximum fecundity, hatchability, male and female adult longevity, gross and net reproductive rate, doubling time and intrinsic rate of increase might be due to the presence of high total protein and lipid recorded in *H. armigera* larvae. This shows that the reduviids appear to require animal protein for development (Taylor and Schmidt, 1996).

The only nymphal of *R. longifrons* did not develop during third generation due to the failure of moulting in first instar. Though, other cotton pests are in favour for the life traits of this predator, *A. gossypii* does not proceeds to its third generation. This might be due to:

i) the small body size which needs to consume enormous number of preys by spending more energy,

ii) low amount of nutrition (carbohydrate, protein and lipid),

iii) feeding stress of aphids for previous two generations and

iv) toxic alleochemicals produced by the aphis (Francis *et al.,* 2001).

Edwards (1962b) found that the reduviid required at least one meal of prey for moulting to occur. Moreover, *R. longifrons* fed with *A. gossypii* does not proceed to the third generation due to the lack/ unsupporting of nutrients part in the prey as reported by Taylor and Schmidt (1996). Furthermore though *A. gossypii* was available in plenty at the experimental area, the predator did not approached them which lead to cannibalism.

CONCLUSION

1. Both nymphs and adults feed all the four cotton pests continuously along with its factitious host *Corcyra cephalonica.*
2. In this study among the four cotton preys reared *R. longifrons* showed maximum survival and fecundity, hatchability on *H. armigera.*
3. From these results we concluded *H. armigera* is a suitable prey for laboratory rearing of *R. longifrons* and it can be used as a biological control agent against *H. armigera* followed by other cotton pests.
4. Macromolecule profile (carbohydrate, protein, and lipid) of pests revealed that lepidopteran pest *H. armigera* has higher quantity of protein and lipids. This too may be responsible for enhancing the reproduction of *R. longifrons.* However, carbohydrate was higher in hemipteran pest *P. solenopsis.*

CHAPTER - 3

3.1. ABSTRACT

The use of natural enemies in the field of agriculture is of great importance because it is gradually replacing the use of chemical pesticides. As an initial step, *ie* the stage preference, prey preference, biological control potential of *Rhynocoris longifrons* (Stål) was evaluated against four cotton pests such as *Aphis gossypii* (Glover), *Dysdercus cingulatus* (Fab.), *Helicoverpa armigera* (Hubner) and *Phenacoccus solenopsis* (Tinsley) under laboratory condition. The stage preference was studied using nymphs and adults of *D. cingulatus* (second, third, fourth, fifth and adult), *H. armigera* (second, third, fourth and fifth) and *P. solenopsis* (first, second, third and adult). Third, fourth and fifth stadium of *R. longifrons* preferred second instar *D. cingulatus* nymphs and *H. armigera* larvae, whereas, adults preferred third instar of *D. cingulatus* nymphs and of *H. armigera* larvae. All the tested nymphal instar and adults of *R. longifrons* preferred *P. solenopsis* adults alone. Host preference of visual method of the predator life stages was higher for *H. armigera* than for *D. cingulatus*, *P. solenopsis* and *A. gossypii*. Prey preference using kairomone shows that nymphal instar and adults of *R. longifrons* significantly approached *H. armigera* crude kairomone extracts (CKE) rather than *D. cingulatus, P. solenopsis* and *A. gossypii* CKE. The Gas chromatography-Mass spectrometry (GC-MS) analysis of the prey (CKE) reveals that *H. armigera, D. cingulatus, P. solenopsis* and *A. gossypii* have more amounts of celidoniol (21.99%), dodicamethyl (72.06%), dodecanoicacid (12.80%) and dotriacontane (14.54%), respectively. These chemicals are responsible for the preference of reduviid against specific pests. Life stages of *R. longifrons* offered with different densities of preferred stages of *D. cingulatus* (1, 2, 4, 8 and 16), *H. armigera* (2, 4, 6, 8 and 10), *P. solenopsis* (2, 4, 6, 8 and 10) and *A. gossypii* (5, 10, 20, 30 and 40).

The *R. longifrons* adult consumed more number of preys of higher prey densities. Biological control potential of *R. longifrons* on these pests shows that the number of preys killed, searching time, handling time depended upon the prey densities and hence this predator can be utilized for the management of cotton pests.

Key words: Bioassay, crude kairomone, functional response, GC-MS, host preference, olfactometer, stage preference

3.2. INTRODUCTION AND REVIEW OF LITERATURE

Cotton is an important crop grown in many parts of the world. One of the major obstacles in cotton cultivation is insect pests infestation. Red cotton bug, *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae) (Schaefer and Ahmed, 1987; Kohino and Bui Thi, 2004; Tanu Sharma, 2010), mealy bug *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) (Williams and Granare de Willink, 1992; Saeed *et al.,* 2007; Arif *et al.,* 2009; Laxman *et al.* 2009; Patel *et al.,* 2010; Rashid *et al.,*2012; Sahito *et al.,* 2011), *Aphis gossypii* Glover (Homoptera: Aphididae) (Ghabeish 2010; Rostami *et al.,* 2012) and *Helicoverpa armigera* Hubner (Grundy and Maelzer, 2000; Grundy, 2007; Downes and Mahon, 2012) infest cotton and cause yield loss (Reed and Pawar, 1982; Kabissa, 1989; Venugopalrao *et al.,* 1993; Ramalho *et al.,* 2012; Yenagi *et al* 2012). These pests are difficult to control by insecticides, because of the highly mobile nature of *D. cingulatus* (Nyamasyo and Karel, 1982), white lipid layer coating of *P. solenopsis* (Arif *et al.,* 2009; Mahalakshmi *et al.,* 2010; Vennila *et al.,* 2010; Ahmed *et al.,* 2012) non contact nature of *A. gossypii* (Boiteau and Osborn, 1997; Herron *et al.,* 2001; Wang *et al.,* 2002) and *H. armigera* (Armes *et al.,* 1992; Forrester *et al.,* 1993; Ahmad *et al.,* 1995; Kranthi, 2007; Leethial and Regupathy, 2007). Therefore, the use of natural

enemies to manage these pests should be considered because natural enemies can be used not only in cultivated cotton fields, but also in vegetative stands containing alternative host plants of these pests (Kaplan and Eubanks, 2002; Ma *et al.,* 2006; Wang *et al.,* 2010).

In biological control programme, the reduviid predator has been widely used worldwide under laboratory (Murdoch and Oaten, 1975; Ghosh and Chandra, 2011) and field conditions (Altieri, 1995; Ravi, 2004; Sahayaraj and Martin, 2003; Sahayaraj and Ravi, 2007b; Balasubramanian, 2008). Reduviids have been recorded as important natural enemies in suppressing the several pests (Ambrose *et al.*, 2008a, 2008b, 2009a; Chandral and Sinazer, 2011; Alexandre *et al.,* 2009) especially Orthpteran (O'Neill *et al.,* 2008), Hemipteran (Ambrose and Kumarasami, 1990; Sahayaraj and Ambrose, 1993; Sahayaraj and Ambrose, 1995; Sahayaraj and Ambrose, 1997) and Lepidopteran (Sahayaraj, 1994; Grundy and Maelzer, 2000, 2002; Sahayaraj, 2000; Sahayaraj *et al.,* 2004; Sahayaraj, 2006; Grundy, 2007; Sahayaraj, 2007) insects. Reduviids are generalized predators. They exhibit a certain degree of host specificity (Ambrose, 1999; Sahayaraj, 2007). The Reduviidae is the largest family of predaceous land Heteroptera and many of its members are found to be potential predators of a number of insect pests (Ambrose, 1999, 2000, 2003).

Generalist insect predators like assassin bugs are often expected to be important for controlling pests in agricultural system (Altieri, 1995). The importance of the predators in the suppression of insect pests is coming into closer focus, based on modern ecological investigations and experiments (Ables, 1978; Schaefer and Ahmed, 1987; Ambrose, 2002; Sahayaraj, 2002b; Sahayaraj *et al.,* 2003b). The intuitive predation is that increasing the density of generalist predators in a crop should increase predation on pests (Chang and Kareiva, 1999; Ambrose and Nagarajan, 2010; Nagarajan *et. al.,* 2010). Since they are ploy phagous they may not be effective against specific pests, but they are

valuable predators in situations where a variety of insect pests occur. To understand predator-prey interactions has been the purpose of numerous studies, especially those related to predator use in biological control in agro-ecosystems.

3.2.1. Stage and host preference

The reduviids are abundant, occur worldwide and are highly successful predators and they play a vital role in the biocontrol of insect pests. Moreover, they exploit the most adverse microhabitats of every ecosystem predating on a wide variety of insect pests. Since they are ploy phagous predators they may not be useful as predators on specific pests but they are valuable predators in situations where a variety of insect pests occur. However, they exhibit a certain amount of host as well as stage preferences. Stage preference of reduviids against chosen pest insects (Joseph, 1959; Holling, 1966; McMahan, 1982; Ambrose, 1995; Sahayaraj and Sivakumar, 1995; Ambrose, 1999; Sahayaraj, 1999; Das and Ambrose, 2008) were available in the literature. Several researchers have investigated the stage and host preferences of assassin bug and confirmed that life stage of reduviid predators also prefers a particular stage of the prey (Sahayaraj and Ambrose, 1994b; Ambrose, 1995; Sahayaraj and Sivakumar, 1995; Ambrose *et al.,* 2009b). The reduviid exhibit a narrow range of host preference it is imperative to understand their host specificity before employing them in biological control warfare (Ambrose and Sahayaraj, 1993; Sahayaraj, 1995a). The stage and host preference of reduviids *A. pedestris R. marginatus, R. kumarii, R. fuscipes, S. versicolor, E. tibialis, C. brevipennis* were evaluated by (Kumaraswami, 1991; Sahayaraj, 1991; Sahayaraj and Ambrose, 1994b). However, stage proference of *R. longifrons* is not available in the literature, besides it has been distributed in various agro-ecosystems and preying on many economically important pests.

3.2.2. Kairomone

Biological control potential of *R. longifrons* against *H. armigera* (Ambrose *et al.*, 2003) has been found to elicit the host searching behaviour of this reduviid predator (Kumar and Ambrose, 1996; Claver *et al.*, 2002). The chemical which governs the prey and predator interaction are generally called infochemicals (Ananthakrishnan, 2002). It includes kairomones and allomones, which influence the prey-predator interaction (Ananthakrishnan, 2002). Several studies have been carried to find out role of kairomone of lepidopteran and hemipteran pests in eliciting a host searching behaviour of many generalist predators such as Pentatomidae (Yasuda, 1997), *Chrysopidae* (Bakthavatsalam *et.al.*, 2000), Anthocoridae (Tapia *et al.*, 2010; Uefune *et al.*, 2010) and Reduviidae (Sahayaraj and Paulraj, 2001c; Sahayaraj and Delma, 2004; Sahayaraj, 2008; Sujatha *et al.*, 2012) of Hemiptera. However, no studies have been carried out on the prey preference of *R. longifrons* life stages to the crude kairomone of *H. armigera*, *D. cingulatus*, *P. solenopsis* and *A. gossypii* identifying the compounds that elicit the prey preference response using olfactory response.

3.2.3. Functional response

One of the fundamental aspects of a predator-prey interaction is the relationship between prey density and predator consumption, to which Solomon (1949) attributed the term "**functional response**". According to Holling (1959, 1961), there are four basic types of functional response: I (linear), II (curvilinear), III (sigmoidal), and IV (dome-shaped). The responses of types I and II are found in most invertebrates, whereas type III is more common in vertebrates, although some arthropods can also show this response when their preferential prey is not available (Hassel *et al.*, 1977; Jervis and Kidd, 1996; Ambrose *et al.*, 2009b).

The functional response of *Rhynocoris* spp. such as *R. marginatus* (Ambrose and Kumaraswami, 1990; Sahayaraj, 1994; Ambrose *et al.,* 1996; Ambrose and Claver, 1996; Ambrose *et al.,* 2000; Sahayaraj, 2000; Sahayaraj *et al.,* 2003b; Claver and Ambrose, 2003; Ambrose *et al.,* 2010), *R. fuscipes* (Ambrose and Claver, 1997; Sahayaraj *et al.,* 2002a; Claver and Ambrose, 2002; Bibin *et al.,* 2010; Nagarajan *et al.,* 2010), *R. kumarii* (Ambrose *et al.,* 1997; Ambrose *et al.,*2008a; Sahayaraj and Asha, 2010), and *R. longifrons* (Kumar and Ambrose, 1996; Claver *et al.,*2002; Ravichndran *et al.,* 2003; Mitradev, 2011) were available in the literature. Few studies have been conducted on the functional response of *R. longifrons* but no detailed study has been carried out on the functional response and predatory rate of both nymphal instars and adults against *A. gossypii, D. cingulatus, H. armigera* and *P. solenopsis*cotton life stages.

The core objective of this study is to fully explore the biological control potential (functional response) of *R. longifrons* at each development stage, as this knowledge will provide important information for determining the stage of predator's release at specific stage because its unnecessary use may suppress other natural enemies in field via intraguild interactions (Roy and Wajnberg, 2008). The detailed objectives are as follows:

OBJECTIVES

1. To record prey stage preference and prey preference and the predatory potential (functional response) of *R. longifrons* on four economically important cotton pests such as *A. gossypii, D. cingulatus, H. armigera* and *P. solenopsis*cotton using visual methods.
2. To confirm the prey preference of this reduviid using preys crude kairomone using 'Y' shape glass olfactometer.
3. To document the chemical composition of the tested prey kairomone.

3.3. MATERIALS AND METHODS

3.3.1. Collection and maintenance of insects

Kindly see section 2.3.1 in page 13 for details about this aspect.

3.3.2. Stage preference

Stage preference of third, fourth, fifth and adult life stages of *R. longifrons* was evaluated with different life stages of *D. cingulatus* (5.1±0.1 mm, 7.3±0.13 mm, 9.2±0.13 mm, 11.4±0.23 mm and 16.2±0.69 mm for second (8.76±0.51 mg), third (11.6±0.12 mg), fourth (27.6±0.16 mg), fifth (46.2±0.23 mg) nymphal instars and adult (55 mg), respectively), *H. armigera* [(5.9±0.1mm, 9.0±0.3 mm, 14.8 mm and 19.9 mm for second (11.3±0.13 mg), third (23.4±0.10mg), fourth (52.1±0.20 mg) and fifth (77.2±0.21mg) instar larvae, respectively] and *P. solenopsis* (0.80±0.1 mm, 0.99±0.1 mm, 1.81±0.2 mm and 2.43±0.3 mm for first, second, third nymphal instars and adult (0.40±0.11 mg), respectively) separately by choice-based experiment. To study the stage preference, *D. cingulatus, H. armigera* and *P. solenopsis* life stages were introduced into separate petridish (9 cm diameter) containing fresh cotton leaves (SVPR 2) and 24 h pre-starved third stadium of *R. longifrons* was released into the petridishes, closed with the lid and the predatory behaviour was observed visually consecutively for 3 hr. Successfully captured, killed and consumed prey stage was recorded as preferred stage of the reduviid. Ten replications with 10 separate and same aged predators were used in the replications. Similar procedure was followed in fourth and fifth stadium and also adult predators.

3.3.3. Host preference by visual method

Once the stage preference, host preference studies were conducted by introducing the preferred stages of *D. cingulatus, H. armigera P. solenopsis* and *A. gossypii* (adults) were introduced into petridish (9 cm diameter and 2 cm height) containing fresh cotton leaf (SVPR 2). The pests were allowed to move undisturbed for 10 minutes, then third,

fourth and fifth nymphal instars and adult of *R. longifrons* were released into the petridishes and the successfully captured hosts were recorded visually. The experiment was replicated ten times.

3.3.4. Host preference by using prey kairomone

3.3.4.1. Crude Kairomone (CK) extraction

The crude kairomone was extracted from preferred life stages of *H. armigera* (HA) (second and third instar larvae), *D. cingulatus* (DC) (second and third nymphal instars), *P. solenopsis* (PS) (adult stage) and *A. gossypii* (AG) (all stages) using hexane + acetone mixture (1:2) using by (Yasuda, 1997). For this purpose, 50, 100, 300 and more than 500 live animals of *H. armigera, D. cingulatus, P. solenopsis* and *A. gossypii*, respectively were introduced into a stopper bottle (250 ml) to which 100 ml of 1:2 hexane + acetone mixture was added and the mixture was allowed to stand for 2 hrs at room temperature using shaker (REMI, India) at 240 rpm. The crude extract was filtered through Whatman No.1 filter paper, then the solvent was evaporated under room temperature and the residue was considered as crude kairomone extract (CKE). Crude kairomone extract was taken in a separating funnel (125 ml) and dissolved with 100 ml of diethyl ether. The hexane and acetone mixture was separated and the impurities were discarded gently. Fifty ml of double distilled water was added into the mixture and vigorously agitated for 30-60 seconds. The lower portion of the hexane and acetone mixture which has a yellow coloured crude kairomone noticed at the bottom was removed and collected in a glass bottle (10 ml). Excess amount of hexane and acetone was evaporated overnight. The residue dissolved with hexane (10 ml) was considered as prey crude kairomone (CK).

3.3.4.2. Bioassay

Crude kairomone from each prey species was extracted by hexane and acetone mixture using the method by Yasuda (1997) and Sahayaraj (2008). Two pieces (3cm diameter) of filter paper (Whatmann No. 1) was impregnated with 50 µl of crude kairomone. The two pieces of filter paper were arranged in 'Y' shape olfactometer (Plate 2). The following combinations of prey kairomone were used for this study: DC+HA (I), DC+PS (II), DC+AG (III), HA+PS (IV), HA+AG (V), PS+AG (VI). Subsequently three 24 hurs pre-starved predators were selected for a replication and introduced into the releasing chamber and the lid gently closed. This procedure was used for third, fourth, fifth nymphal instar and adult stages of predator. The piece of filter paper with kairomone first approached by the predator was recorded visually for 10 min. Five replication were tested and the preferred prey kairomone combination was considered as the choice of the majority of the tested individuals. From the results the percentage of predators preferring the kairomone of a particular pest was calculated (Sahayaraj, 2008). The kairomone preference was studied using the formula,

$$\frac{\text{No. of predators responding to the specific /particular prey kairomone}}{\text{Total no. of predators released}} \times 100$$

The predatory behaviour was observed in terms of approaching time.

3.3.4.3. GC- MS analysis of preys crude kairomone

GC- MS analysis was conducted with GC-MS shemadzu qp 2010 plus Japan using the column CBP 5 (25m×0.25 1.0×0.2µm film thickness). The prey extract was concentrated to 0.5 ml and 0.1µl was injected into the column. The column temperature was first set at 70°C with 5 min standing time and programmed to increase to 260°C at the rate of 10°C/ min. The final temperature of 260°C was held for 15 min. The carrier gas

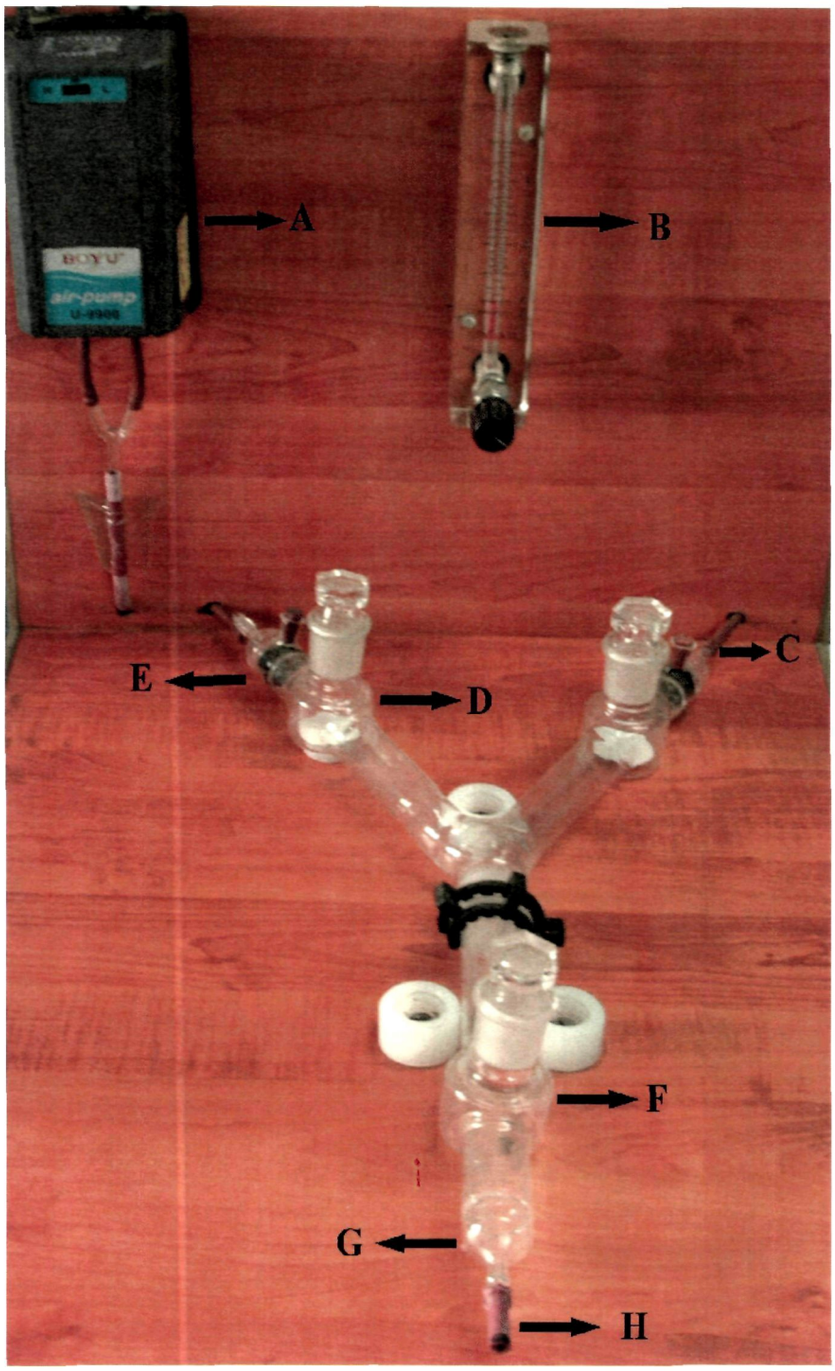

Plate 2. 'Y' shaped olfactometer used for the analysis of whole body crude kairomones preference by a reduviid predator *R. longifrons* third, fourth and fifth stadium nymphs and adults (male and female). Key: A) Air pump, B) Airflow meter, C) Air inlet, D) Experiment chamber E) Activated charcoal filter, F) Releasing chamber, G) Glass filter and H) Air outlet

was helium at a column pressure of 70 kpa with a flow rate at 1 ml/min. The compound present in the extract was identified by comparing the extract with the compounds from WILEY library based on their retention time and mass spectra.

3.3.5. Functional response

Five densities of different cotton pests such as *D. cingulatus* (1, 2, 4, 8 and 16/ predator), *H. armigera* (2, 4, 6, 8 and 10/predator), *P. solenopsis* (2, 4, 6, 8 and 10/ predator) and *A. gossypii* (5, 10, 20, 30 and 40/predator) were introduced into a petriplate (9 cm diameter × 2 cm height) separately (each petriplate containing different densities) and allowed to move undisturbed for 10 min (a SVPR 2 cotton leaf was placed at the bottom of the petriplate). Life stages of predator was selected (24 hrs starved) and introduced into preys contain petriplate. The predator subsequently introduced singly to the setup and sequential events of feeding was recorded visually until the entire sequence of predatory behavioural elements had been completed. The sequential pattern of predatory behaviour (Sahayaraj, 2007) *i.e.* i) searching time, ii) handling time, iii) number of prey killed was recorded for 3 hrs by visual observation. The number of ingested or killed and remaining preys was recorded after 24 h (T). The predator search efficacy was calculated from the number of dead (Na) and offered prey density (N) through the formula,

$$E = Na/N \quad \text{---------- (1)}$$

The handling time (T_h) was estimated through the Holling 'disc' equation. From the estimated handling time, we calculated total handling time (T_h total), searching time (TS), attack ratio (a') and maximum number of ingested or killed prey/ predator (Na max). The calculation was done using the following formula (modified Holling, 1959)

$$Th_{total} = Th \times Na \quad \text{---------- (2)}$$

$Ts = T - (Th_{total})$ ---------- (3)

$a' = Na/ (Na \times Ts)$ -------- (4)

$Na_{max} = T/Th$ ---------- (5)

3.4. STATISTICAL ANALYSIS

The comparative mean values were subjected to chi-square (host preference) and ANOVA (prey kairomone preference) by SPSS version 11.5 with significance expressed at 5% level. The data for predator consumed on *D. cingulatus, P. solenopsis, A. gossypii* and *H. armigera* were analysed through linear regression (SPSS 11.5). The data of stage and hostspreference were subjected to binomial distribution using MS-excel. The binomial distribution values (BDV) are expressed at 5% leavel.

3.5. RESULTS

3.5.1. Stage preference

The third, fourth and fifth nymphal instars of *R. longifrons* preferred second instar *D. cingulatus* (0.00008, 0.0009648 and 0.000006 BDV) nymphs and *H. armigera* larvae (0.000006, 0.010475 and 0.00096 BDV), whereas adult predator preferred third stadium of *D. cingulatus* (0.0000002) and *H. armigera* (0.0009648) (Fig. 3A and 3C). However, nymphal instars (0.000006, 0.0000002 and 0.000964 BDV) and adult (0.000006 BDV) of *R .longifrons* selected only adult stages of *P. solenopsis* (Fig. 3B). Hence the result indicated that size of the prey has a major role in the predation build up of a predator.

3.5.2 Host preference

3.5.2.1. Host preference by visual method

When the predators were released into the Petri dish, *R. longifrons* showed more response to *H. armigera* ($P<0.05$) by chi-square as compared to that of *D. cingulatus*, *P.*

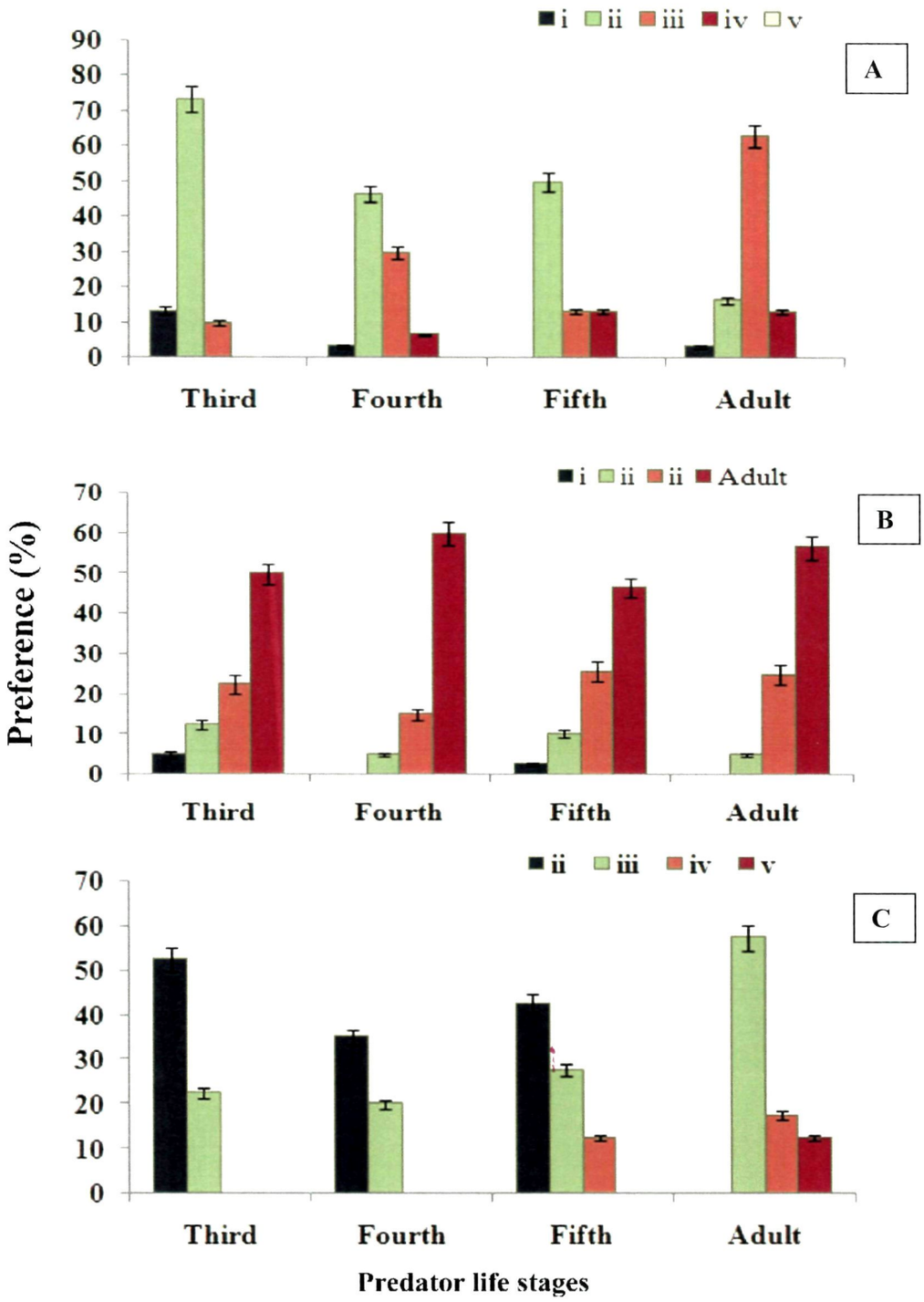

Figure 3. Stage preference of *R. longifrons* third, fourth, fifth nymphal instars and adult on *D. cingulatus* (A) (ii,iii,iv,v and adult), *P. solenopsis* (B) (i,ii,iii and adult) and *H. armigera* (C) (ii,iii,iv and v).

solenopsis and *A. gossypii.* Chi-square for all the nymphal instars (0.010475) and adult (0.0009648) preference were significant (P<0.05) for *H. armigera,* whereas both third, and fourth nymphal instar of *R. longifrons* showed maximum (P<0.05) response to *D. cingulatus* (Table 17).

3.5.2.2. Prey crude kairomone preference

The response of the *R. longifrons* against various preys crude kairomone is shown in table 18 and the cumulative data is represented in figure 4. The response was higher in third, fourth, fifth and adult stages of *R. longifrons* for *H. armigera* crude kairomone extract (CKE) when compared to *D. cingulatus, P. solenopsis* and *A. gossypii* (Fig. 4). However, individual non- choice test shows that the significant difference was observed in third instar *P. solenopsis* to *A. gossypii* combination (t= 3.13; df= 3; P< 0.05) and *H. armigera* as against *A. gossypii* combination (t= 0.775; df= 3; P< 0.05) which was significant in fourth instar. The fifth instar of *R. longifrons* also showed preference on *D. cingulatus* over *A. gossypii* (t= 3.162; df= 4; P < 0.05) combination. A similar statistical significance was also observed in *H. armigera – A. gossypii* combination. Adult predator preferred *D. cingulatus* and *H. armigera* (t= 2.44; df= 4; P< 0.05) over *P. solenopsis* (Table 19). The cumulative approaching time of *R. longifrons* towards the odour source of *A. gossypii, D. cingulatus, H. armigera* and *P. solenopsis* are presented in Table 18. Results reveal that third and fifth stadium and adult predator invariably took less time to approach *A. gossypii* odour impregnated filter paper.

3.5.2.3. GC- MS analysis of prey crude kairomone

Figure 5 shows that the GC-MS of crude kairomone of *H. armigera, D. cingulatus, P. solenopsis* and *A. gossypii* and their chemical compositions presented along with their percentage of area (Table 20). The predominant bioactive compounds identified

Table 17. Host preference of *R. longifrons* on four cotton pests, *A. gossypii, D. cingulatus, H. armigera* and *P. solenopsis* life stages

Parameters	**Stage of predator**			
	III	**IV**	**V**	**Adult**
	Aphis gossypii			
Positive choice	1	2	0	0
Negative choice	14	13	15	15
χ^2	11.26	8.06	15	15
Significant	P>0.05	P>0.05	P>0.05	P>0.05
	Dysdercus cingulatus			
Positive choice	6	4	2	3
Negative choice	9	11	13	12
χ^2	0.60	3.26	8.06	5.40
Significant	P<0.05	P<0.05	P>0.05	P>0.05
	Helicoverpa armigera			
Positive choice	6	6	7	10
Negative choice	9	9	8	5
χ^2	0.60	0.60	0.06	1.66
Significant	P<0.05	P<0.05	P<0.05	P<0.05
	Phenacoccus solenopsis			
Positive choice	2	5	3	0
Negative choice	13	10	12	15
χ^2	8.06	1.66	5.40	15
Significant	P>0.05	P<0.05	P>0.05	P>0.05

* Significant at P< 0.05 level

Table 18. Cumulative mean (± standard error) time (min) spent by the reduviid, *R. longifrons* to approach crude kairomones of *A. gossypii*, *D. cingulatus*, *H. armigera* and *P. solenopsis* in a Y-shape olfactometer.

Predator Life stage	Time taken to approach the prey whole body crude kairomone (min)			
	A. gossypii	*D. cingulatus*	*H. armigera*	*P. solenopsis*
Third stadium	1.02 ± 0.16^{a}	1.44 ± 0.16^{ab}	1.063 ± 0.11^{abc}	1.10 ± 1.16^{abcd}
Fourth stadium	1.26 ± 0.22^{a}	1.69 ± 0.27^{ab}	1.47 ± 0.31^{bc}	0.93 ± 0.15^{acd}
Fifth stadium	0.92 ± 0.16^{a}	1.19 ± 0.22^{ab}	1.18 ± 0.18^{bc}	0.94 ± 0.17^{abc}
Adult male and females	0.92 ± 0.18^{a}	1.32 ± 0.16^{ab}	1.31 ± 0.17^{bc}	1.03 ± 0.22^{abcd}

Same alphabets in a row is not statistically significant at 5% level

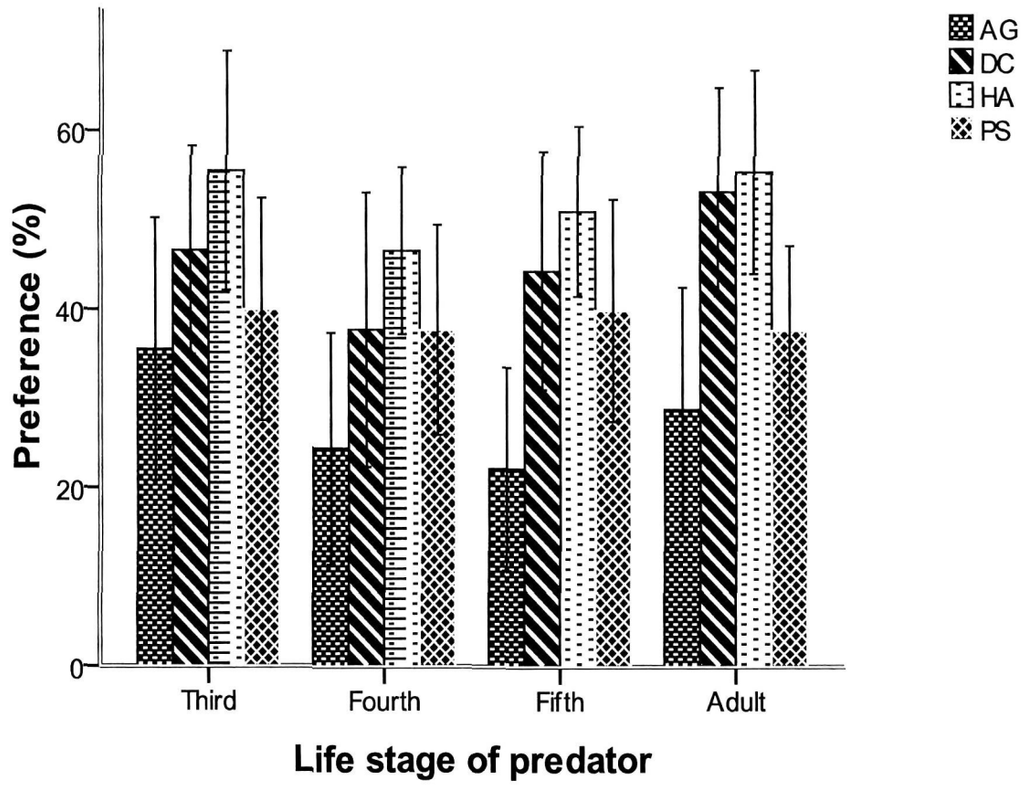

Figure 4. Cumulative preference percentage of *R. longifrons* third, fourth and fifth stadium nymphs and adults (male and female) choosing the whole body crude kairomones of *A. gossypii* (AG), *D. cingulatus* (DC), *H. armigera* (HA) and *P. solenopsis* (PS) in a Y-shape olfactometer.

Table 19. Individual mean (± standard error) visit (%) of the reduviid, *R. longifrons* third, fourth and fifth stadium nymphs and adults (male and female) to the whole body crude kairomone of *A. gossypii* (AG), *D. cingulatus* (DC), *H. armigera* (HA) and *P. solenopsis* (PS) in a Y-shape olfactometer

Life stage of the predator	Series 1		Series 2		Series 3		Series 4		Series 5		Series 6	
	DC	HA	DC	PS	DC	AG	HA	PS	HA	AG	PS	AG
Third stadium	40.0±12.4	53.3±8.1	53.2±8.2	33.3±10.0	46.6±8.1	26.6±12.4	46.6±13.3	33.3±0.0	66.6±10.5	33.3±10.5	53.3±13.3	46.6±13.2*
Fourth stadium	26.6±12.4	40.0±6.6	40.0±12.4	33.3±10.0	53.3±13.3	20.0±13.3	46.6±8.1	33.3±10.5	53.3±8.1	26.6±6.7	40.0±6.6*	26.6±12.4*
Fifth stadium	33.3±10.5	53.1±8.2	46.6±13.3	33.3±10.0	53.3±8.1	20.0±8.1*	53.2±8.1	40.0±6.6	46.6±8.1	13.3±8.1*	46.6±13.3	33.3±10.5
Adult	40.0±6.6	53.0±8.1	53.2±8.1	33.0±0.00*	66.6±10.5	26.6±12.4	53.2±8.1	33.3±0.0*	60.0±12.5	26.6±12.4	46.6±13.3	33.3±10.5

*All results are significant at 5% level

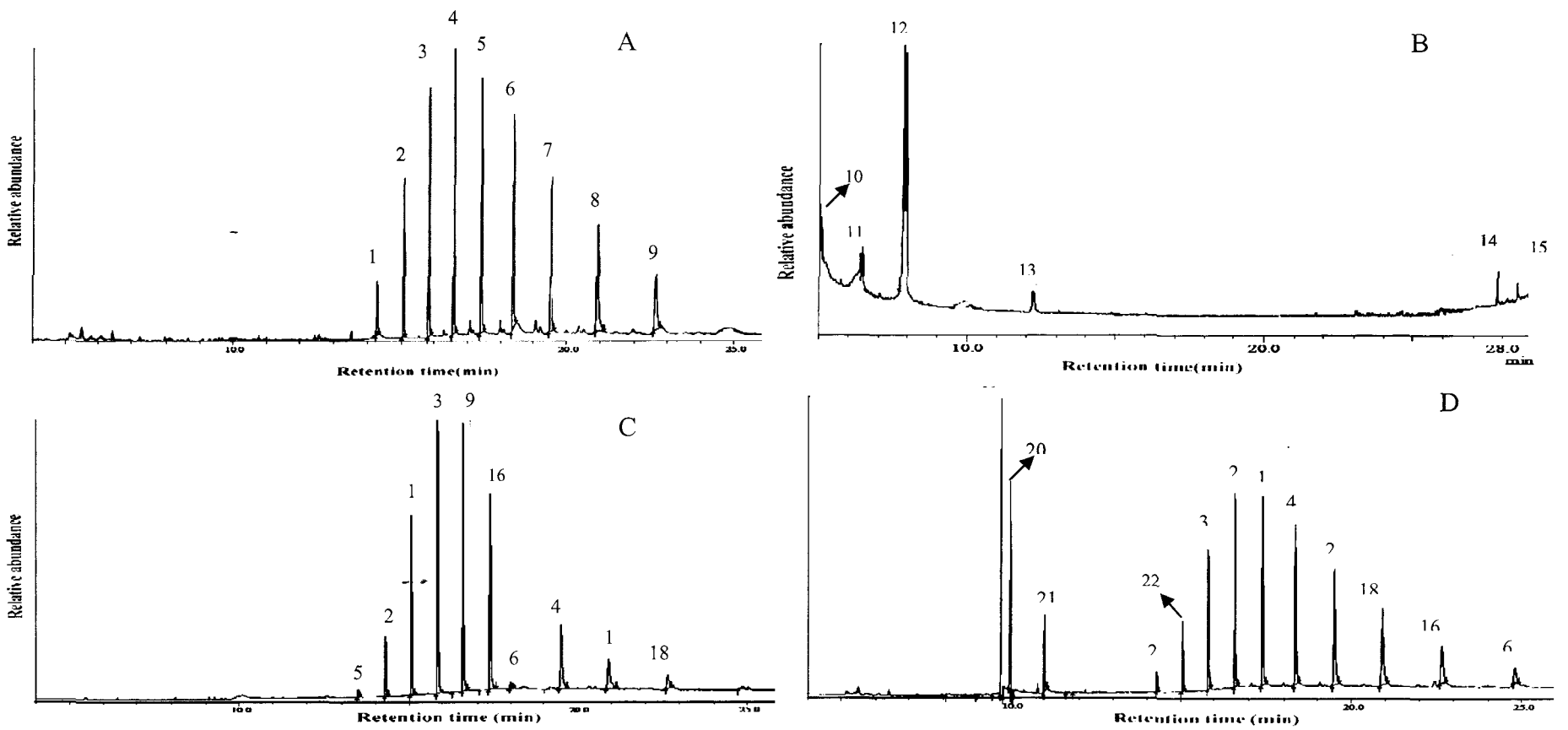

Figure 5. GC-MS spectrum of *A. gossypii* (A), *D. cingulatus* (B), *H. armigera* (C), and *P. solenopsis* (D) whole body crude kairomone. Key: 1. Tetracontane, 2. Hexatriacontane, 3. Tetracosane, 4. Dotriacontane, 5. Eicosane, 6. Tetrapentacontane, 7. 3-Methylheptadecane, 8. Squalane, 9. Celidoniol, 10. Cyclotetrasiloxane, 11. 2- Pentadecene, 12. Dodecamethyl, 13. 9-oxa-12-1-dodecene-6,11-diyne, 14. 1-Pentanone, 15. Hexadecane, 16. Hexacosane, 17. Hexacontane, 18. Pentatriacontane, 19. Dodecanoicacid, 20. Diethyl Phthalate, 21. Tetradecanoicacid and 22. Heneicosane; retention time (min) plotted in 'X' axis and relative mobility (%) plotted in 'Y' axis.

Table 20. GC-MS detection of *H. armigera, D. cingulatus, P. solenopsis* and *A. gossypii* crude kairomone compounds

Crude kairomone source	Identified compounds	Retention time (min)	Area (%)
H. armigera	Eicosane	13.50	0.62
	Hexatriacontane	14.28	3.99
	Tetracontane	15.05	12.74
	Tetracosane	15.82	20.63
	Octadecane	16.30	0.36
	Celidoniol	16.58	21.99
	Nonacosane	17.09	0.96
	Hexacosane	17.41	18.43
	Tetrapentacontane	17.99	0.86
	Tritricontane	19.08	0.62
	Dotriacontane	19.52	9.13
	Hexacontane	20.92	6.05
	Pentatriacontane	22.65	2.49
	Tetratetracontane	24.80	1.12
D. cingulatus	Cyclotetrasiloxane	5.03	5.80
	2- Pentadecene	6.22	4.13
	Dodecamethyl	7.86	72.06
	9-oxa-12-1-dodecene	12.23	4.77
	1,2-di -3 -pentadecenylbenzene	14.99	2.09
	1-Heptane	25.9	2.26
	1,3 - Dioxolane	26.8	0.95
	1- Pentanone	27.8	3.99
	Hexadecane	28.4	2.25
A. gossypii	unknown peak	9.99	3.25
	Tetracontane	14.28	2.57
	Hexatriacontane	15.06	6.94
	Tetracosane	15.83	11.85
	Dotriacontane	16.58	14.54
	Eicosane	17.41	14.45
	Tetrapentacontane	18.37	14.14
	3-Methylheptadecane	19.52	12.23
	Squalane	20.92	10.34
	Celidoniol	22.65	5.90
	2-Tetradecyl-1-Octadece	24.80	3.78
P. solenopsis	Hexadecanoic acid	8.11	3.04
	Molybdenum, Tetrakis(ETA.3-2 propenyl)	8.96	1.40
	2-Dodecylcyclobutanone	9.46	1.65
	Dodecanoic acid	9.70	12.80
	1.Alpha.-18O-1,25-dihydroxycholecalciferol	9.78	1.58
	Diethyl Phthalate	9.99	8.76
	Hexagermane	10.06	1.55
	Tetradecanoic Acid	10.99	3.68
	4-Nitrophenyl laurate	11.62	1.13
	Eicosanoic acid, 2-hydroxy-1	11.84	0.79
	Hexatriacontane	14.28	0.80
	Heneicosane	15.05	3.03
	Tetracosane	15.82	6.30
	Hexatriacontane	16.58	9.26
	Tetracontane	17.40	10.32
	Dotriacontane	18.36	10.67
	Triacontane	19.51	9.29
	Pentatriacontane	20.91	7.85
	Hexacosane	22.64	4.17
	Tetrapentacontane	24.79	1.94

were celidoniol (21.99%), dodecamethyl (72.06%), dodecanoicacid (12.80%) and dotriacontane (14.54%) in *H. armigera, D. cingulatus, P. solenopsis* and *A. gossypii,* respectively. Tetrapentacontane, dotriacontane, tetracontane, tetracosane, hexatriacontane these compounds are commonly present in the *H. armigera, P. solenopsis* and *A. gossypii* CKE (Table 20).

3.5.3. Functional response

It is a typical density dependant function of the tested predators that responded to the increasing prey density by killing more number of preys than it killed at lower prey densities. The functional response of III, IV, V nymphal instars and adult of *R. longifrons* were recorded. Results revealed that the attack ratio decreased with increase of prey density (Table 21-24). The functional response of *R. longifrons* on different densities of *D. cingulatus* (y=0.39+0.44x; r=0.98) is shown in Table 21. Maximum number of preys attacked in 16 densities was recorded for the adult predator on *D. cingulatus* which is higher than third (y=1.43+0.16x; r=0.92), fourth (y=0.99+0.27x; r=0.99) and fifth (y=1.02 +0.19x; r=0.93) nymphal instars followed by *P. solenopsis* (y=-0.04+0.49x; r=0.98; y=0.98+0.23x; r=0.98; y=0.64+0.21x; r=0.64 and y=0.1.10+0.22x; r=0.88 for adult, third, fourth and fifth) and *H. armigera* (y=0.15+0.31x; r=0.91; y=0.76+0.10x; r=0.82; y=0.66+0.17x; r=0.79 and y=1.30+0.09x; r=0.96 for adult, third, fourth and fifth). However, the third (y=0.64+0.22x; r=0.94) instar predator consumed maximum number of *A. gossypii* at 40 density rather than fourth, fifth and adult (y=2.01+0.20x; r=0.97; y=0.64+0.17x; r=0.97 and y=1.30+0.13x; r=0.91 for third, fourth, fifth and adult) (Fig. 6).

The adult and third instars of predators took lesser time for the handling (0.12 h). Similar trends were observed for *R. longifrons* adult on *H. armigera* (0.20 h and 0.44 h for fourth and adult, respectively) (Table 22), *P. solenopsis* (0.06 h and 0.04 h for third

Table 21. Functional response parameters of *R. longifrons* life stages against preferred life stages of *D. cingulatus*

Stage of the predator	Prey densities (N)	Maximum Na	Searching Efficiency (E)	Handling time (T_h)	Total T_h	TS	Predicted Na	Rate of discover (a')
III	1		1.0	0.29	0.29	0.71	1.0	1.40
	2		1.0	0.25	0.50	0.75	2.0	1.33
	4		0.58	0.37	0.85	0.63	3.9	0.90
	8		0.38	0.33	1.02	0.67	7.9	0.56
	16	3.8	0.24	0.12	0.45	0.88	15.9	0.26
IV	1		1.0	0.40	0.40	0.60	1	1.66
	2		0.60	0.27	0.34	0.73	1.9	0.82
	4		0.45	0.43	0.77	0.57	3.9	0.78
	8		0.43	0.43	1.46	0.57	7.9	0.73
	16	3.8	0.24	0.41	1.56	0.59	15.9	0.38
V	1		1.0	0.36	0.36	0.64	1	1.56
	2		0.80	0.07	0.11	0.93	1.9	0.86
	4		0.60	0.52	1.25	0.48	3.9	1.25
	8		0.40	0.22	0.70	0.78	7.9	0.51
	16	5.4	0.34	0.30	1.62	0.70	15.9	0.47
Adult	1		1.0	0.25	0.25	0.75	1	1.33
	2		0.80	0.25	0.40	0.75	1.9	1.06
	4		0.58	0.12	0.28	0.88	3.9	0.64
	8		0.38	0.20	0.60	0.80	7.9	0.46
	16	8.0	0.50	0.27	2.16	0.73	15.9	0.68

Third and fourth instar predator offered second stadium of *D. cingulatus* whereas other stages provided with *D. cingulatus* third stadium.

Table 22. Functional response parameters of *R. longifrons* life stages against preferred life stages of *H. armigera* larvae

Stage of the predator	Prey densities (N)	Maximum Na	Searching Efficiency (E)	Handling time (T_h)	Total (T_h)	TS	Predicted Na	Rate of discover (a')
III	2		0.60	0.51	0.61	0.49	2.0	1.22
	4		0.25	0.28	0.28	0.72	3.9	0.35
	6		0.20	0.44	0.52	0.56	5.9	0.36
	8		0.18	0.28	0.39	0.72	7.9	0.25
	10	2.0	0.20	0.49	0.98	0.51	9.9	0.39
IV	2		0.75	0.47	0.70	0.53	2.0	1.41
	4		0.45	0.60	1.05	0.40	3.9	1.10
	6		0.30	0.20	0.35	0.80	5.9	0.36
	8		0.25	0.39	0.78	0.61	7.9	0.40
	10	2.3	0.23	0.41	0.94	0.59	9.9	0.39
V	2		0.70	0.47	0.65	0.53	2.0	1.32
	4		0.30	0.50	0.60	0.50	3.9	0.60
	6		0.20	0.55	0.66	0.45	5.9	0.44
	8		0.22	0.69	1.24	0.31	7.9	0.71
	10	2.8	0.28	0.62	1.73	0.38	9.9	0.74
Adult	2		0.50	0.51	0.51	0.49	2.0	1.02
	4		0.33	0.46	0.52	0.54	3.9	0.61
	6		0.33	0.58	1.16	0.42	5.9	0.78
	8		0.25	0.58	1.16	0.42	7.9	0.60
	10	3.8	0.37	0.44	1.65	0.56	9.9	0.66

Third and fourth instar predator offered second stadium of *H. armigera* whereas other stages provided with *H. armigera* third stadium

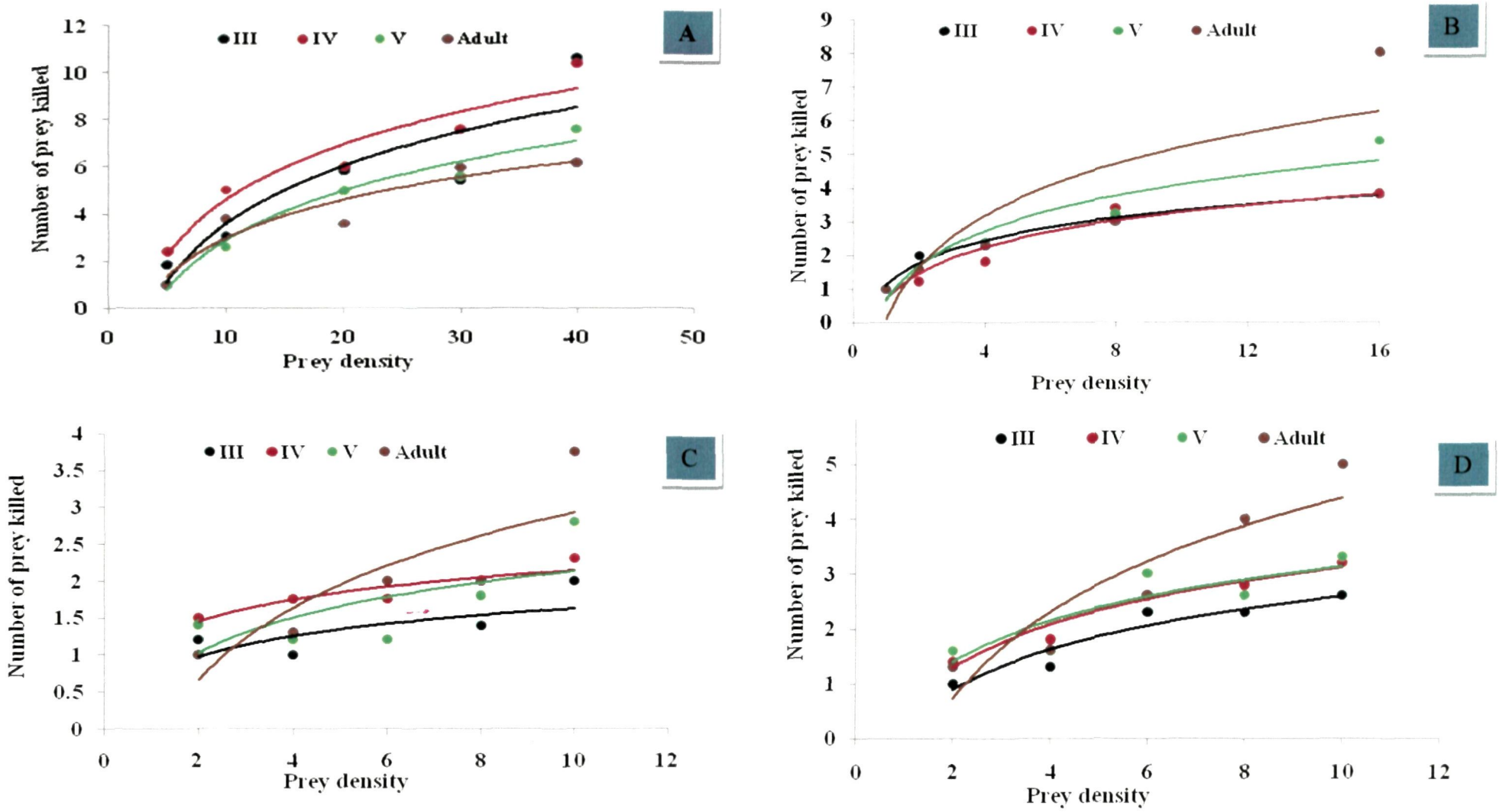

Figure 6. Curve linear graph for functional response of *R. longifrons* life stages on four cotton pests *A. gossypii* (A), *D. cingulatus* (B), *H. armigera* (C), *P. solenopsis* (D)

and adult, respectively) (Table 23) and *A. gossypii* (0.15 and 0.09 for fourth and fifth, respectively) (Table 24). The correlation between prey density and attack ratio was significant at 5% level in all stages of the *R. longifrons* against four cotton pests. The 'r' value was more than 0.9 in all the cases. However, correlation between prey density and handling time was significant for fifth instar of *R. longifrons* against *H. armigera* (r=0.865) and *P. solenopsis* (r=0.617).

3.6. DISCUSSION

3.6.1. Stage preference

Generalist insect predators like reduviids are often expected to be important in controlling agricultural pests (Altieri, 1995; Grundy and Maelzer, 2002; Ambrose, 2003; Rocha and Redaelli, 2004; Sahayaraj, 2004, 2007). It was predicted and also observed that life stages of reduviid predator preferred particular stage of the prey (Sahayaraj, 2011). All the nymphal instars and adult stages of predator preferred large size of *D. cingulatus, H. armigera* and *P. solenopsis.* Similar report was also observed by Sahayaraj (1994). Sahayaraj and Ambrose (1994b), George (2004), Balasubramanian (2008) reported that stage acceptances of *R. marginatus* and *A. pedestris* could be attributed to the dynamics of prey-predator interaction which is principally governed by the size. These results indicate that the larger predator accepted the larger-sized prey and the smaller predator accepted the smaller size prey (Sahayaraj, 1999; Chandral and Sinazer, 2011). *Rhynocoris longifrons* preferred soft bodied lepidopteran larvae followed by hemipteran pests. The soft as well as comparatively larger lepidopteran pests were preferred to the harder hemipteran pests, depending upon the chemical nature, high motility and size of the prey.

Table 23. Functional response parameters of *R. longifrons* life stages against preferred life stages of *P. solenopsis*

Stage of the predator	Prey densities (N)	Maximum Na	Searching Efficiency (E)	Handling time (T_h)	Total T_h	TS	Predicted Na	Rate of discover (a')
III	2		0.50	0.15	0.15	0.85	1.9	0.58
	4		0.33	0.4	0.52	0.59	3.9	0.54
	6		0.38	0.06	0.13	0.94	5.9	0.40
	8		0.29	0.20	0.40	0.80	7.9	0.35
	10	2.6	0.26	0.17	0.44	0.83	9.9	0.31
IV	2		0.71	0.38	0.53	0.62	1.9	1.12
	4		0.45	0.53	0.95	0.47	3.9	0.95
	6		0.43	0.49	1.27	0.51	5.9	0.84
	8		0.35	0.21	0.58	0.79	7.9	0.44
	10	3.2	0.32	0.64	2.0	0.36	9.9	0.88
V	2		0.80	0.12	0.19	0.88	1.9	0.90
	4		0.40	0.05	0.08	0.95	3.9	0.42
	6		0.50	0.12	0.36	0.88	5.9	0.56
	8		0.32	0.19	0.49	0.81	7.9	0.39
	10	3.3	0.33	0.15	0.49	0.85	9.9	0.38
Adult	2		0.65	0.04	0.05	0.96	1.9	0.67
	4		0.40	0.16	0.26	0.84	3.9	0.47
	6		0.43	0.19	0.49	0.81	5.9	0.53
	8		0.50	0.09	0.36	0.91	7.9	0.54
	10	5.0	0.50	0.13	0.65	0.87	9.9	0.57

Third,fourth, fifth instar and adult predator offered adult stage of *P. solenopsis*

Table 24. Functional response parameters of *R. longifrons* life stages against preferred life stages of *A. gossypii*

Stage of the predator	Preydensities (N)	Maximum Na	Searching Efficiency (E)	Handling time (T_h)	Total T_h	TS	Predicted Na	Rate of discover (a')
III	5		0.36	0.31	0.56	0.69	4.9	0.52
	10		0.30	0.27	0.81	0.73	9.9	0.41
	20		0.29	0.37	2.14	0.63	19.9	0.46
	30		0.18	0.26	1.40	0.74	29.9	0.24
	40	10.6	0.26	0.16	1.69	0.84	39.9	0.30
IV	5		0.48	0.15	0.36	0.85	4.9	0.56
	10		0.50	0.44	2.2	0.56	9.9	0.89
	20		0.30	0.15	0.90	0.85	19.9	0.35
	30		0.25	0.24	1.82	0.76	29.9	0.32
	40	10.4	0.26	0.37	3.85	0.63	39.9	0.41
V	5		0.20	0.16	0.16	0.84	4.9	0.23
	10		0.26	0.24	0.62	0.76	9.9	0.34
	20		0.25	0.09	0.45	0.91	19.9	0.27
	30		0.18	0.36	2.01	0.64	29.9	0.28
	40	7.6	0.19	0.30	2.28	0.70	39.9	0.27
Adult	5		0.20	0.40	0.40	0.60	4.9	0.33
	10		0.38	0.19	0.72	0.81	9.9	0.46
	20		0.18	0.37	1.33	0.63	19.9	0.28
	30		0.19	0.15	0.87	0.85	29.9	0.22
	40	6.2	0.15	0.20	1.24	0.80	39.9	0.18

Third,fourth, fifth instar and adult predator offered adult stage of *A.gossypii*

3.6.2. Host preference by visual bioassay

The results of the experiments on the prey preference of *R. longifrons* show that the predator was able to attack and feed on all four cotton pests species offered (Plate 3). All developmental stages of the predator showed a clean preference for *H. armigera*, as compared to the other prey species offered. However, considerable number of *D. cingulatus* and *P. solenopsis* were also consumed. Several researchers reported that *R. longifrons* preferred and fed on *H. armigera* (Ambrose *et al.,* 2003; Ravi, 2004; Ganesh Kumar, 2011) and *D. cingulatus* (Ganesh Kumar, 2011). However, there are no reports of the predation of *A. gossypii* and *P. solenopsis* by *R. longifrons.*

3.6.3. Prey kairomone preference

Our results demonstrate that *R. longifrons* preference of positive anemotax is in response to crude kairomone extract of *H. armigera, D. cingulatus, P. solenopsis* and *A. gossypii.* The mean percent visit (Table 18) and locomotory response (Table 19) were characterized by an increased degree of quick approaching time compared to the control situation. *Helicoverpa armigera* odour source significantly attracted to elicit anemotax (Table 18) in the presence of *D. cingulatus, A. gossypii* and *P. solenopsis* odour is the host preference of source as an alternative source for this reduviid. Most of the compounds identified by GC-MS analysis are common in cuticular lipids of insects as reported by Roux *et al.* (2007). The host preference of nymphs and adults of the predatory reduviid bug *R. longifrons* preferred lepidopteran pest followed by hemipteran (Sahayaraj and Ambrose, 1994b). The nymphs and adults of *R. longifrons* highly preffered kairomone of lepidopteron pest. This result is in accordance with that of Sahayaraj and Delma (2004) and Sahayaraj (2008) in the reduviid predator *R. marginatus* on *H. armigera, S. litura* and *M. Pustulata.* Sujatha *et al.* (2012) reported that *R. fuscipes* on *A.*

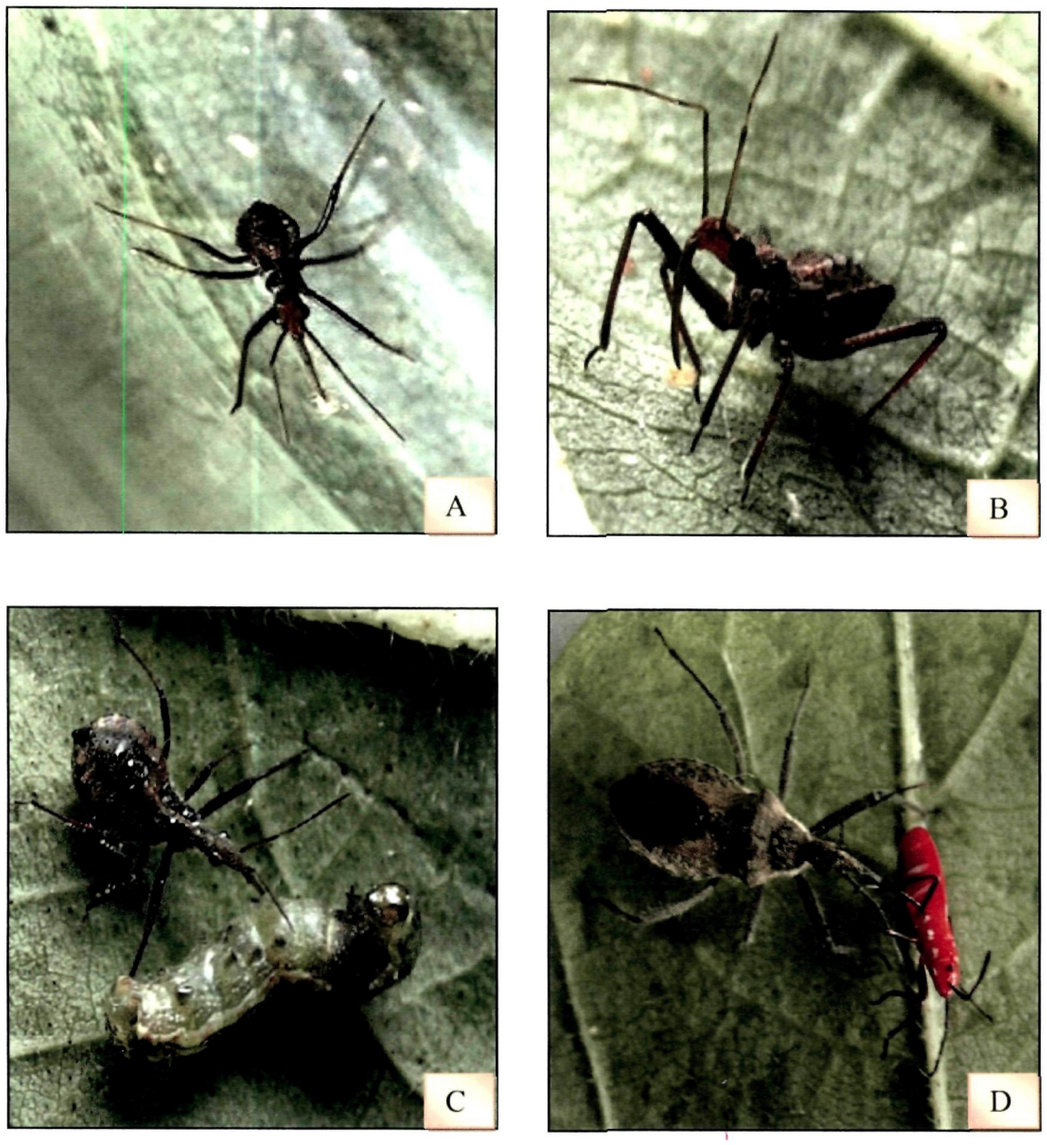

Plate 3. Feeding behavior of *R. longifrons* nymphs (A, B, C) and adult (D) against *P. solenopsis* adults (A), *A. gossypii* adults (B), *H. armigera* larvae (C) *D. cingulatus* nymphs (D).

janata, D. cingulatus and *S. litura* kairomone extracts. Several studies carried out in this regard in other predatory insects like *Eriopis connexa* and *Hippodamia variegata* on *Acyrthosiphon pisum* (Aphidiae) (Tapia *et al.,* 2010). Dauphin *et al.* (2009) reported the foranging behaviours of *Trissolcus basalis* (Hymenoptera: Scelionidae) using host kairomone of *Nezara viridula* (Heteroptera: Pentatomidae). *Trichogramma chilonis* on kairomone of *H. armigera* and *Corcyra cephalonica* (Ananthakrishnan *et al.*, 1991). Similar kind of observation was also recorded by Yasuda and Wakamura (1996), who had shown that the predatory sting bug *Ecocantheconia furcellata* (Wolff) (Heteroptera: Pentatomidae) was attracted to the larval extract of the *Spodoptera litura.*

3.6.4. GC – MS analysis of prey crude kairomone

The GC-MS results of *H. armigera* kairomone are closely similar to those of Sahayaraj (2008), who implicated the compounds such as octodecane, eicosane, dotriacontane previously tetracosane and hexatriacontane (Ananthakrishnan *et al.,* 1991; Singh *et al.,* 2002) were also identified from the kairomone of *H. armigera.*

In this study we have identified other minor peaks like hexacosane, tetracoasne, hexadecane, henicosane, octadecane etc. These chemical compounds were also identified by Seenivasagam and Paul (2011) in larval body extract of *Plutella xylostella* for behaviour of *Costesia plutella.* Decane, eicosane and contane are the important kairomonal compounds of larvae (Seenivasagam and Navarajan Paul, 2011), and these type of hydrocarbons were recorded from *A. gossypii.* Moreover, eicosane, hexacosane were also recorded from *H. armigera* and *P. solenopsis,* respectively. It indicates hydrocarbons as kairomones for predators (Singh *et al.,* 2002). Kairomonal stimulatants of *H. armigera* (celidoniol), *D. cingulatus* (dodicamethyl), *P. solenopsis* (dodecanoic

acid) and *A. gossypii* (dotriacontane) may be the reason for the preference of reduviid against specific pests. Even the aphis aqueous extract acted as kairomonal stimulatant of *Coccinella septempunctata* (Shonouda, 1999), based on our results and the existing literature. We suggest that the odour emitted directly by tested insect species is at least partially / fully responsible for the reduviids response.

3.6.5. Functional response

Before using a predator in biological control programme, it is essential to know its biological control efficiency. One of the most important methods to assess the efficacy of natural enemies is studying the behavioural characteristic, including foraging behaviour. Studying predator behaviour is an important key to understand how insects live and influence the population dynamics of their prey (Dixon, 2000). One of the important behavioural patterns considered for assessing the bioefficacy is functional response. It describes the way of natural enemy measured attribute of natural enemies of pests (Hassell, 1978). Holling (1959, 1966) considered three type of functional response.

The functional response of a predator is crucial factor in the population dynamics of prey – predator systems. The functional response model helped to evaluate two vital parameters: i) handling time (*i.e.* the time taken by a predator to encounter and consume a single prey) and ii) attack rate (rate at which a predator searches). Several studies have been carried out on the functional response of reduviid predator against various economically important pests. The reduviid predator usually shows a type II functional response, as was observed in other reduviids, for example *Rhynocoris marginatus* (Sahayaraj, 1994; Sahayaraj *et al.,* 2003b; Ambrose *et al.,* 2010); *Sphedanolestes variabilis* (Ambrose *et al.,* 2009a); *R. longifrons* (Claver *et al.,* 2002); *Cosmoclopius nigroannulatus* (Rocha and Redaelli, 2004); *Rhynocoris fuscipes* (Sahayaraj *et al.,* 2002a; Bibin *et al.,* 2010; Nagarajan

et al., 2010; Majesh *et al.*, 2011); *Acanthaspis quinquespinosa* (Ambrose *et al.*, 2008b) and *Zelus renardii* (Shrestha and Parajulee, 2004).

3.6.5.1. Predatory rate

A positive relationship between prey density and predation rate is often assumed to imply that predation of *R. longifrons* against chosen cotton pest is stabilizing. The high predation rate of the early instars (third and fourth) of predator against very small size prey, *A. gossypii* is of particular interest. It shows the size of the predator in relation to that of its prey which appears to be the major factor determining success in capturing and consuming prey. Over 24 h, the third, fourth and fifth nymphal instars and adult predators accounted for 26. 5%, 32%, 33.74% and 50% of the total *A. gossypii, P. solenopsis, D. cingulatus* and *D. cingulatus,* respectively. These stage dependant predations are similar to other reduviid predators like *A. pedestris* (Sahayaraj and Ambrose, 1994c; Sahayaraj, 1995c), *Allaeocranum quadrisignatum* (Ambrose and Sahayaraj, 1993a), *Coranus nodulosus* (Sahayaraj and Ambrose, 1993), *E. tibialis* (Sahayaraj, 1995b), *Neohaematorrhophus therasii* (Sahayaraj and Ambrose, 1996), *R. kumarii* (Ambrose *et al.,* 1997; Sahayaraj and Asha, 2010). It indicates that adult predator is most important for biological control of *D. cingulatus, H. armigera* and *P. solenopsis* whereas third and fourth nymphal instar can be utilized for the management of *A. gossypii.* The low predation rates observed in the third and fourth instar against *H. armigera* and fifth instar nymph and adult predator against *A. gossypii* are likely due to their low attack rates and longer handling times (Table 24). The higher proportion of prey consumption at low densities for tested life stages of *R. longifrons* tested was a typical result for a Type II response (Atlihan and Kaydan, 2010) and it indicates that predator would be more effective at controlling the pests at lower densities. Similar observation was recorded by Sahayaraj (2000) and prey size (Cogni *et al.*, 2002). Biocontrol potential of *R. longifrons* on aphids, the adult had little consumption compared

with nymphal instars. This result is in accordance with that of Sahayaraj *et al.* (2003b). Holling (1959) mentioned that the attack rate constant can be considered independent from density.

The higher predation rates observed in the higher prey density are probably because of their shorter prey handling time. This is understandable because at higher prey density, the predator not spent more time for searching its victim, finds its prey within shorter vicinity; hence they need shorter time to search, handle and kill their prey. Predatory rate of *R. longifrons* life stages against preferred life stages of *D. cingulatus, H. armigera* and *P. solenopsis* gradually increased while predator grew older a common phenomenon in reduviid predators (Kumaraswami, 1991; Sahayaraj, 1991). But it is not true for all the preys offered to R. *longifrons* life stages. For instance, *R. longifrons* third instar consumed more number of *A. gossypii* (10.6 preys/predator) than fourth and fifth nymphal instars and adults.

3.6.5.2. Searching time

A decrease of estimates and observed search time as *D. cingulatus, H. armigera, A. gossypii* and *P. solenopsis* densities increased was found all life stages of *R. longifrons*. According to Hassell *et al.* (1976), this decrease in search time occurs because at high densities preys are more easily caught. The short search time evidenced in *R. longifrons* life stages can also be attributed to shorter handling time at high prey density. Our laboratory measured functional response of *R. longifrons* may not exactly correspond to the field situation. However, our study has a value as the first step in advocating *R. longifrons* as a biological control agent of cotton pests.

3.6.5.3. Handling time

The handling time is a good indication of consumption rate and effectiveness of a predator, because it reflects the cumulative effect of time taken during capturing, killing, subduing and digesting the prey. The total handling time (T_h) per prey per day estimated increased as prey density increased in *C. nigroannulatus* (Rocha and Redaelli, 2004); *A. quinquespinosa* (Ambrose *et al.,* 2008b); *A. pedestris* (Ambrose and Sahayaraj, 1996); *R. kumarii* (Sahayaraj and Asha, 2010); *R. fuscipes* (Nagarajan *et al.,* 2010); *R. marginatus* (Sahayaraj, 1994; Sahayaraj and Ambrose, 1994b; Sahayaraj, 2000; Sahayaraj *et al.,* 2003b); *S. variabilis* (Ambrose *et al.,* 2009b); *E. tibialis* (Sahayaraj, 1995b); *N. therasii* (Sahayaraj and Ambrose, 1996) and an opposite trend recorded in *R. fuscipes* (Ambrose and Claver, 1997). Besides, handling time can influence other components like attack rate and search efficiency (Beddington, 1975). At high *D. cingulatus* density, the T_h gradually increased from third instar to adult indicates, adults consumed/ predate at maximum food/ prey in order to maintain its reproduction. However, *R. longifrons* fourth instar and fifth instar spent more time handling against *A. gossypii, P. solenopsis* and *H. armigera,* respectively, producing slower consumption acceleration, regulating in almost line trend recorders curve. Although it may be more realistic to measure predation under field conditions (Sahayaraj and Ambrose, 1994b; Shrestha and Parajulee, 2004; Ambrose *et al.,* 2009a; Ambrose and Nagarajan, 2010; Atlihan and Kaydan, 2010; Ghosh and Chandra, 2011), our study confined to a very small arena, Petri dish, under laboratory condition allowing us to predict the efficiency of each developmental stage of *R. longifrons* at varying densities of *A. gossypii, D. cingulatus, H. armigera* and *P. solenopsis* in field conditions.

3.7. CONCLUSION

1. Based on the results of the present study, it can be concluded that although *R. longifrons* showed a clear preference for *H.* armigera, it also accepted the three other cotton pest species offered.
2. As *R. longifrons* is a ploy phagous predator, it is possible that predator can be utilized in cotton pest management.
3. This study suggests that the reduviid predator *R, longifrons* could be employed as a biocontrol agent against *H. armigera, D. cingulatus, P. solenopsis* and *A. gossypii.* Using this predator for controlling agricultural pests can minimize environmental hazards.

CHAPTER - 4

4.1. ABSTRACT

The biocontrol potential of reduviid *Rhynocoris longifrons* on cotton pests *Dysdercus cingulatus* (second and third instar nymphs) and *Phenacoccus solenopsis* (adult), *Helicoverpa armigera* (second and third instar larvae) and *Aphis gossypii* (all stages) were carried out under pot condition. The *R. longifrons* adult showed higher predatory rate against *H. armigera* than *D. cingulatus* and *P. solenopsis* while the predator under study was released either in the morning and/or in the evening. *Rhynocoris longifrons* life stages preferred hiding under pebbles rather than fallen leaves and cotton plants which indicates the acclimatization of the predator. The augmentative release of *Rhynocoris longifrons* were evaluated against selected cotton pests of farmers field at irrigated and rain fed condition. The results revealed that the predator highly reduced *D. cingulatus* (53.80 %) population and *A. gossypii* (11.8 %) at rain fed condition and *P. solenopsis* (26.0%) under irrigated condition. Between the fields tested, the high cotton yield and cost benefit ratio was recorded in *R. longifrons* released plots (837.0 kg h^{-1}) rather than control plot (715.5 kg h^{-1}) in irrigated condition.

Key words: Hiding, irrigation, pot study, rainfed, pest population, production

4.2. INTRODUCTION AND REVIEW OF LITERATURE

World cotton production was 24.49 million tons in 2010-2011. In 2011-2012 it is expected to be 23.13 million tonnes. In India upto April 2012, the cotton production is 298.3 lack bales (Cotton Corporation of India, 2012). The world average cotton yield is projected to on par with last year (2011) yield at 748 kg/hectare (Cotton Corporation of

India. 2012). The production loss is due to the infestation of pests and infection of pathogenic microbes. The pests and diseases complex in cotton agro-ecosystem is diverse and innumerous. In augmentative biological control, natural enemies are periodically introduced, and this method is commercially applied on large areas in various cropping systems worldwide (Gutierrez *et al.,* 1999). It is a popular biological control approach amongst professional and progressive farmers, and has been stimulated by the current international attitudes regarding reduction of pesticide use. Augmentative biological control practice has been in vogue worldwide and more than 150 species of natural enemies are now commercially available (Ricardo and Pratissoli, 2009). However, generalist predators, particularly predatory bugs, have been largely ignored for augmentation in cotton pest management (King and Powell, 1992; Goldstein and Whalen, 1993; Kehrli and Wyss, 2001; Prabhakar and Roy, 2000; Bayoumy, 2011; Jalalizand *et al.,* 2011; Reddy and Rosalie, 2011; Sushilkumar and Ray, 2011; Van Lenteren, 2012; Venkatesha and Dinesh, 2012). Field testing is an important step in evaluating the use of natural enemies as augmented biological control agents (Cloutier and Bauduin, 1995; Grundy, 2004; Sahayaraj and Ravi, 2007b).

Biological control by predators such as assassin bugs helps in the regulation of insect pest population in Integrated Pest Management (IPM). Biological control refers to the regulatory action of parasites, predators or pathogens to maintain the density of an organism (pest) at a lower level than would occur without these natural enemies. Reduviids are exclusively predatory mostly on insect pests (Kalsi and Seal, 2011; Sahayaraj, 2002b; Grundy, 2004). Previously reduviids like *Pristhisancus plagipennis* (Walker) (Grundy and Maelzer, 2000; Grundy, 2004), *Rhynocoris marginatus* Fabricius (Ambrose and Claver, 1999; Sahayaraj, 1999; Sahayaraj and Martin, 2003; Sahayaraj and Ravi, 2007b), *Platymeris longicollis* Distant (Antony *et al.*, 1979), *Ectomocoris tibialis*

(Distant) (Sahayaraj and Ambrose, 1997), *Rhynocoris kumarii* Ambrose and Livingstone (Claver and Ambrose, 2001b), were released augumentatively and their biological control potential in various agro-ecosystems was evaluated.

The Indian assassin bug *Rhynocoris longifrons* is a natural enemy of *D. cingulatus, M. pustulata, H. armigera* and *S. litura* under laboratory (Ambrose *et al.*, 2003; Ravi, 2004; Ganeshkumar, 2011; Mitradev, 2011) and the authors suggested that *R. longifrons* can be used for biological control augmentation against insect pests. However, no one has taken the initiate to release this bug augmentative in field crops. To our knowledge, this is the first report about the augmentative biological control of cotton pests using *R. longifrons*. The aim of this study is to evaluate the bioefficacy of *R. longifrons* under pots and field in two different crop conditions (rainfed and irrigated) and subsequently to find out cost benefit ratio.

4.3. MATERIALS AND METHODS

4.3.1. Collection and maintenance of insects

Adults of *R. longifrons were* collected from cotton fields and scrub jungle bordering of agro-ecosystem in Tirunelveli district, Tamil Nadu. They were maintained on factitious host *Corcyra cephalonica* under laboratory conditions 32 ± 2.0° C temperature, 56.0±5% relative humidity (Rh) and photoperiod of 11:13 hr (L:D) in 1 litre transparent plastic containers. More than fifteen generations were reared under laboratory condition. Laboratory emerged nymphs and adults were used for the study.

All the pest species were collected from the same cotton field from which the reduviid was collected. *Aphis gossypii, D. cingulatus*, *H. armigera* and *P. solenopsis* were

maintained in groups in plastic trough (7 litre) (32 cm diameter × 15 cm height) and transparent plastic container (50 ml) on young leaves, flowering and bolls.

4.3.2. Bioefficacy evaluation under pot condition

This experiment was conducted within a small plot (10 × 4 meters m^3). The biocontrol potential of *R. longifrons* life stages (III, IV, V stadium and adults) on chosen cotton pests such as *A. gossypii, D. cingulatus, H. armigera* and *P. solenopsis*. Experiments were carried out in pots (made up of cement) (36 cm diameter and 22 cm height) using 30 days-old healthy cotton plants (SVPR 2) covered with nylon net as shown in plate 4. They were maintained inside the screen house (36×21.5 sq. Feet) of Crop Protection Research Centre, St. Xavier's college. Third, fourth and fifth stadium nymphs and adults (without discriminating the sex) were used in the study. Two sets of experiments were carried out in the (i) morning hours and (ii) in the evening hours. The predator preferred life stages of *D. cingulatus* (second and third instar), *H. armigera* (second and third instar larvae), *P. solenopsis* (adults) and *A. gossypii* (fifth instar nymphs and adults) released at different optimum densities of *D. cingulatus* (6, 6, 8 and 8 preys for third, fourth, fifth and adult predator, respectively), *H. armigera* (6, 6, 6 and 8 preys for third, fourth, fifth and adult predator, respectively), *P. solenopsis* (6, 6, 8 and 10 preys for third, fourth, fifth and adult predator, respectively) and *A. gossypii* (10, 10, 8 and 8 preys for third, fourth, fifth and adult predator, respectively) on the tender leaves of cotton plant. Then it was allowed to acclimatize for 12 hours. Then, predator life stages were released individually on the tender leaves using Camlin brush. After 12 hrs (6 pm), the predatory rate were recorded (number of preys killed by a predator). Six replications were maintained for each test of the individual pest density. In the second set of experiments, pests were released at 6 am, allowed to acclimatize for 12 hrs. The predators were released at 6 pm. The predatory rate was recorded after 12 hr (next day 6 am).

Plate 4. Bioefficacy evaluation of *R. longifrons* under pot condition

4.3.3. Hiding behaviour of the predator under screen house

The relevance of this study was aimed to record the hiding places of the predator when it was released into the cotton during augmentative programme. The hiding behaviour of *R. longifrons* carried out under screen house (36 × 21.5 sq. feet) in 30 day-old cotton plant (MCU- 5). Two plots were maintained (10 × 4 meters) with 52 plants at spacing of 35×75 cm and oriented east and west. Ten 24 hr pre starved fourth stadium predators were released at 6.00 am, 8.30 am, 11 am and 3.30 pm separately using Camlin brush in the north east corner on to the ground level. After every 2 hr, the number of insects settled at the base of the plant, fallen leaves, small pebbles in the plant and other objects (if any) were recorded. Similar procedure was followed for fifth stadium and adult of *R. longifrons*. After 24 hours the insects were collected back.

4.3.4. Experimental design for augmentative release of *R. longifrons*

The experiment was conducted in farmer's field within a small plot of irrigated (75×30 sq. feet) cotton (SVPR 4 cultivar) at Kothankulam (N 9° 28' 47.37'), Virudhunagar district, Tamil Nadu and rainfed (30 × 15 sq. feet) cotton (SVPR 4) in K. Duraisamiyapuram (E 77° 35' 22.16'), Tuticorin district, Tamil Nadu, India. The experiments were conducted during 2011 Khariff (July to September) and 2012 Post monsoon (December to February) for irrigated and rainfed condition, respectively. The treatment plots were arranged in a randomized complete - block design (RBD) with five 450 sq. feet replicates of each treatment. These pesticide-free cotton fields were divided into five plots (15×30 sq. feet). Predator-free standard was used as a control. A pre counting of pest and natural enemy's population were completed two days before releasing the predators. First, second, third, fourth and fifth instars *R. longifrons* (fifty each) were released onto the cotton foliage on 40^{th}, 55^{th} and 70^{th} day after the seedling emergence (DASE). In addition, two egg masses approximately with 50 eggs hereafter

called egg card were also released. In total, 750 (9000 h^{-1}) *R. longifrons* life stages were released and 150 eggs during the study period. Nymphs for each treatment were released singularly onto the terminal shoots of the crop foliage using a Camel-hair brush in the morning hours. The growing points and squares of the upper two-thirds of the growing 10 randomly selected cotton canopy were searched for larvae, nymphs and adult bugs. Flowers and bolls throughout the cotton were also inspected for larvae. The number of reduviid predators, ants, coccinellids, wasps and spiders were recorded before (2days) and after the release of *R. longifrons* expressed in number of predator per plant.

4.3.5. Cost Benefit Ratio (CBR) and Percent avoidable loss

At the completion of the growing season, cotton in each treatment replicate was harvested. The harvested cotton was cleaned, weighted and sold (kg/ha^{-1}) in the local market. The cost benefit ratio (CBR) was calculated and worked out according to Balasubramanian (2008) methodology/procedure. The percentage of avoidable loss was also calculated using the formula of Krishnaiah (1977):

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

$$\text{Percent avoidable loss} = \frac{\text{Mean yield from protected plots} - \text{Mean yield from unprotected plots}}{\text{Mean yield from protected plots}}$$

4.4. STATISTICAL ANALYSIS

The insect sample (pests and predators) data together with yield weight were subjected to ANOVA. The prey consumption of morning and evening time data of pot study were analyzed with 't' test. Least significant differences were calculated to determine difference at $P<0.05$.

4.5. RESULTS

4.5.1. Bioefficacy evaluation under pot condition

The *R. longifrons* adult showed higher predatory rate against *H. armigera* during morning (t=3.505; df=5; P<0.05) than evening (t=1.380; df=5; P>0.05) hours. Similar observation was made in third and fourth stadium of *R. longifrons* which showed higher predatory rate against *P. solenopsis* (t=6.708; df=5; P<0.05 and t=2.988; df=5; P>0.05 morning and evening, respectively). However, no difference was observed against *D. cingulatus* (t=2.246; df=5; P>0.05 and t=1.091; df=5; P>0.05 for morning and evening, respectively. Third and fourth instar of *R. longifrons* on *A. gossypii* was higher (t=2.712; df=5; P<0.05 and t=1.464; df=5; P<0.05 morning and evening, respectively). Invariably during day time the adult predator showed higher consumption when compared with that in the evening (t=4.392; df= 5;P<0.05; t=3.796; df=5; P<0.05 and t=1.168;df=5;P>0.05) *H. armigera*, *D. cingulatus,* and *P. solenopsis*, respectively) (Table 25).

4.5.2. Hiding behaviour of the predator under screen house

The hiding behaviour of *R. longifrons* revealed that the percentage of animals hiding under pebbles was higher between 6 am and 10 am. Fifth stadium reduviids always prefer to hide under pebbles, whereas fourth stadium predator first hid under fallen leaves then moved into pebbles within an hour, again left the place and returned to the fallen leaves. However, adults, immediately after the release, preferred to hide under the pebbles, but moved towards the base of the plant; climbed on to plants searched various places and if no preys available, they came back on to the ground and settled under the pebbles (Fig. 7 and Table 26), otherwise, consume the preys left the place and returned to its original place.

Table 25. Biocontrol potential of *R. longifrons* third, fourth and fifth stadium and adult (male and female) on *A. gossypii*, *D. cingulatus*, *H. armigera* and *P. solenopsis* in pot study

Stages of predator	Predator releasing time			
	Dawn Hours		Dusk Hours	
	Total number of prey consumed	Predatory rate	Total number of prey consumed	Predatory rate
		D. cingulatus		
Third	0.66±0.21*	0.33±0.1	0.50±0.34*	0.25±0.17
Fourth	0.50±0.22*	0.25±0.17	0.33±.21*	0.16±0.10
Fifth	1.33±0.42 ^{NS}	0.44±0.14	1.0±0.25*	0.33±0.08
Adult	2.16±0.30*	0.71±0.10	1.50±0.56*	0.49±0.18
		P. solenopsis		
Third	1.83±0.30^{NS}	0.91±0.15	2.0±0.44 ^{NS}	1.0±0.22
Fourth	2.0±0.57 ^{NS}	1.0±0.28	1.5±0.34 ^{NS}	0.75±0.17
Fifth	2.0±0.81 ^{NS}	0.65±0.26	1.83±0.70 ^{NS}	0.61±0.23
Adult	2.5±0.76 ^{NS}	0.82±0.24	2.5±0.99 ^{NS}	0.82±0.32
		H. armigera		
Third	0.83±0.30*	0.41±0.15	1.33±0.33 ^{NS}	0.66±0.16
Fourth	1.0±0.36*	0.50±0.18	2.16±0.70 ^{NS}	0.49±0.20
Fifth	1.66±0.42*	0.55±0.14	1.16±0.40*	0.38±0.13
Adult	2.66±0.42*	0.88±0.13	1.50±0.61 ^{NS}	1.08±0.35
		A. gossypii		
Third	1.66±0.61*	0.83±0.30	1.0±0.68 ^{NS}	0.50±0.34
Fourth	0.50±0.34 ^{NS}	0.25±0.17	1.0±0.63 ^{NS}	0.50±0.31
Fifth	0.83±0.54 ^{NS}	0.27±0.18	-	-
Adult	0.16±0.16*	0.06±0.055	0.33±0.33 ^{NS}	0.11±0.11

* Significant at 5% level, NS- Not significant

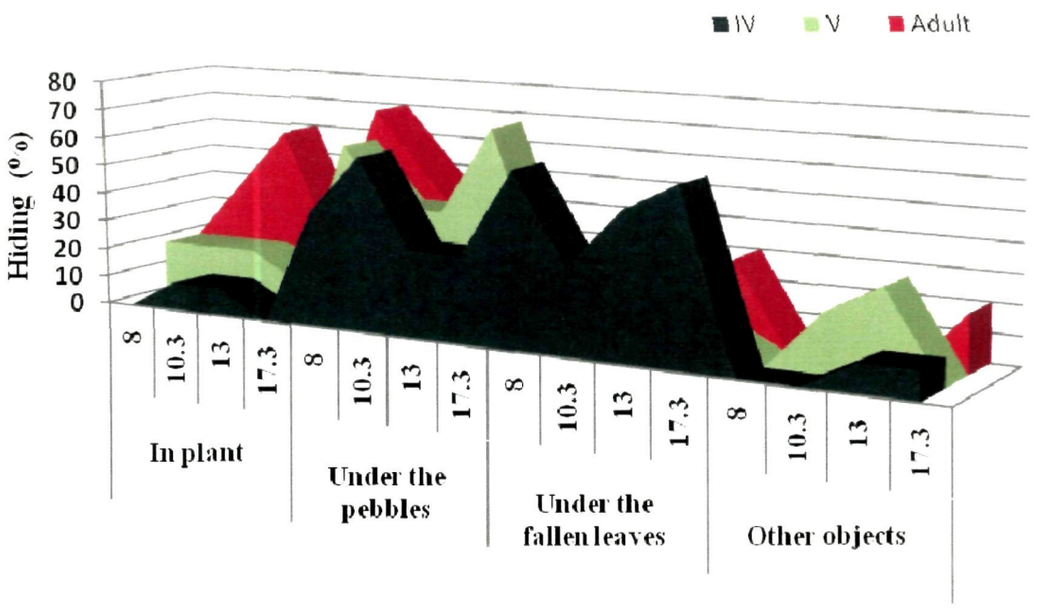

Figure 7. Hiding behavior of *R. longifrons* fourth (IV) and fifth (V) stadium nymphs and adult (male and female) released from morning 6 am to 3.30 pm under screen house condition

Table 26. Hiding location selection (%) of *R. longifrons* life stages released from 6.00 AM to 3.30 PM and observed after two hours of release under screen house condition (n=10)

Predator stage	Hiding location	Observation time				Mean
		8.00	10.30	13.00	17.30	
IV	In plant	0	10	10	0	5.0±2.9
V		20	20	20	10	17.5±2.5
Adult		20	40	60	20	35.0±9.5
IV	Under the pebbles	40	60	30	30	40.0±7.0
V		60	40	40	70	52.5±7.5
Adult		70	50	30	30	45.0±9.5
IV	Under the fallen leaves	60	30	50	60	50.0±7.0
V		20	20	10	10	15.0±2.9
Adult		10	10	10	30	15.0±5.0
IV	Other objects	0	0	10	10	5.0±2.9
V		0	20	30	0	12.5±7.5
Adult		0	0	0	20	5.0±5.0

4.5.3. Augmentative releases of *R. longifrons* irrigated and rain fed cotton field

Bioefficacy of *R. longifrons* was evaluated in field condition from May 2011 to August 2011 and also from December 2011 to February 2012 under irrigated conditions and rain fed condition, respectively. The most abundant pest insects (young ones and adults alone) in cotton field were *A. gossypii, D. cingulatus, P. solenopsis* and *H. armigera* in both cultivable condition. Hence we mainly concentrated on these pests throughout our observations. In the irrigated field, *A. gossypii* incidence varied from 109.6 to 65.7/plants ($F= 1.246$; df= 92, 57; $P< 0.05$); 0.90 to 0.04/ plant for *D. cingulatus* ($F= 0.165$; df= 6, 143; $P< 0.05$); 0.49 to 0.01/ plant for *H. armigera* larvae ($F= 1.107$; df= 146, 3; $P<0.05$). *Phenacoccus solenopsis* ($F= 1.702$; df= 26,123; $P< 0.005$) population increased during the third release when compared with first release (Fig. 8, Table 27). However, the release of the predator did not decrease *P. solenopsis* population during the third releases. In contrast, predators highly reduced the pest population after second release (11.8%, 17.9%, 26.0% and 50% for *A. gossypii, D. cingulatus, P. solenopsis* and *H. armigera*, respectively) (Fig. 12).

In rainfed condition, *R. longifrons* controlled the *D. cingulatus* population only after the third release ($F= 3.252$; df= 9,140; $P<0.05$), and the same trend was also been recorded for *A. gossypii* ($F= 1.926$; df= 99,50; $P<0.05$) and *P. solenopsis* ($F= 2.024$; df= 21, 128; $P< 0.05$) (Fig. 9, Table 27). The total *D. cingulatus* population reduction was higher (53.80 %) than *A. gossypii* (13.1 %) under rainfed condition rather than irrigated cotton field condition (17.9% and 11.8% for *D. cingulatus* and *A. gossypii*, respectively) and *P. solenopsis* (26.0%) population reduction was also observed in irrigated cotton field condition (Fig. 12).

Figure 10 (B) shows the population of the natural enemies for irrigated condition cotton field. The results reveal that reduviid population was higher in *R. longifrons*

Table 27. Augmentative release of *R. longifrons* at thrice on pests population before and after release in two cotton field condition

Field condition	Pest	Control				Treatment			
		First release	Second release	Third release	Mean	First release	Second release	Third release	Mean
Irrigation	*D. cingulatus*	0.90±0.14*	0.14±0.03*	0.13±0.03*	0.48±0.06	0.68±0.10*	0.14±0.03 NS	0.04±0.01*	0.39±0.06
	H. armigera	0	0.49±0.12 NS	0	0.004±0.003	0	0.01±0.007 NS	0	0.002±0.002
Rain fed	*D. cingulatus*	0	0	0.17±0.07*	0.05±0.02	0	0	0.080±0.05 NS	0.02±0.01

* Significant at 5% level , NS- Not significant

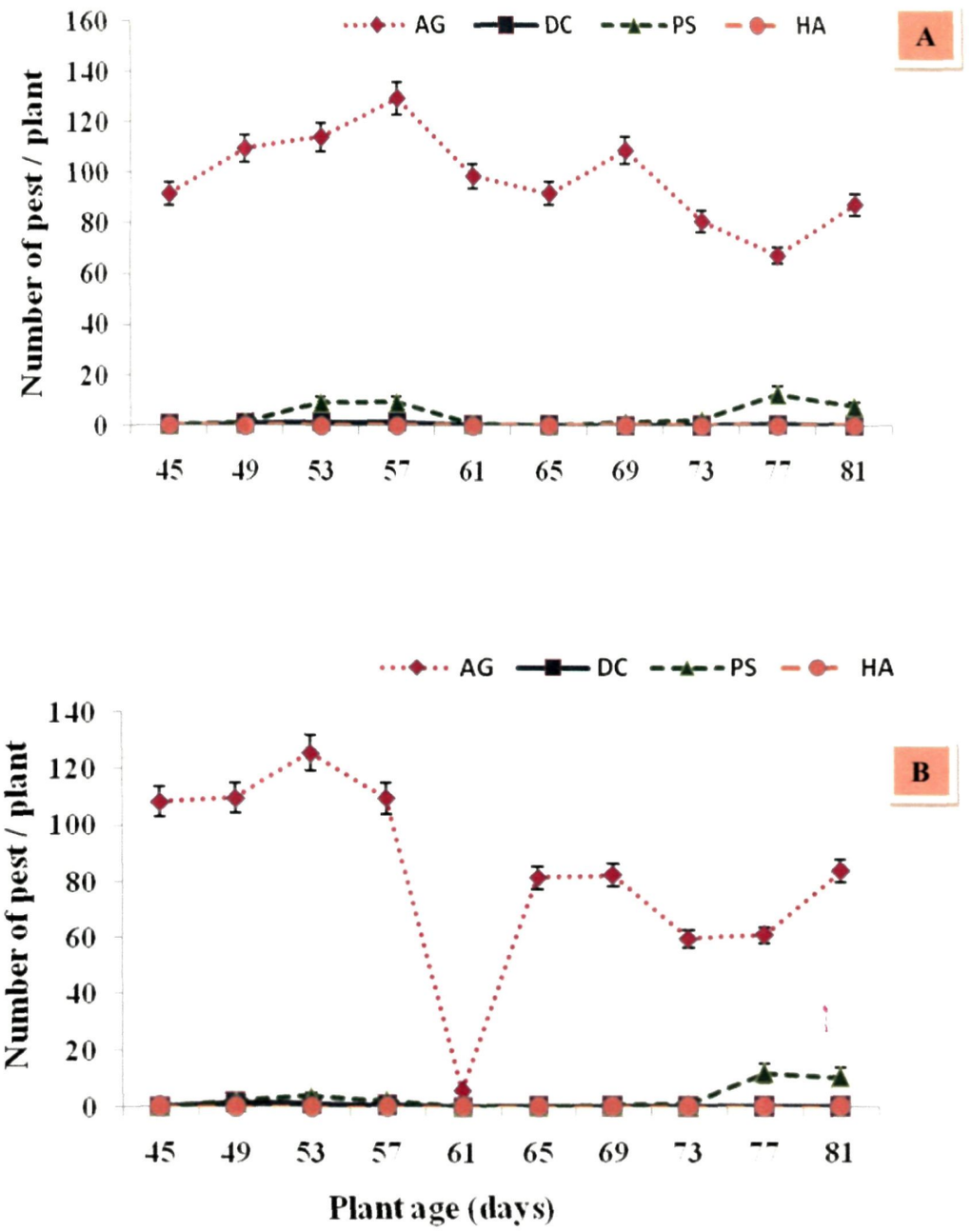

Figure 8. Augmentative releases of *R. longifrons* on the *A. gossypii* (AG), *D.* cingulatus (DC), *P. solenopsis* (PS) and *H. armigera* (HA) population (number/plant) under irrigated condition. Control (A) and Experiment (B).

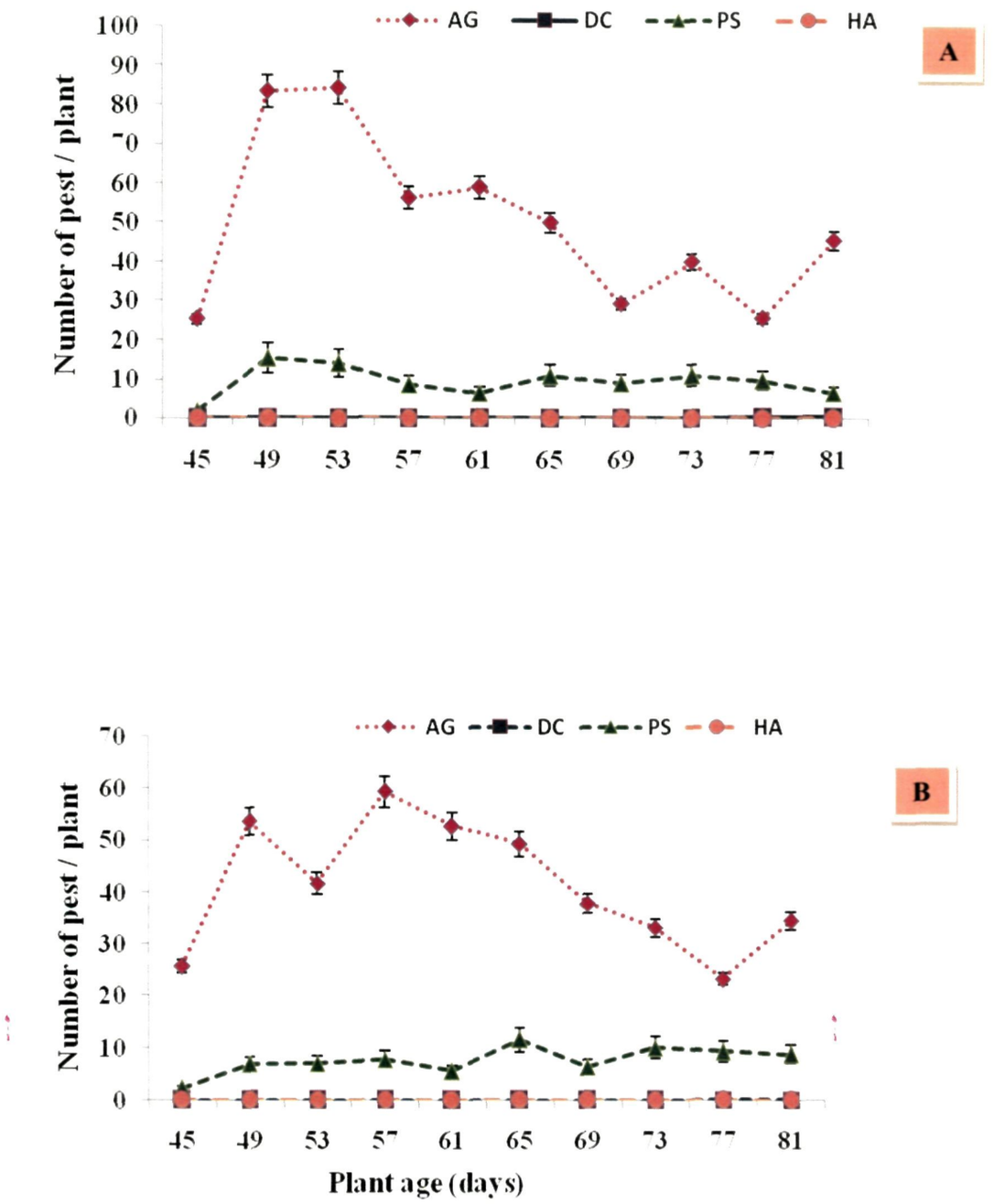

Figure 9. Augmentative releases of *R. longifrons* on the *A. gossypii* (AG), *D.* cingulatus (DC), *P. solenopsis* (PS) and *H. armigera* (HA) (number/plant) under rainfed. Control (A) and Experiment (B).

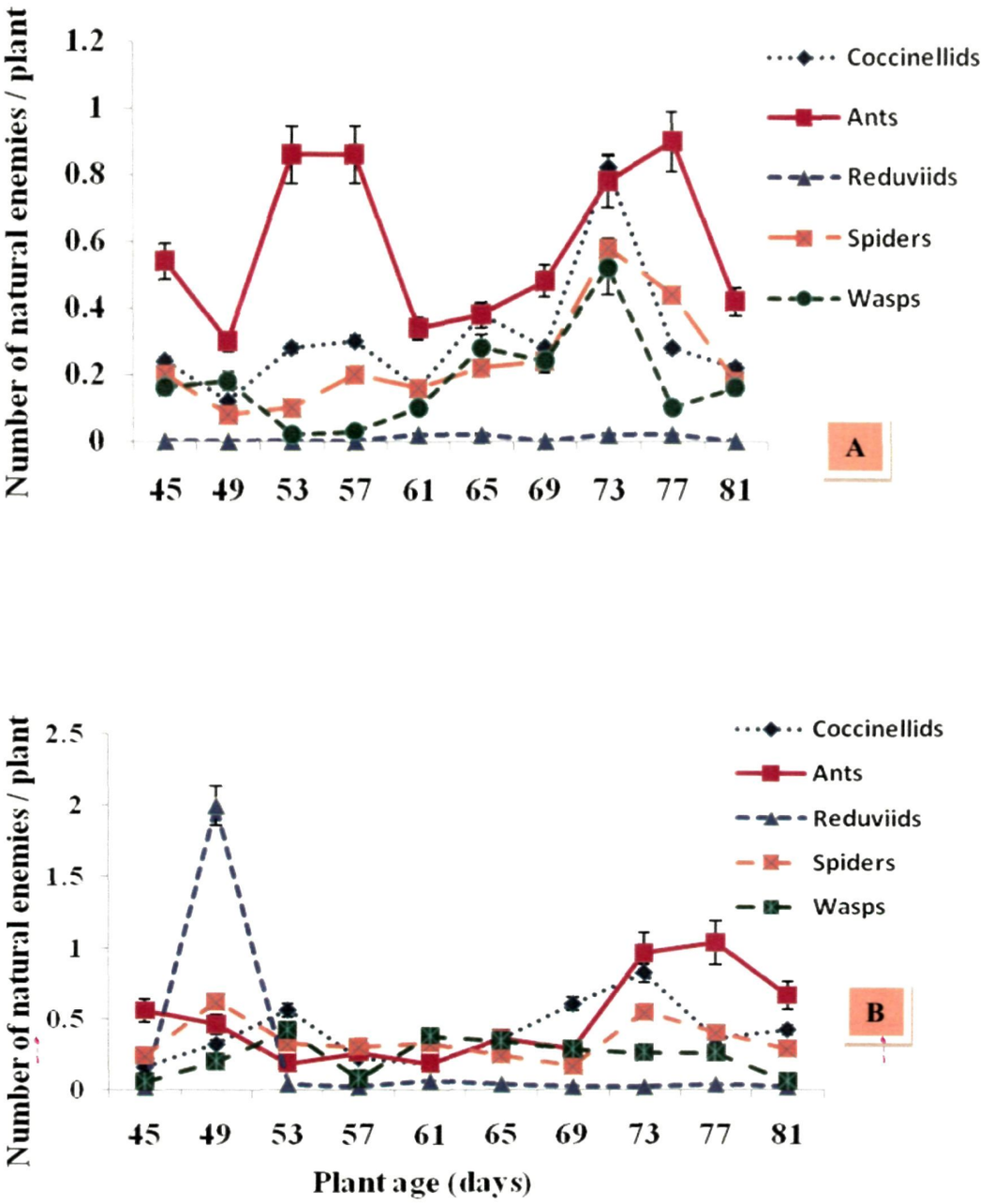

Figure 10. Augmentative releases of *R. longifrons* on the natural enemies population (number/plant) under irrigated condition. Control (A) and Experiments (B).

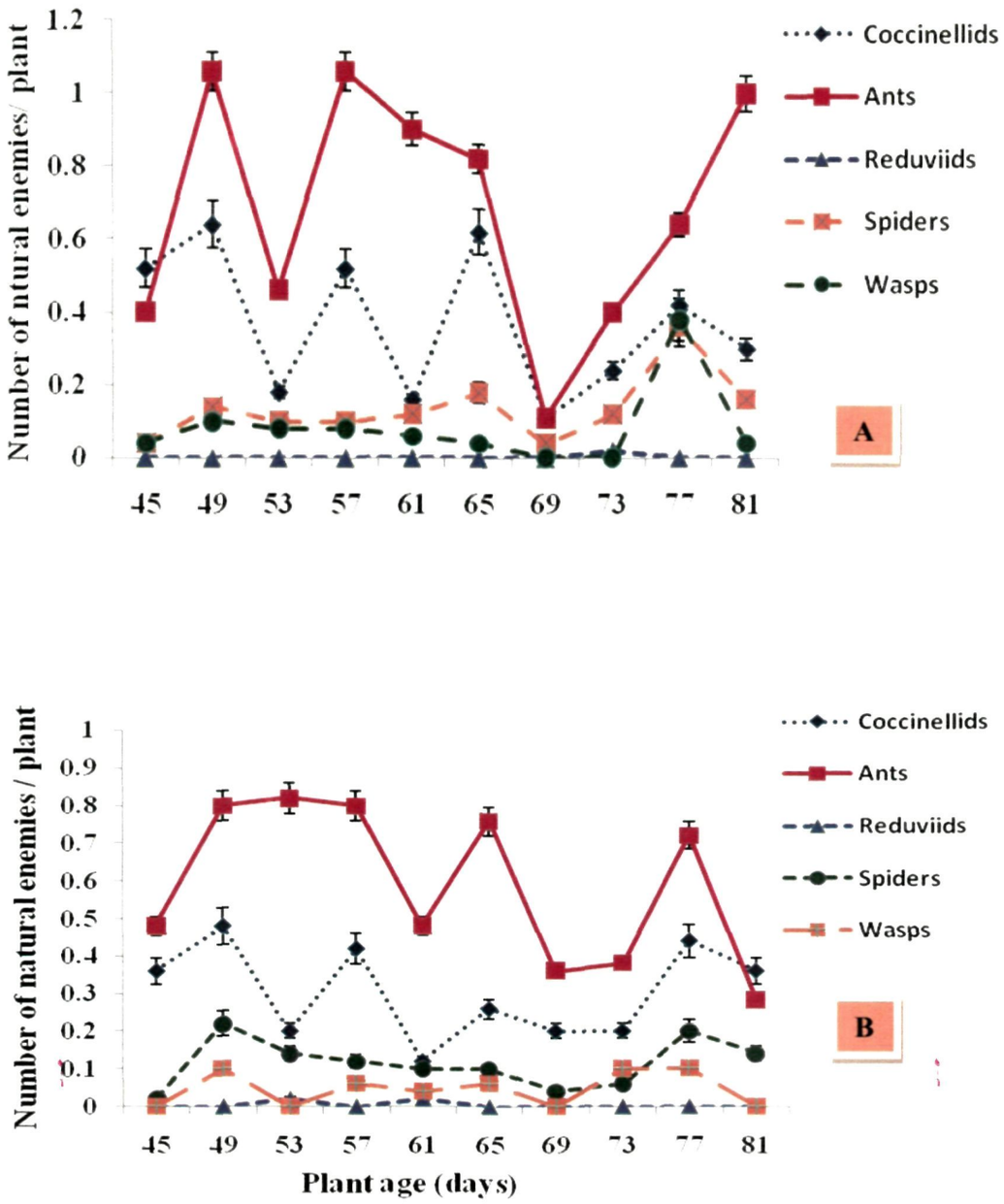

Figure 11. Augmentative releases of *R. longifrons* on the natural enemies population (number/plant) under rainfed condition. Control (A) and Experiments (B).

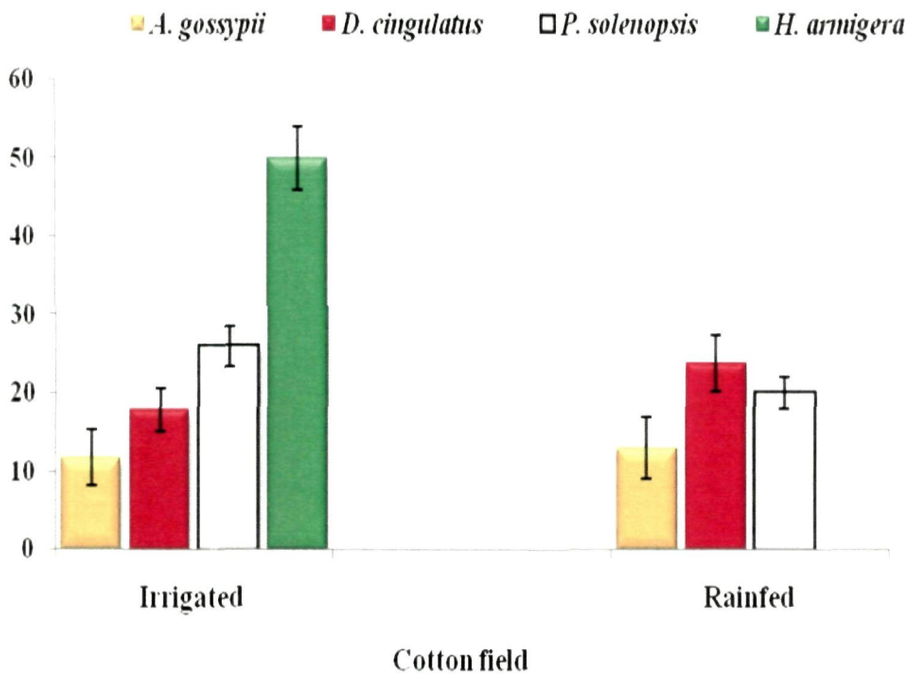

Figure 12. Augmentative release of *R. longifrons* on cumulative *A. gossypii, D. cingulatus, H. armigera* and *P. solenopsis* populations reduction (%) at irrigated and rainfed condition

released cotton fields than in the control. Similar result was also observed in the rainfed condition cotton field (Fig. 11 B).

4.5.4. Cotton production, Cost Benefit Ratio (CBR) and Percent avoidable loss

The production of cotton was higher in predator released field (837.0 and 753.35 kg/h^{-1} for irrigated and rain fed condition, respectively). Similarly, the cost benefit ratio was higher in the predator released field (1: 1.28) than in the control field (1: 1.17) in irrigated field condition and also in rainfed (1:1.24) condition (Table 28). The percent avoidable loss was high in irrigated condition cotton field (14.5%) when compared with rainfed condition cotton field (4.9%).

4.6. DISCUSSION

Ectrichodiines insects are diurnal (Ambrose, 1999), whereas Peiratinae and Emesineae are nocturnal as light attracts reduviid genus. No information is available about under field condition, whether reduviids are nocturnal or diurnal. We took an initiative to find out the preliminary observation regarding the same. The results reveal that based upon the availability of prey and stage of the predator, predatory rate changed from day time to night time. However, fourth, fifth and adult stages of *R. longifrons* consumed more number of *D. cingulatus, P. solenopsis* and *H. armigera* during day time.

4.6.1. Hiding

The hiding behaviour of reduviid predator reveals that more number of adult individuals hide under pebbles, followed by those hiding under the leaves (Ambrose, 1999), under shrubs, under the boulder, ground, herbs, shrubs, on the bark and foliage of trees in that order (Sahayaraj, 2007). Reduviid, *Zelus longipes* hid inside foliage (Kalsi and Seal, 2011) and *Scipinnia repax* (Harland and Jackson, 2004; Jackson, *et al.,* 2010) in order to find its prey. Similarly preying mantids (a generalist predator) hid behind leaves

Table 28. Augmentative release of *R. longifrons* on cost benefit ratio analysis under irrigated and rainfed cotton cultivation.

Pest	Field condition			
	Irrigated condition		Rain fed condition	
	Treatment	Control	Treatment	Control
Plough (Rs.)	3705.00	3705.00	2470.00	2470.00
Seed (Rs.)	-	-	-	-
Sowing (Rs.)	2034.00	2034.00	1729.00	1729.00
Manure (Rs.)	5775.00	5775.00	3087.00	3087.00
Harvesting (Rs.)	3800.00	3800.00	3705.00	3705.00
Transportation (Rs.)	-	-	3978.00	3978.00
Weeding (Rs)	5928.00	5928.00	6175.00	6175.00
Production cost of *R. longifrons* (Rs)	1444.0	-	1444.0	-
Total expenses (Rs.)	22686.00	21242.00	22588.00	21144.00
Total Income (Rs.)	29096.00	24872.00	28134.00	26652.00
Cotton production (kg/h^{-1})	837.0	715.5	753.35	716.30
CBR	1.28	1.17	1.24	1.26
The percent avoidable loss (%)	14.5		4.9	

and inflorescences (Sampaio *et al.*, 2008) as observed in our results, whereas *R. longifrons* preferred to hide under small pebbles naturally present in the cotton agroecosystem. The predator preference to inhabit under the litters indicates we would not alter the natural ecosystem for the habitat selection. However, Claver *et al.* (2003) and Ganesh Kumar (2011) altered the natural condition and provided large size stone or palm leaves or banana leaves to facilitate the hiding of reduviids. *Platymerus rhadamanthus* Gerst (Vanderplank, 1958) prefer to hide in shady parts of the crown of the coconut palm as 10 – 12 % of *R. longifrons* preferred shady places in cotton agroecosystem too.

4.6.2. Pot study

In the pot study our result revealed that *R. longifrons* consumed higher number of *H. armigera* (Lepidopteran pest) than *A. gossypii, D. cingulatus* and *P. solenopsis* (Hemipteran pests). The similar trend was observed by Ganesh Kumar (2011). He reported that *R. longifrons* consumed more number of prey in *Spodoptera litura*, *H. armigera* and *Achaea janata* than hemipteran pest *D. cingulatus* and coleopteran pest *Mylabris pustulata*. Wyss *et al.* (1999) and Muzammil (2010) have observed that aphidophagous predator showed potential control against *A. gossypii*, this results was resemblance with our pot study. Size dependant predatory potential was observed, third instar *R. longifrons* consumed more number of *A. gossypii* than other stages of predator.

4.6.3 Augmentation

Phenacoccus solenopsis (pest) population encountered during irrigated condition was higher, ranging between 6 and 0.92 (pest) per plant than that observed under the rainfed condition. Monthly augmentative release of *R. longifrons* at 9250 predators h^{-1} succeeded in suppressing *D. cingulatus* and *H. armigera* population on irrigated and rainfed cotton, respectively. Although not statistically significant, the efficacy of *R. longifrons* release was not most pronounced on *P. solenopsis*. It was 26% (irrigated) and

20% (rainfed) respectively lower than non release cotton field. Similarly, the field release of *Pristhisancus plagipennis* (Grundy and Maelzer, 2000; Grundy, 2004), *P. laevicollis* (Antony *et al.,* 1979) and *R. marginatus* (Sahayaraj, 1999, 2002b; Sahayaraj and Martin, 2003; Sahayaraj and Ravi, 2007b) were successful in reducing various pests such as *S. litura*, *H. armigera* and *Aphis craccivora* in cotton and groundnut field. Sahayaraj and Martin (2003) reported that *R. marginatus* controlled *H. armigera* and Aphids population more significantly than other pests of groundnut. Grundy and Maelzer (2000) and Grundy (2004) reported that *Pristhisancus plagipennis* has reduced whatever cotton pest available in the agroecosystem.

Results of this study suggest that *R. longifrons* can survive, develop and reproduce in the cotton agroecosystem after the release. Muzammil (2010) reported the field release of *Chrysoperla carnea* (Neuroptera: Chrysopidae) reduced the *A. gossypii* population (75.02%) in cotton field. We also observed that *R. longifrons* reduced *A. gossypii* population in cotton field by 11.8% and 13.1% in irrigated and rain fed condition, respectively which indicates that this predator can be released either alone or along with other BIPM components. No adverse interaction between *R. longifrons* and indigenous predators like ants, spider and coccinellids were detected. Hence reduviids can be utilized as part of a multiple species release programme as suggested for *Dephastus catalinae* (Horn) (Coccinellidae) (Heinz *et al.,* 1999). The CBR is very low (1:1.22) while compare to microbial insecticides like HaNPV (1:3.50) or Bt (1:1.23) in cotton (Praveen and Dhandapani, 2001; Balakrishnan *et al.,* 2004). But, for reduviids the CBR is 1:1.28 or 1:1.24 which indicates the possibilities of integrating this generalist predator in cotton pest management. Similarly cotton production was increased by the release of *P. plagipennis* (Grundy, 2004; 2007; Grundy and Maelzer, 2000).

4.7. CONCLUSION

1. From hiding behaviour of *R. longifrons,* we concluded that the predator hide during day time and predate more number of prey.
2. Similarly in pot study same trend was observed this clearly indicates that the predator may be highly potential in morning hours at field condition.
3. Prey suppression ability of the predator gradually increased up to the ninth day, and then the preys stabilized their population. *Rhynocoris longifrons* increased the cotton production and enhanced the cost benefit ratio increased in predator released cotton field.
4. It is concluded that reduviid predators can be integrated in cotton Bio-intensive Integrated Pest Management Programme (BIPM).

SUMMARY

SUMMARY

Chapter 1 deals with the survey of reduviid predators and their dominant as well as common preys of cotton in relation with climatic factors [temperature, rain fall, relative humidity (RH) and wind velocity] in Tirunelveli, Thoothukudi, Kanyakumari, Theni, Virudhunagar, Sivagangai and Madurai districts of Tamil Nadu, India. Six reduviid (*Acanthaspis* sp., *Ectomocoris* sp., *Lophocephalus* sp and *Rhynocoris* spp.) genus were recorded from cotton fields (MCU 5,MCU 7, SVPR II, SVPR IV, Bunny and local variety). More number of reduviids (65) was recorded at Kanyakumari district, followed by Tirunelveli district (43). *Acanthaspis pedestris, Rhynocoris fuscipes, Rhynocoris longifrons* and six dominant pests were collected common and abundant in all the districts.

Biology and life table traits of *R. longifrons* and estimation of biochemical of their pests were highlighted in chapter 2. *Helicoverpa armigera* was the most suitable prey for this predator. Because it reduce the total developmental period (45 days), enhanced the reproduction, enhanced sex ratio (1:0.54), minimized nymphal survival rate (54%). The protein (13 mg/100 mg) and lipid content were higher (25.1 mg/100 mg) in *H. armigera,* whereas the carbohydrate content was higher (22 mg/100 mg) in *P. solenopsis*.

Chapter 3 deals with the bioefficacy of *R. longifrons* on four cotton pests such as *D. cingulatus, H. armigera* and *P. solenopsis*. Stage preference of third, fourth and fifth nymphs preferred second instar nymphs of *D. cingulatus* and second instar larvae of *H. armigera* and adult of *R .longifrons* preferred third instar nymphs of *D. cingulatus* and third instar larvae of *H. armigera*. Nymphal instar and adults of *R. longifrons* preferred *P. solenopsis* adults. Host preference was observed by visual and

kairomone response was favour to *H. armigera* followed by other cotton pests. GC-MS study of *H. armigera, D. cingulatus, P. solenopsis* and *A. gossypii* kairomone. The predominant bioactive compounds identified were celidoniol (21.99%), dodicamethyl (72.06%), dodecanoicacid (12.80%) and dotriacontane (14.54%) for *H. armigera, D. cingulatus, P. solenopsis* and *A. gossypii,* respectively. The functional response of *R. longifrons* on different densities of *D. cingulatus* (y=0.39+0.44x; r=0.98) was showed. Maximum number of prey (8 prey/ predator) attacked in 16 number densities was recorded for the adult predator on *D. cingulatus* was higher than third (3.8 prey/ predator), fourth (3.8 prey/ predator) and fifth (5.4 prey/ predator) nymphal instars, whereas the third instar predator consumed maximum number of *A. gossypii* (10.6 preys/ predator) at 40 number densities.

The hiding behaviour, pot evaluation and augmentative release of *R. longifrons* in cotton field were investigated in chapter 4. The adult *R. longifrons* showed maximum predatory rate against the *H. armigera* (2.66 preys/predator) followed by *D. cingulatus* (2.16 preys/ predator) and *P. solenopsis* (2.5 preys/ predator) in both morning and evening study under the pot evaluation. In variably during day time the predator showed more consumption rate when compared with evening. In hiding behaviour of *R. longifrons,* most of the predator life stages were preferred pebbles followed by fallen leaves and plants. Laboratory reared *R. longifrons* was augmentative released in to the cotton field and evaluated its impact in terms of pest and cotton production. It reduced the cotton pests such as *D. cingulatus* (17% and 53 % for irrigated and rain fed, respectively), *P. solenopsis* (26.0% and 20.7% for irrigated and rain fed, respectively), *A. gossypii* (11.8% and 13.1% for irrigated and rain fed, respectively) and *H. armigera* (50 % for irrigated cotton field) and the cotton production was higher in irrigated condition (837.0 kg/ h^{-1}) than the rainfed (753.35

kg/ h^{-1}) and increased the yield of cotton and CBR (1.28 and 1.24 for irrigated and rain fed, respectively). The percent avoidable loss was high in irrigated condition cotton field (14.5 %) when compared with rainfed condition cotton field (4.9%).

FUTURE RECOMMENDATIONS

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1. Mass production technology could be invented
2. Bioefficacy of *R. longifrons* will be evaluated against other economically important agricultural pests.
3. Prey-predator interaction of this reduviid will be studied using molecular tools.
4. The compatibility of *R. longifrons* with botanicals and chemical pesticides will be evaluated laboratory and field conditions.

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ANNEXURE

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Evaluation of nymphicidal and ovicidal effect of a seaweed, *Padina pavonica* (Linn.) (Phaeophyceae) on cotton pest, *Dysdercus cingulatus* (Fab.)

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Impact of brown seaweed, *Padina pavonica* (Linn.) chloroform, and benzene extracts were evaluated against an economically important cotton pest, *Dysdercus cingulatus* (Fab.) (Pyrrhocoridae). The result reveled that between the two solvents, benzene extracts of *P. pavonica* caused more nymphal mortality. It reduce *D. cingulatus* egg hatchability than chloroform extract. Benzene extract also reduced the survival rate of *D. cingulatus* eggs from 53.33 to 0.00 per cent for 0.025 to 0.4% concentration of *P. pavonica* benzene extract respectively. *P. pavonica* extracts also significantly reduced the total body protein (22 to 39%) and DNA (27 to 30%) content. Presence of saponin, steroids and phenolic compounds in the extract might be the reason for these activities. It is concluded that *P. pavonica* possess both nymphicidal and ovicidal activity. It can be utilized for the management of sucking pests of cotton and other crops.

[Keywords: Seaweed, Cotton pest, Nymphicide, Ovicide, Macro molecule]

Introduction

Dysdercus cingulatus (Fab.) is a serious pest of cotton and distributed all the cotton growing region of India[1-3]. It is difficult to control by insecticide because it is highly mobile, Polyphagous[4] and Polymorphic[5] pest of many Malvaceae crops. Terrestrial plants like *Catharanthus roseus* G. Don, *Parathenium hysterophorus* Don and *Nephroleps* extracts were have insecticidal activity against red cotton bug[6-8]. Moreover, neem based pesticide like neem gold[9] also shows nymphicidal activity against this pest. Ovicidal activity of *Pedalium murex* (Linn.)[10] on *D. cingulatus* was reported earlier.

Extracts of the red alga *Polcamium cartilagineum,* and *P. violaceum* from California exhibited insecticidal activity against tobacco horn worm[11]. Insecticidal activity of two halogenated monoterpenes, isolated from the red alga, *Plocamimum cartilagimeum*, and two derivatives (dibromomortensene and dihydromertensene) also showed insecticidal activity on tomato moth, *Tuta absolute* and green bug, *Schizaphis graminum*[12]. Studies also reveal that some *Padina* species (Phaeophyceae) showed antibacterial, antifungal, phytotoxic and insecticidal activities[13]. However, information about the insecticidal activity of *Padina Pavonica* (Linn.) was not available in the literature. Present study was conducted to evaluate the impact of chloroform and benzene extracts of *P. pavonica* on nymphal and egg mortality, nymphal development, morphogenesis and total body protein and DNA content of *D. cingulatus*. In addition, preliminary photochemical screening of the seaweed was also recorded.

Material and Methods

The seaweed, *P. pavonica* was collected from Thoothukudi, and Kanyakumari districts coasts of Tamilnadu, India. To remove salts and micro algae, *P. pavonica* leaves were washed thrice with fresh water and once with distilled water. Then shade dried for two weeks continuously and dried seaweed was partially powdered using domestic blender. 100 mg of partially powder weed (PPW) was packed in Soxhlet apparatus and refluxed with chloroform 800 ml (60-80°C), benzene 800 ml (50-60°C) for 12 hours each continuously. The extracts were dried over sodium sulphate, solvents received under reduced pressure and stored at 20°C until further use. Chloroform and benzene extracts were labeled as PPCE and PPBE respectively. Five concentrations each (0.1, 0.2, 0.4, 0.8 and 1.6% for chloroform), (0.025, 0.05, 0.1, 0.2 and 0.4% for benzene), were prepared using respective solvents and used for this experiments. The qualitative photochemical screening of *P. pavonica* was carried out using standard procedures[14,15].

Nymphs and adult of *D. cingulatus* were collected from cotton fields of Tirunelveli district, and Theni district, Tamilnadu, India. They were maintained under laboratory conditions (28 ± 2°C, 70 –75% RH, 11L and 13D hrs photoperiod) in plastic container (300 ml capacity) on water soaked cotton seeds. The laboratory emerged third instar nymphs and laboratory laid eggs were used for the experiments.

Uniform sized third instar *D. cingulatus* were randomly selected from the stock culture and three insects were placed in the plastic container (360 ml capacity). Ten cotton seeds were soaked in different concentration of the extracts separately for overnight and provided to the test insects. Insects were allowed to feed *P. pavonica* extract soaked cotton seeds for 96 hours continuously. Twenty replications (n=60) were maintained for each concentrations. Mortality was corrected using Abbott's formula[16] if any mortality was recorded in the control category. Then the data was subjected to probit analysis[17]. After 96 hrs, alive nymphs were provided with water soaked cotton seeds till their death.

Freshly laid *D. cingulatus* eggs were collected from the laboratory culture for the experiment. Whatmann no.1 filter paper was placed at the bottom of the Petri dishes (9cm diameter) and placed 10 eggs over the paper. *P.pavonica* extracts were sprayed over the egg (2ml/10 eggs/Petri plate). During spraying eggs were rotated and see that all parts should be adequately contacted with the extract. The control category was sprayed with water alone. Six replications (n=60) were maintained for each concentration and control categories.

Immediately after the death, insects were kept in hot air oven at 50°C for three to four hours for protein estimation. Five gram of the dried sample was grinded well using mortar and pastel with 1 ml of phosphate buffer (pH 7.2). Centrifuge the sample at 1000 rpm about 15 minutes, collect the supernatants and utilized for the estimation of total body protein[18,19] using BSA as standard. Pipette out 0.1, 0.2, 1.0 ml of the standard protein solution into a series of test tubes. The volume of the standard was making up to 1ml by adding distilled water. A test tube with 1 ml of distilled water was served as blank. Add five ml of protein reagent to each test tube including the blank. Mix well and allow standing for 10 minutes. Similarly test insect sample was also treated and allow them for incubation between five to 10 minutes. Finally the absorbance was red at 595 nm against blank as check. The optical density was compared with the standard graph to estimate protein quantity and expressed in mg/100 mg dried sample. For DNA extraction, insect stored in -20° C was used. DNA was successfully extracted and quantified by Sambrook[19] method.

Results

When PPBE was treated with *D. cingulatus* egg, the survival rate was gradually diminished from lower concentration to higher concentration (0, 16.6, 36.6, 40.0, 53.3 and 90 percentage for 0.4, 0.2, 0.1, 0.05, 0.025 per cent concentrations and control respectively). However, irrespective of PPCE, eggs precede their embryogenesis up to third day, and then it was stopped.

First and second instar nymphs of *D. cingulatus* seldom present on the floor of the cotton field. Then they dispersed in to the cotton plant and start to damage the plant. Hence we selected third instars *D. cingulatus* nymphs for toxicity evaluation. PPBE was highly toxic (LC_{50}=0.004%) than chloroform extract (LC_{50}=0.039%). Similar trend was also recorded for LC_{30} and LC_{90} (Table 1).

Table 1—Impact of *Padina pavonica* extracts on regression equation, LC_{50} values and fiducial limits parameters of *D. cingulatus*

Exposure Time (in hour)	Regression Equation	LC_{30}	LC_{50}	LC_{90}	Variance	Chi-square
		Chloroform extract				
24	Y = 5.349 x+ 6.46	1.1754	1.387	1.6364	0.0013	0.00
48	Y = 3.419 x+ 1.85	0.8392	1.011	1.2184	0.0017	26.05
72	Y = 2.293 x+ 0.89	0.4815	0.621	0.8009	0.0032	20.72
96	Y = 1.728 x+ 3.00	0.0359	0.039	0.045	0.0123	0.0941
		Benzene extract				
24	Y = 1.083 x + 3.36	0.1649	0.330	0.6605	0.0237	10.22
48	Y = 0.754 x + 4.71	0.0130	0.024	0.0760	0.3830	31.62
72	Y = 1.096 x + 4.75	0.0180	0.017	0.018	0.0611	15.29
96	Y = 1.063 x + 5.40	0.0030	0.004	0.0084	0.3220	1.85

Total body protein of *D. cingulatus* was significantly reduced by PPCE (20.4mg/100mg) and PPBE (16.1mg/100mg) ($P<0.05$) compared to the control (26.4 mg/100mg). Similar impact was also observed in total body DNA content (3.84µg/µL and 4.02µg/µL for chloroform, benzene respectively) of *D. cingulatus* were as in control the total body DNA content was 5.52µg/µL.

The preliminary phytochemical analyses of *P. pavonica* chloroform and benzene extracts revealed that steroids, saponins and phenolic compound were present in both the solvent extracts. Whereas triteriphenoids, alkaloids, tannin and flavonoids were absent. Reducing sugar and xanthoprotein were absent in PPCE extract and carbohydrate was absent in PPBE. The qualitative test was done for identifying different components in the plant extracts by using[14,15] methods.

D. cingulatus nymphs took 16.6 days to attain in to adult. It was insignificant ($P<0.05$) at 0.1 and 0.2 percent PPCE treated seed feed *D. cingulatus*. Other three concentrations of PPCE, *D. cingulatus* developed only up to fourth instars and their nymphal period was significantly ($P<0.05$) prolonged both in 0.8 and 1.6 percent concentrations (Table 2). In PPBE, *D. cingulatus* developed up to fourth instar except at 0.4 percent concentration of *P. pavonica*. All the concentrations extends the nymphal period significantly ($P<0.05$) except at 0.025 percent PPBE (Table 2).

Discussion

Impact of chloroform and benzene extracts of *Padina pavanica* were evaluated against *D. cingulatus* nymphs and eggs. Red alga *Plocamimum cartilagineum*, caused 91% mortality after 48 hrs exposure[12]. However, this study revealed that benzene (86%) and chloroform (96%) extracts caused more than 85 per cent mortality at 72 hrs and 48 hrs after the exposure respectively. Similarly *Osmundae pinnatifida* showed low insecticidal activity[20]. Brown algae of the family Dictyotaceae produce a new diferpene dictyo crenulol, which possesses insecticidal activity against tomato moth *Tuta absoluta* [21]. In addition to the defoliators, sucking pest like *Aphis fabae* was also controlled by *Plocamium cartilagineum*[22,23]. Present study revealed that in addition to the nymphicidal activity, *P. pavonica* either reduced or increase the nymphal developmental period. This is showed that *P.pavonica* extracts interphere with physiology of *D. cingulatus*.

D. cingulatus eggs are elliptical, creamy white in colour with a thick chorion. During embryogenesis creamy white colour changes to yellow on early development (fourth day) and to orange on fifth day or on nearly sixth day. Application of PPCE & PPBE of *P. pavonica* elicited a dreadful impact on *D. cingulatus* embryogenesis. All the eggs were changed their colour from creamy white to yellow and then shrinked completely. It showed that all the tested concentration benzene extract of *P. pavonica* showed higher ovicidal activity. However, chloroform extracts also showed ovicidal activity in dose dependent manner. This might be due to the degrees of morphogenetic malformation in the recipient pest embryo was found to be dose dependent and also showed the anti-juvenile hormonal compounds present in the *P. pavonica* chloroform extract to stop

Table 2—Chloroform and benzene extracts of *Padina pavonica* on the nymphal developmental period (in days) of *D.cingulatus*

Solvents	Concentration	Up to fourth instar	Up to fifth instar	Up to Adult
Chloroform	Controls	6.62 ± 0.58	11.60 ± 0.7	16.62 ± 0.6
	0.1	-	-	16.83 ± 0.3 ^{NS}
	0.2	-	-	16.91 ± 0.1 ^{NS}
	0.4	-	11.71 ± 0.3 ^{NS}	-
	0.8	-	12.80 ± 0.1*	-
	1.6	-	13.60 ± 0.3*	-
Benzene	0.025	-	11.80 ± 0.2*	-
	0.05	-	12.32 ± 0.1*	-
	0.1	-	3.13 ± 0.5*	-
	0.2	-	14.32 ± 0.4*	-
	0.4	7.81 ± 0.7*	-	-

NS - indicates note significant ; * - show significant at 5% level.

the embryonic development of *D. cingulatus*. The effects were higher when the benzene extract was used in high concentrations (0.05 to 0.4%) and was brought into contact with the embryo in very early as reported in *D. cingulatus* by[24] and the authors reported that juvenile hormone is essential for the normal development of *D. cingulatus*. Bioactive principles present in *P. pavonica*, blocked either at germ bund stage, or at blastokinesis stage. Similar impact was also reported by in *D. cingulatus* when exposed to root extracts of *Pedalium murex*[10].

Proteins are an integral part of the cuticle and play an important role in metamorphosis and insect growth. Our study shows that *P. pavonica* extracts significantly reduced the total body protein. Previously it was reported that seed extracts of *Annona*[25], leaves extracts of *Lantana wightiana, Premna tomentosa* and *Synedrella nodiflora*[26] reduced the total body protein content of the tested insect. The plant extracts usually reduce the macromolecular content and they were fed along with the natural food of the host[27]. *Porteresia coarctata* Takeoka leaf extract at different concentration showed significant reduction in protein and DNA content in the fat body and midgut tissues[28].

Benzene extract caused mortality, arrest the nymphal development, highly reduce total body protein content whereas chloroform extract interfere with the embryonic development and reduce whole body DNA content (30.43%). Both extracts can be used in pest management mainly sucking type of mouth parts.

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Bioefficacy of *Rhynocoris fuscipes* on three cotton hemipteran pests

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Abstract

Rhynocoris fuscipes (Fab.) (Hemiptera: Reduviidae) is a reduviid predator of many agricultural crop pest. A laboratory experiments was conducted to evaluate the bioefficacy of this predator using functional response as a tool. Third, fourth and fifth instar nymphs and adults of *Rhynocoris fuscipes* on three hemipteran pests such as, *Dysdercus cingulatus* (Fab.), *Aphis gossypii* (Glover) and *Phenacoccus solenopsis* (Tinsley) were evaluated. Predatory rate gradually increased from the third instar nymph to adults. *Rhynocoris fuscipes* killed and consumed more number of preys in high densities (4.4, 4.2, and 5.8 preys/ predator on *D. cingulatus, A. gossypii and P. solenopsis* respectively). Rate of discovery gradually diminished when the predator grew older. Handling time was also diminished when the predator grew older. When the predator preys upon these pests, *R. fuscipes* showed an exponential (Type II) functional response. Type II functional response is more pronounced in *D. cingulatus* than other hemipteran pests. Such different patterns showed that this needs to adapt distinct strategies according to the kind of prey available in the field. Biological control potential of *Rhynocoris fuscipes* on these three hemipteran pest shows that the, searching time, handling time and number of prey killed depended upon the prey densities and predator life stages hence this predator can be utilized for the management of the chosen pests. *Rhynocoris fuscipes* can be utilized to manage these hemipteran pests' particularly *D. cingulatus*.

Key words: Cotton pests, Biological control, *Rhynocoris fuscipes*.
Short title: Bioefficacy of *Rhynocoris fuscipes* on cotton hemipteran pests.

Introduction

Predation is assumed to be one of the significant biotic mortality factors reducing insect pest populations, and using them in insect pest management programs has been receiving increased attention because of the current need to reduce the exclusive use of insecticides for pest control (DeBach & Rosen 1991, Riudavents & Castane 1998, Sarmento *et al.* 2007). Functional response of a predator is one of the important factors regulating population dynamics of predator–prey systems, and functional response curves can be used to infer basic mechanisms underlying predator–prey interactions, clarify coevolutionary relationships, and enhance biological control (Houck & Strauss 1985).

Reduviids are one of the important groups of predatory insects because of their biocontrol potential and they have been receiving attention as biological control agents mainly of hemipterous species (Ambrose & Sahayaraj 1993, Sahayaraj & Ambrose 1993, 1995, 1997, Sahayaraj *et al.* 2003, Sahayaraj 2007, 2008, Sahayaraj & Asha 2010) and other insect pests (Grundy, 2007). Generally reduviids showed type II functional response curves irrespective of the predator age/stage or prey offered.

The red cotton bug, *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae),

mealy bug *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) and aphids *Aphis gossypii* (Glover) (Hemiptera: Aphididae) are the cosmopolitan species, widely distributed in tropical, subtropical and warm regions. These pests cause serious damage to cotton by feeding on developing cotton bolls and ripe cotton seeds and transmitting fungi that develops on the immature lint and seeds (Ahmed & Khan 1980). *Rhynocoris fuscipes* (Fab) (Hemiptera: Reduviidae) is a multivoltine, voracious harpactorine reduviid predator, common throughout Asia. It feeds on more than 40 insect pests of agricultural importance. In spite of its high population in many agro-ecosystems, there is no information on its consumption capacity and efficacy. Present study was carried out to determine the functional response type of life stages of *Rhynocoris fuscipes* using different densities of three major hemipteran pests of cotton in laboratory set up.

Materials and methods

Insect collection and rearing- The reduviid predator and cotton pests were collected from cotton agro-ecosystems in and around Tirunelveli and Kanyakumari districts, Tamil Nadu, India. The collected insects were maintained under the laboratory condition (31±1° C, 75±5% RH and 11-13 hours (L: D) in plastic containers (20 cm diameter) using *C. cephalonica* larvae. Laboratory emerged life stages of *R. fuscipes* were used for the experiment. Thus, individuals of the same age and size as well as with normal response were used to standardize individuals used in experiments.

Stage preference- Stage preference of third, fourth, fifth nymphal instars and adult of *R. fuscipes* against *D. cingulatus* and *P. solenopsis* life stages (except first instars) were performed under laboratory condition. For stage preference evaluation, preys were introduced in to the Petri dish (9 cm) containing cotton leaves, allowed to acclimatize for 10 minutes without any disturbances. Each nymphal instars and adult of predators were introduced in to the Petri plates separately and successfully captured and killed stages of the prey were considered as a preferred prey.

Functional response- The experiments were conducted with densities of 1, 2, 4, 8, 10 *D. cingulatus;* 1, 2, 4, 8, 16 *P. solenopsis* and 1, 2, 4, 8, 16 *A. gossypii* in Petri dishes (140×18 mm). The pests were transferred into the Petri dishes with a fine soft brush. A single *R. fuscipes* nymph of 3rd and 4th instars, adult female and male were starved for 24 h and released into the dishes 2 h after the preys were introduced. This interval ensured that preys had dispersed throughout the dish. After 24 h, the predators were removed and the number of consumed and/or killed prey was counted (n=10–12). Treatments were carried out at 31±1° C, 75±5% RH and 11-13 hours (L: D). The predator search efficacy was calculated from the number of dead (Na) and offered prey density (N) by Na/N (Holling 1959). The linear regression graphs were plotted for the feeding efficacy of the predator.

Statistical analysis- The linear regression equation was used to find out the relationship between the prey consumption rate of the predator in different densities using SPSS version 11.5.

Results

Stage preference- The third instars nymphs of *D. cingulatus* were preferred by 3rd and 4th instars nymphs of the predator. The V instars of the predator maximum fed up on fourth instars of *D. cingulatus* and adults of the predator maximum preferred fifth instars of *D. cingulatus*. Invariably all the stages of the predator predate up on adults of *P.* solenopsis. These results showed that life stages of *R. fuscipes* preferred different stages of the pest tested. The results also suggest that both fifth instar and adult predators were more successful in encountering the large sized *D. cingulatus* and *P.* solenopsis (Table 1).

Functional response- The percentage of prey consumed by each predatory stage in the Petri

dishes declined monotonically with the initial prey density and the logistic regression suggested Type II functional response for all stages (Figure 1). This was because the estimate of the linear coefficient was significantly <0 and the quadratic coefficient were positive (Table 1). Therefore, the "random-predator" equation for Type II was used to estimate the attack rate coefficient (α) and the handling time (Th) (Table 1). This model fits the observed data reasonably well. Handling time seemed to decrease with increasing prey density in both fifth nymphal instars and adults, but this relation was not defined in third and fourth nymphal instars of *R. fuscipes* while provided with *D. cingulatus*. The third (y=1.24+0.22x, r=0.921), fourth (y=0.89+0.32x; r=0.99) and fifth (y=0.96+0.34x; r=0.98) nymphal instars and adult (y=0.92+0.36x; r=0.98) of *R. fuscipes* on *D. cingulatus* showed maximum Na values as 3.33, 3.96, 4.23 and 4.41 at 10 prey densities respectively. The same result was also observed in *P. solenopsis* at the prey densities of 1, 2, 4, 8 and 16 for preys per predator and y=1.30+0.28x; r=0.93; y=1.22+0.23x; r=0.92; y=0.99+0.26x; r=0.93 and y=1.40+0.31x; r=0.94 for third, fourth, fifth and adults stages of the predator respectively (Fig. 1). The handling time (Th), which is sometimes a good indicator of the predation rate, was the shortest for adult females and the longest for the 3rd instar nymphs of reduviid. Although differences among the handling times of adult females, males and 4th instar larvae were not statistically significant, the general trend usually remained towards higher performance for adult females.

Discussion

The effectiveness of the predator under controlled condition depends up on the type and number of prey consumed. Moreover, before utilizing a natural enemy for biological control, it is important to assess its ability to capture and consume relevant stages of the targeted insect pests. The biocontrol potential of *R. fuscipes* on *D. cingulatus, P. solenopsis* and *A. gossypii* were recorded in this study. Results revealed that the particular stage of the predator preferred the desired stage of the pests. Moreover, younger reduviid preferred younger prey and vice versa. Stage preference exhibited by the life stages of *R. fuscipes* could be attributed to the dynamics of prey – predator interaction which is governed by the size of both predator and prey (Sahayaraj & Ambrose 1994). Our results showed resemblance with the studies were carried out with different reduviids predators on cotton stainer, *D. cingulatus* such as *Rhynocoris marginatus* (Ambrose & Kumaraswami 1990), *Ailaeocranum quadrisignatum* (Ambrose & Sahayaraj 1993), *Ectomocoris tibialis* (Sahayaraj 1995, 1997).

Decline in the proportion of prey consumption with the increasing prey density for all predatory stages indicates that the functional response data were described well by a Type II asymptotic curve. The potentiality of *R. fuscipes* was previously analyzed on *Riptortus clavatus* (Ambrose & Claver 1995), *Spodoptera litura* (Ambrose & Claver 1997, Ambrose & Maran 2000). The feeding efficacy of the predators was increased while the prey density increased. This exhibits a typical type II model Holling's disc equation. The feeding efficacy was positively correlated with prey density but the attack ratio was higher in low prey densities. The attack rate coefficients obtained for the different stages tested did not differ statistically. This indicates that the attack rate coefficient did not change between different predator stages. Other reports on the attack rate coefficients of reduviids are in agreement with the results described here (Ambrose & Sahayaraj 1993, Sahayaraj & Ambrose 1997, Sahayaraj *et al.* 2003, Sahayaraj 2008, Sahayaraj & Asha 2010). The handling time is a good indicator of consumption rate and effectiveness of a predator because it reflects the cumulative effect of time taken during capturing, killing, subduing, and digesting the prey (Sahayaraj & Asha 2010). The higher handling time value obtained for 3rd instar larvae was due to a lower consumption rate, especially for aphids. Because of lower consumption by 3rd instar larvae, any estimate of the voracity of preys should consider the life stage. This experiment clearly revealed that different stages of the predator can be utilized in pest

management programme. However, before recommending this reduviid for biological control programmes, it is essential to evaluate its potentiality under augmentation level.

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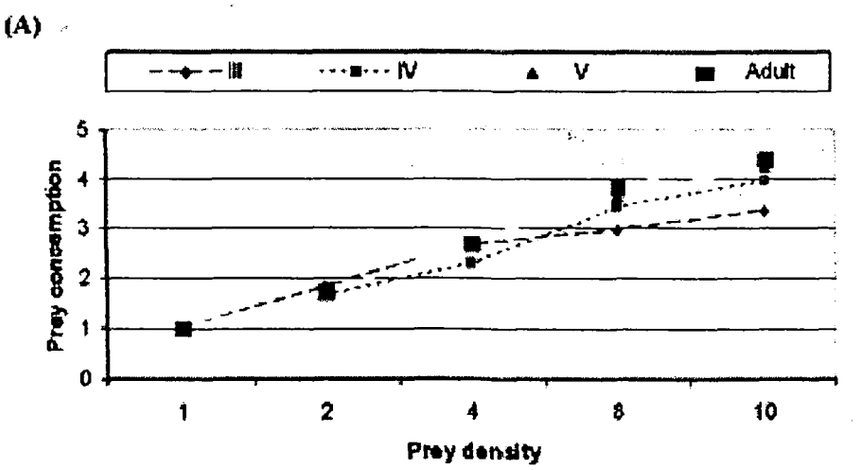
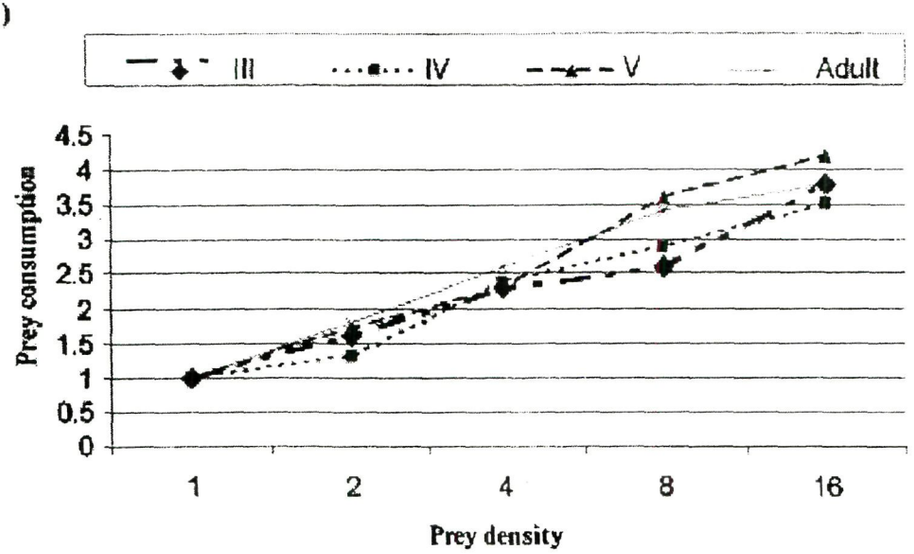


(B)



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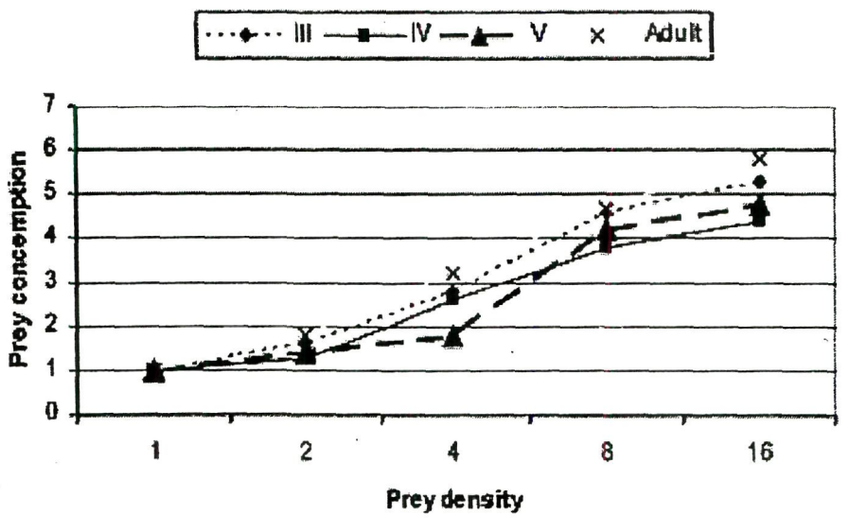

Figure 1: Predatory potential of *R. fusipes* on *D.cingulatus* (A), *A. gossypii* (B) and *P. solenopsis* (C).

Table 1: Stage preference of *R. fuscipes* on *D. cingulatus* and *P. solenopsis.*

Stage of the predator	Stage of the pests preferred (in %)						No Response
	I	II	III	IV	V	Adult	
D. cingulatus							
III	0.00	13±0.06	36.5±0.03	28.4±0.05	8.0±0.12	0.00±0.00	14.1±0.12
IV	0.00	5.8±0.03	43.4±0.13	26.6±0.06	12.6±0.03	3.6±0.13	8.0±0.13
V	0.00	6.8±0.04	24.4±0.05	33.3±0.23	23.4±0.05	6.67±0.21	5.5±0.03
Adult	0.00	3.4±0.21	20.4±0.03	23.6±0.03	34.5±0.12	12.5±0.14	5.6±0.03
P. solenopsis							
III	0.00	0.00	39.6±0.02	-	-	47.8±0.12	12.3±0.03
IV	0.00	0.00	19.3±0.03	-	-	80.0±0.04	0.00
V	0.00	0.00	8.3 ± 0.03	-	-	86.4±0.03	4.8±0.12
Adult	0.00	0.00	16.8±0.03	-	-	78.6±0.04	4.8±0.03

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Artificial Rearing of the Red cotton bug, Dysdercus cingulatus using Cotton seed-based Artificial diet (Hemiptera: Pyrrhocoridae)

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Preparation and application of an artificial diet on insects often encounter a lot of barriers. The successive proportion of the diet affects the biology and reproduction of the insects. *Dysdercus cingulatus* (Fabricius 1775), an economically important insect pest infesting most of the Malvaceae plants, has been utilized in many biological researches. Laboratory rearing on natural hosts is a not economically viable, laborious and time consuming. To overcome these constrains, an artificial diet was developed using its natural feed, the cotton seed. *D cingulatus* developed quickly (17 days) with heavier adult female (148 mg/animals) and maximum fecundity (57 eggs/female) when reared with artificial diet. However, artificial diet reduces nymphal survival rate (10%), adult longevity (3 and 2 days for male and female respectively) and male-biased sex ratio. Furthermore, artificial diet significantly enhances hatchability. Oviposition index and relative growth rate of the pest might be due to the higher carbohydrate, protein and lipid content observed in the artificial diet. It is therefore suggested that the proposed artificial diet be used for artificial rearing of the pest.

Keywords: *Dysdercus cingulatus* (Fabricius 1775) – artificial diet – chemical composition – cotton pest – growth – life history – reproduction

1 Introduction

The red cotton bug (RCB) or cotton stainer, *Dysdercus cingulatus* (Fabricius 1775) is a serious pest of cotton, lady's finger, sambhal, hollyhock, hibiscus [KOHNO & NGAN 2004]. It is distributed in various parts of Asia, Australia, Egypt, Africa etc. In cotton agro-ecosystem, nymphs and adults feed on developing and mature cotton, *Gossypium hirsutum* (Linnaeus 1758) seed. Seed weight, oil content and seed viability decline as a result of RCB infestation. Severe attacks on bolls of two weeks old can kill developing seeds leading to boll shedding. Where feeding is less, though damaged bolls are retained and yield and quality of lint are also reduced as a secondary effect of feeding. Furthermore, it also acts as vector for transmitting the fungal pathogens in cotton [KSHEMKALYANI et al 1989, KARIMI et al 2010].

It was reported by WILSON et al [2008] that fuzzy cotton seed used for stock feed is an important alternate source of food for cotton stainers. Storing fuzzy seed in exposed places where cotton stainers can access this food source over long periods has to be avoided.

The control of rotten cotton and cotton volunteers is important for limiting cotton stainer's access to alternate food source. In general, rearing of phytophagous insects on natural food is not only time consuming but also involves a lot of man power. Further, frequent handling of the culture results in high mortality of insects. The success over the past century is due to the ability to rear insects on artificial diet [COHEN 2004].

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Moreover, it was proposed that insects, reared on artificial diet are used as feed for animals [IAN & GREG 2010] including their natural enemies and for sterile insect technologies. Furthermore, the availability of a large numbers of insects is a pre-requisite for a sound integrated pest mangment (IPM). *D cingulatus* has been used for rearing *Antilochus coqueberti* [KOHNO et al 2004] and also for other research purposes. No information is available in the literature about the artificial rearing of this economically important pest. Therefore, in the present work, the artificial diet developed for the Asian species has to be tested for the first time on *D cingulatus*. In particular, the effect of cotton seed-based artificial diet on *D cingulatus* development and reproduction have to be determined.

2 Material and methods

2.1 Insect collection and maintenance

The stock colony of *D cingulatus* was maintained in a plastic trough (upper 30 cm diameter, height 10 cm, lower 27 cm diameter) at room temperature of 28 ± 2 °C and 70 ± 5 % RH with 11L: 13D cycle in the laboratory. It has been previously collected from cotton fields in Tirunelveli district of Tamil Nadu in India. Laboratory emerged first instar nymphs were placed individually in plastic containers (9 cm height x 6 cm diameter) with a cotton leaf for the first two instars, then, transferred in groups of 5 into plastic rearing boxes (13 cm height x 8 cm diameter) on an artificial liquid diet (partially defined chemically) and maintained in the above mentioned laboratory conditions. Ten replications were maintained for each category. Adults were maintained in pairs (1M: 1F) in plastic container (9 cm height x 6 cm diameter) using artificial diet till their death. Insects provided with water soaked cotton seeds were considered as control.

2.2 Preparation of artificial diet and insect rearing

50 g cotton seeds soaked overnight in dark was ground with domestic blender by adding 50 ml sterilized distilled water. The mixture was passed through a muslin cloth and the filtrate has been used as source ingredient. The red cotton bugs artificial diet (AD) composed of 50 g source ingredient, 50 mg of Proteinex (Wockhardt Ltd, Mumbai), 25 mg of yeast powder (Himedia, Mumbai), 1 ml of honey (Dabur, Mumbai), 10 mg of vitamin tablet – Supradyn (Piramal Health Care, Mumbai), 7 mg of ascorbic acid (Piramal Health Care, Mumbai), 25 mg of milk powder (Nestle, New Delhi), 1 mg of streptomycin sulphate (Piramal Health Care, Mumbai) and a 100 ml of distilled water. The ingredients were blended to obtain a homogenous texture. The artificial diet was prepared in the same day and kept thereafter in a freezer at -4 °C. A known amount of cotton (50 mg) was rolled as a ball. 200 ml of the diet was wetted with cotton ball and provided to the insects. AD was replaced with fresh diet once in two days throughout the life time.

2.3 Parameters recorded

The development time and daily weight gain of *D cingulatus* on AD and cotton seeds were recorded with the survival during each instar.

The relative growth rate (RGR) [ISIKIBER & COPLAND 2002] of *D cingulatus* was calculated for each of its third, fourth and fifth nymphal instar separately by the following formula: RGR = (Fwt - Iwt)/ [(Fwt + Iwt)/2] x D. **Fwt** final weight of the insect, **Iwt** initial weight of the insect and **D** days taken for each nymphal development. In adults, the adult longevity, pre-oviposition, oviposition, and post-oviposition periods; fecundity and egg hatchability were recorded. Total carbohydrate [THOMAS 1956], protein [LOWRY et al 1951] and lipid [BRAGDON 1951] contents of AD and cotton seed source ingredient were estimated.

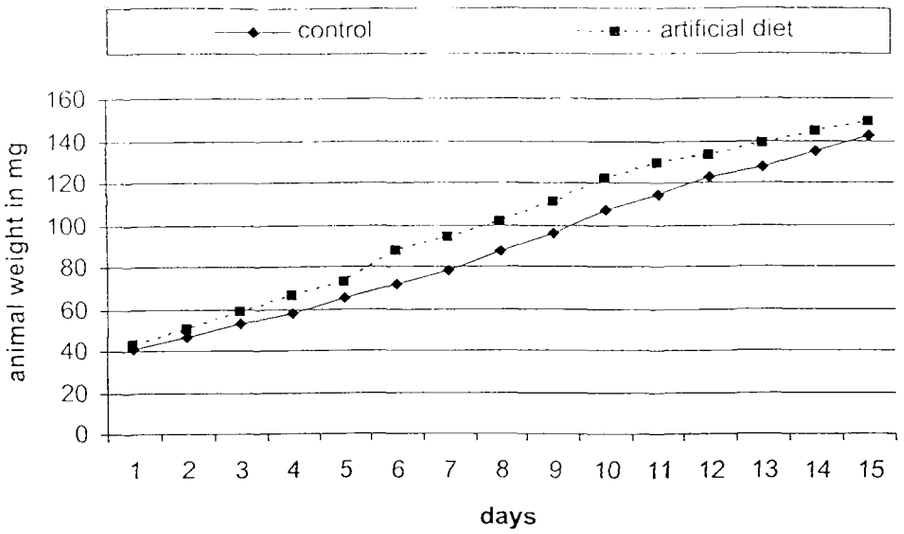

Fig 2: Influence of artificial diet and natural food (control) on the weight gain (in mg) of *Dysdercus cingulatus* (Fabricius 1775) [Hemiptera: Pyrrhocoridae] reared from third instars to before mating.

4 Discussion

In this study, an AD was developed for the nymphs and adults of *D cingulatus* for the first time. Under natural conditions, nymphs and adults of *D cingulatus* are found to feed on the cotton bolls and seeds [Kohno et al 2004]. Since usage of cotton bolls as a source ingredient for the artificial diet is economically non viable, commercially available cotton seed has been used as source ingredient. Enhanced development and reproduction of RCB with AD indicates the AD fulfils all the nutritional requirements for both nymphs and adults.

Variations in the quantity and quality of suitable food can have important effects on insect development. Usually, when food ingestion decreases, the duration of development is extended and the insects become smaller and lighter. The sufficiency of larval food is correlated within the quality of nutrients stored for egg production; however more direct effects of nutrients levels occur in insects that feed as adults [Lee et al 2008]. The sex ratio of hemipteran pests has been influenced by nutritional availability of the host plants [Kaushalya et al 2008], as found out the present observation. To maintain a colony of *D cingulatus* by using artificial diet in the laboratory, it is important to determine whether adult females can consistently lay fertile eggs. When bugs were provided with the artificial diet, females laid significantly more eggs and more than 98% of the eggs hatched, suggesting that the artificial diet developed in the present study is suitable for rearing nymphs and adults of *D cingulatus*.

Tab 3: Total carbohydrates, protein and lipids (mg/ml) of cotton seed extract (Source ingredients) and artificial diet.

Diets	Macromolecules (mg/ml) Levels		
	Total Carbohydrate	Total Protein	Total Lipid
Cotton seeds	0.54±0.02	82±0.03	0.08±0.02
Artificial diet	0.68±0.020.	1.08±0.03	0.09±0.02

This successful rearing of *D cingulatus* nymphs and adults on the artificial diet can help reduce the cost of mass-rearing, because it would reduce the quality and quantity of cotton fibre required for rearing the insect.

In summary, the artificial diet was prepared by using commonly available, economically feasible ingredients rich in nutrients and it had positive effects on the nymphal development and oviposition of *D cingulatus*. The diet can be utilized for the mass production of *D cingulatus*. To the best of our knowledge this is the first successfully developed artificial diet for mass rearing of *D cingulatus*.

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Biosafety Evaluation of *Tephrosia purpurea* Stem-based Formulation (Telp 3% EC) Against Three *Rhynocoris* species

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ABSTRACT

To utilize a plant-based insecticide, the study of biosafety of botanical insecticide to polyphagous natural enemies are imperative. A laboratory trial was conducted to investigate the biosafety of *Tephrosia purpurea* stem-based formulation (Telp 3% EC) against three reduviid predators such as, *Rhynocoris fuscipes*, *Rhynocoris marginatus* and *Rhynocoris longifrons* adults using Y-shaped olfactometer considering olfactory response as a tool. Telp 3% EC was impregnated in Whatman No. 1 filter paper, Bt cotton leaves (BT bunny) and groundnut leaves (TMV 4). The Access Proportion Index (API) was calculated in different time intervals like 20, 40 and 60 min. Olfactory response results revealed that impregnation of Telp 3% EC in Bt cotton leaves does not deter *Rhynocoris longifrons* olfactory response whereas, groundnut leaves deter both *Rhynocoris fuscipes* and *Rhynocoris marginatus* olfactory response. It has been concluded from the results that Telp 3% EC can be incorporated along with reduviid predators in BT cotton pest management. However, detailed studies are necessary to confirm the observation.

Key words: Biosafety, *Rhynocoris* spp., olfactory response, Telp 3% EC

INTRODUCTION

Reduviidae constitute an important group of predatory insects in various parts of the world. *Rhynocoris fuscipes* (Fabricius), *Rhynocoris marginatus* Fab. and *Rhynocoris longifrons* (Stal) (Hemiptera: Reduviidae) are brightly coloured, entomophagous, harpactorine reduviids, found in the agroecosystems, semi-arid zones, scrub jungles and reported to be predating on insect pests of Lepidoptera, Hemiptera, Isoptera, Orthopetera etc. (Ambrose, 1999; Sahayaraj, 2007).

Generally, biopesticides are considered safe to natural enemies of the target pest (Schmutterer, 1990; Ascher, 1993). Earlier studies demonstrated that application of biopesticides does not reduce of the biological control efficacy of *R. marginatus* (Sahayaraj, 2001). Sahayaraj and Paulraj (1999), Sahayaraj and Karthikraja (2003) and Sahayaraj *et al.* (2003) reworded the impact of different plant extracts against *R. marginatus* life stages.

Tephrosia purpurea (Dil.) Pers, (Fabaceae), is used traditionally for digestible, anthelmintic, alexiteric, leprosy, ulcers, antipyretic, alternative, cures diseases of liver, spleen, heart, blood, tumours, asthma, dyspepsia, diarrhoea, rheumatism, asthma and urinary disorders. Species of the Genus *Tephrosia* has been used as insecticide for instance *T. candida* DC., *T. purpurea* Pers., *T. vogelii* and *T. noctiflora* Bojer ex. Baker (Klocke, 1989). In addition, the bark of *Tephrosia*

purpurea has insecticidal activity against the third instar larvae of *Plutella xylostella* (You-Zhi *et al.*, 2011) and *Corcyra cephalonica* (Jadhav, 2009). The roots and seeds of this plant reported to have insecticidal, piscicidal and vermifugal properties (Hegazy *et al.*, 2009). However, no information has been available about the impact of this plant extracts or its formulation on any natural enemies including reduviid predators. In view of this lacuna, effect of *Tephrosia purpurea* stem based formulation (Telp 3% EC) was investigated under laboratory conditions against three important reduviids such as *R. fuscipes*, *R. marginatus* and *R. longifrons*.

MATERIALS AND METHODS

Insect rearing: Life stages of reduviids like *R. fuscipes*, *R. marginatus* and *R. longifrons* were collected from the agroecosystems (groundnut and cotton) in Tamil Nadu, India from January 2012 to March 2012. They were maintained in the laboratory conditions (28±2°C, L:D, (13:11 h) photoperiod and relative humidity of 73±4%) using the methodology of Sahayaraj (2002). Laboratory emerged adults were selected randomly from the stock culture and used for the study.

Preparation of botanicals and bioassay: A 3% Emulsifiable Concentration (EC) of *Tephrosia purpurea* stem extract was prepared by mixing 3 mL of Telp 3% EC in 70 mL of distilled water. Biosafety evaluations were conducted in a customized Y-shaped glass olfactometer (2.5 cm internal diameter, 20 cm stem length, 20 cm arms length). The olfactometer was clamped on to a tripod in a horizontal position. Activated charcoal (Sigma), filtered air stream (Universal Lab Product) (200 mL min^{-1}) was supplied to each arm of the olfactometer by using an electric pump (Boy U, U-9900, China). Each air stream then passed through a glass chamber (4×8 cm) containing test material (a piece of 7.0 cm^3 filter paper dosed with 100 µL of EC formulation).

The 100 µL of 3% Telp was impregnated with Whatmann No. 7 filter paper (3 cm diameter), dried at room temperature and placed into the test chamber of Y-shape Olfactometer and at the other end a filter paper (3 cm diameter) with distilled water was placed. Six adult (sex is not considered) were released one after other into the release chamber of the Y-shaped olfactometer of the stem. Number of insects found on the treated and untreated filter paper was recorded after 20, 40 and 60 min continuously. The experiment was replicated six times with different uniform sized individuals of the same species. Same procedure has been followed for the emulsifiable concentration impregnated with cotton leaf (BT bunny) and groundnut leaf (TMV 4). The insects preferred either control or treated filter paper or neither. If the insects chose neither of the chambers then it was considered that insect made no choice. From the observation recorded the access proportion index API was performed (Yasuda and Wakamura, 1996). Same procedure was followed for other two reduviids.

RESULTS AND DISCUSSION

Reduviids are the dominant invertebrate predators in a variety of agroecosystems including cotton and groundnut where varieties of insecticides (synthetic, botanical, microbial) have been practiced by the farmers. In this study we studied the biosafety of a botanical formulation, Telp 3% EC against three common reduviids like *R. marginatus*, *R. fuscipes* and *R. longifrons*.

***Rhynocoris marginatus*:** Access behaviour of *R. marginatus* against Telp 3% EC is represented in Table 1. Results revealed that *Rhynocoris marginatus* oriented towards the Telp impregnated filter paper, cotton leaf and groundnut leaf without changing its usual olfactory response. However,

Table 1: Access behaviour of *R. marginatus*, *R. fuscipes* and *R. longifrons* adults against *T. purpurea* stem-based formulation (Telp 3% EC) impregnated filter paper, cotton leaf and groundnut leaf

		Exposure time (min)		
Reduviid	Telp impregnated material	20	40	60
R. marginatus	Filter paper	0.12	-0.05	0.31
	Cotton leaf	-0.07	-0.31	-0.08
	Groundnut leaf	-0.175	0.125	0.40
R. fuscipes	Filter paper	0.48	-0.015	0.93
	Cotton leaf	0.00	-0.18	0.07
	Groundnut leaf	0.13	0.02	0.03
R. longifrons	Filter paper	0.33	0.38	0.44
	Cotton leaf	0.10	0.93	1.00
	Groundnut leaf	0.80	0.60	0.60

at 0.8%, *R. marginatus* does not showed any positive or negative (move opposite to the test material) response during the experiment time. Olfactory response of *R. marginatus* purely depends up on leaf which impregnated with botanical formulation, Telp 3% EC. For instance at 20, 40 and 60 min observations, Telp 3% EC slightly deter (-0.08 to -0.31) the reduviid predator. At the same time, at 60 min, Telp 3% EC affect the reduviid behaviour, while the reduviid, while the botanical impregnated in filter paper (API = 0.31) or groundnut leaf (API = 0.4).

Rhynocoris fuscipes: As observed in *R. marginatus*, *R. fuscipes* also showed positive chemotosis activity against Telp 3% EC. Results showed that all the subjected *R. fuscipes* adults did not show either repellent or attraction activity while Telp 3% EC impregnated in cotton leaf. However, more than 13 and 18% of predator attracted towards groundnut leaf and filter paper, respectively impregnated with Telp 3% EC at 20 min observation. These responses had been changed with at 40 min observation. For instance, 3.0, 7.0, 93.0% of *R. fuscipes* was attracted towards groundnut leaf, cotton leaf and filter paper indicated that Telp 3% EC can be integrated along with reduviid predator in Biointensive Integrated Pest Management (BIPM) Programme.

Rhynocoris longifrons: The olfactory response of *R. longifrons* seems to be the same, as showed by other reduviid predators i.e. *R. fuscipes* and *R. marginatus*. Table 1 shows the olfactory response of *R. longifrons* against Telp 3% EC impregnated filter paper, cotton leaf and groundnut leaf. This predator at any observation periods does not show any deterrent activity against Telp 3% EC of *Tephrosia purpurea*.

Previous studies by Jhansilakshmi *et al.* (1998) and Sahayaraj and Ravi (2007) also reported that botanicals can be integrated along with natural enemies. Botanical insecticides cause the death of the natural enemies (lethal effects) or change several other features of their biology and ethology without killing the individuals (sub-lethal effects). For instance, neem insecticides were found to be only slightly harmful to *Rhynocoris marginatus* (Sahayaraj *et al.*, 2003), *Geocoris punctipes* (Hemiptera) (Myers *et al.*, 2006), *Harmonia conformis* (Boisduval) (Coleoptera) and *Mallada signata* (Schneider) (Neuroptera) (Qi *et al.*, 2001), *Harmonia axyridis* (Kraiss and Cullen, 2008), coccinellids (Swaminathan *et al.*, 2010), *Philodromus cespitum* (Walckenaer) (Rezac *et al.*, 2010); *Phytoseiulus persimilis* and *Amblyseius cucumeris* (Spollen and Isman, 1996) (Araneae) and natural enemies in general (Lowery and Isman, 1995; Isman, 2006; Sakthivel *et al.*, 2012).

Podisus maculiventris (Hemiptera) had, however, slightly reduced survival and reproduction (Vinuela *et al.*, 2000). Similarly, a commercial formulation of azadirachtin (Align) affects the fertility of *Chrysoperla carnea* (Stephens) (Neuroptera) (Medina *et al.*, 2004).

CONCLUSION

The study concluded that when *Tephrosia purpurea* stem-based formulation (Telp 3% EC) sprayed in cotton and groundnut, *R. longifrons* did not show any repellent activity than *R. fuscipes* and *R. marginatus*. However, the repellency of *R. fuscipes* and *R. marginatus* were insignificant, indicates these predators can be integrated along with Telp 3% EC formulation in cotton and groundnut pest management.

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COMPARATIVE BIOLOGY AND LIFE TABLE TRAITS OF RHYNOCORIS LONGIFRONS STÅL (HEMIPTERA: REDUVIIDAE) ON FACTITIOUS HOST AND FOUR NATURAL COTTON PESTS

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ABSTRACT

The biological traits and life table parameters of Rhynocoris longifrons was quantified three generations continuously using Dysdercus cingulatus (Hemiptera: Pyrrhocoridae) nymphs, Aphis gossypii (Homoptera: Aphididae) nymphs and adults, Helicoverpa armigera (Lepidoptera: Noctuidae) larvae and Phenacoccus solenopsis (Hemiptera: Pseudococcidae) adults as preys. Helicoverpa armigera the most suitable prey for this predator, because it decreased the total nymphal developmental time and mortality and enhanced the female adult longevity, oviposition days, fecundity and hatchablity. The female predator lived longer than the male. Eggs were laid in batches with minimum of 6 and a maximum of 20 eggs in an egg batch. Innate capacity for increase (rc), intrinsic rate of increase (rm), finite rate of increase (λ) and weekly multiplication (WM) of the predator were high when H. armigera served as food. However, *P. solenopsis* provided the predator to take significantly longer periods to attain adulthood (63 days) and they were dead. Macromolecule profile (Protein, Carbohydrate and Lipid) of pests revealed that lepidopteran pest H. armigera has higher quantity of protein and lipids. This too may be responsible for enhancing the reproduction of R. longifrons. However, carbohydrate is maximum in hemipteran pest P. solenopsis. The laboratory rearing of *R. longifrons, H. armigera* can be used as a prey rather than by *D. cingulatus.*

Key words: Biology, cotton pests, life table, *Rhynocoris longifrons*

INTRODUCTION

Cotton is an important crop grows in many parts of the world. One of the major obstacles hindering cotton cultivation is insect pests infestation. Red cotton bug, Dysdercus cingulatus (Fab.) (Hemiptera: Pyrrhocoridae) (Kohino and Bui Thi, 2004; Tanu Sharma, 2010), mealy bug Phenàcoccus solenopsis (Tinsley) (Hemiptera: Pseudococcidae) (Williams and Granare de Willink, 1992; Rashid et al.,2012) and Aphis gossypii Glover (Homoptera: Aphididae) (Rostami et al., 2012) and Helicoverpa armigera Hubner (Grundy, 2007; Downes and Mahon, 2012) infest cotton and causing yield loss. Reduviids are important predatory insects that could be harnessed for biological control of insect pests (Ambrose, 1988). Studies on the biology and the construction of the life table for a predator species is an important component in the understanding of its population (Carey, 1993; Sahayaraj and Paulraj, 2001b; Sahayaraj and Jeyalakshmi, 2002; Ambrose et al., 2006, 2007b; Sahayaraj and Sujatha, 2011) in agroecosystems and their bordering ecosystems like semi-arid zones, scrub jungles and forests of different kinds.

Adequate literature is available on the biology and the life table of Rhynocoris spp. The genus Rhynocoris consists of more than 20 species. Most of them have been distributed in agro-ecosystem (Putschkov, 1994; Paiero and Marshal, 2003; Sahayaraj and Raju, 2004; Beal and Tallamy, 2006; Rahimi et al., 2010). Rhynocoris longifrons (Stål) is a multivolatine, voracious harpactorine reduviid predator inhibiting tropical rain forests, scrub jungles, semi-arid zones bordering agro-ecosystems (Ambrose et al., 2003). Rhynocoris longifrons are more obscurely coloured, latern areas of head behind eyes black, membrane fusious, abdominal margine palely spotted and head a little longer than pronotum. The length of this predator is 10 – 12 mm and weight is approximately 40 to 57 mg. It feeds on some important insect pests such as Helicoverpa armigera Hubner (Ravichandran et al., 2003; Ravi, 2004), Odontotermes obseus Rambur Clavigralla gibbosa Spinola (Claver et al., 2002; Ambrose et al., 2003), C. cephalonica (George et al., 2000; Ambrose et al., 2007b; Shirley and Prasanna Kumar, 2010), Nezara viridula L., Exelastis atomosa (L.) (Ambrose and Claver, 2001). The predatory potential and biological parameters were studied against on S. litura and H. armigera larvae (Kumar and Ambrose, 1996; Ambrose et al., 2003; Ravichandran et al., 2003; Ambrose et al., 2007b; Sahayaraj and Ravi, 2007a; Kumar et al., 2009; Ganesh Kumar, 2011). However, no one reported the comparative biology of this predator against Dysdercus cingulatus (Hemiptera: Pyrrhocoridae) nymphs, Aphis gossypii (Homoptera: Aphididae) nymphs and adults, Helicoverpa armigera (Lepidoptera: Noctuidae) larvae and Phenacoccus solenopsis (Hemiptera: Pseudococcidae). The present work was undertaken to study the biology, and life table traits of R. longifrons against A. gossypii, D. cingulatus H. armigera and P. solenopsis.

MATERIALS AND METHODS

Collection and rearing of insects

Life stages of Rhynocoris longifrons was collected from the cotton agro-ecosystem bordering the scrub jungle of Kanyakumari and Tirunelveli districts, Tamil Nadu and maintained individually in plastics containers (15 cm diameter × 7 cm height) under laboratory condition at 31. 2 ± 1.0 °C, 56.0 ± 5.0 % with photoperiod of 11L: 13D hr on a larval Corcyra cephalonica Stainton. All the pest species were collected from the same cotton field from which the reduviid was collected. Aphis gossypii, *D. cingulatus*, H. armigera and P. solenopsis were maintained in groups in plastic trough (32 cm diameter × 15 cm height) and plastic container (50 ml) on young leaves, flowering and bolls.

Biology

The eggs laid by the predator in the laboratory were allowed to hatch in Petri dishes (9 cm diameter × 2.0 cm height) with wet cotton swabs for maintaining humidity. The swabs were changed once in two days in order to prevent fungal attack. Only one egg mass was placed in each petridish so that uniform cohorts of nymphs could be reared and the incubation period was recorded. Laboratory emerged nymphs were maintained in plastic boxes (16 cm diameter × 8 cm height and the lid moulded with nylon mesh for aeration) and divided into five diets: 1) A. gossypii (all stages approximately 150 insects per leaf), 2) D. cingulatus [40 second (8.76 mg) and 50 third (11 mg) instar nymphs], 3) H. armigera [40 second (11 mg) and 50 third (23 mg) instar larvae respectively], 4) P. solenopsis [120 to 150 of adults (0.40 mg)] and 5) C. cephalonica [30 and 40 of second and fifth instar larvae respectively] host.

The sex ratio was computed (number of female/total number of adults emerged) on the basis of laboratory emerged adult. *Rhynocoris longifrons* adult (1 male: 1female) were transferred to another plastic container (6 cm height × 4.5 cm diameter) and maintained till their death. The following adult fitness traits were determined: pre oviposition period (the period from adult female eclosion to the age at first oviposition commenced), total number of eggs laid by a female, minimum and maximum number of eggs present in each batch of egg and post-oviposition period (last oviposition upto death), oviposition index (total number of eggs laid /total oviposition days), percentage of nymphs hatched, and adult longevity of male and female. Nymphs emerging from the ten randomly selected egg batches were maintained for each prey species and the predators were reared in the laboratory for three generation continuously (except *A. gossypii).*

Life table

The life table parameter of the predator was calculated by Birch (1948), the equation was later elaborated by Southwood (1978) and Carey (1993).

Σ erm lxmx=1

Where 'e' is the base of natural logarithms, 'x' the age of the individual in days, lx the number of individual alive at age 'x' as a proportion of one and 'mx' the number of female or the net reproductive rate (Ro) was the rate of multiplication of population in each generation. The approximate value of cohort generation time (Tc) was calculated from the birth of parents of birth of offspring (Tc= Σx lxmx/Ro). The value of innate capacity for increase (rc) was calculated using rc= log e Ro / Tc. The arbitrary value of rm and the values of negative exponent of ermx as were certained from this experiment often by outside range. For this reason both sides of the equation were multiplied by a factor of Σe7-rmx lxmx = 1096.6 (Birch, 1948). The precise generation time (T) was then calculated from the formula: T= log e Ro/rm. The finite rate of increase (λ) was determined as (antilog erm /female/ day). Doubling time (DT), the time required to double in number was calculated as log 2/rm and weekly multiplication (WM = antilog erm) was also calculated.

Macromolecular profile of preys

The macromolecules like total body carbohydrate, total body protein and total body lipid (Bragdon, 1951; Lowrey et al., 1951; Sadasivam and Manikam, 1997) contents of D. cingulatus, A. gossypii, P. solenopsis and H. armigera were quantified using standard procedure.

Statistical analysis

The analysis of variance (ANOVA) was used to determine the difference among four cotton preys and also with factitious host C. cephalonica. It was applied to all the biology parameters like nymphal total developmental period, adult longevity, fecundity, number of eggs/batch and hatchability. Deference of significance was expressed at 5 percent level using SPSS 11.5 software.

RESULTS

Nymphal total developmental period

The incubation periods of R. longifrons varies from 7 to 8 days. Rhynocoris longifrons lay their eggs either in single, or in small cluster of 4 to 18 eggs. The total nymphal developmental time of the predator fed on H. armigera was significantly ($F = 6.906$; $df = 13,121$; $P < 0.05$) shorter than D. cingulatus ($F = 1.846$; $df = 12, 94$; $P < 0.05$) and P. solenopsis ($F = 4.912$; $df = 8, 76$; $P < 0.05$), when compared to C. cephalonica (Fig. 1). However, the nymphal survival rate maximum (63) was recorded while the predator is reared with A. gossypii rather than other cotton pests used in this study (Table 1).

Sex ratio and adult longevity

In general, male (0.48) and female biased (0.59) (Table 1) sex ratio was recorded in R. longifrons. The longevity of R. longifrons female fed with H. armigera was significantly longer ($F = 1.056$; $df = 7, 44$; $P < 0.05$) than A. gossypii ($F = 0.892$; $df = 7, 23$; $P < 0.05$) and P. solenopsis ($F = 0.554$; $df = 9,40$; $P < 0.05$). Similar results were also recorded for R. longifrons male ($F = 1.941$; $df = 14, 37$; $P < 0.05$ and $F = 4.488$; $df = 10, 19$; $P < 0.05$ for H. armigera and P. solenopsis, respectively).

Oviposition periods

Table 1 shows that the pre-oviposition period of the predator fed on H. armigera ($F = 0.564$; $df = 6, 53$; $P < 0.05$) was shorter than D. cingulatus ($F = 0.757$; $df = 6, 50$; $P < 0.05$), P. solenopsis ($F= 0.302$; $df= 4, 26$; $P> 0.05$) and A. gossypii ($F = 0.985$; $df = 4,23$; $P < 0.05$) fed groups. Similar observation was also observed for the oviposition period of the R. longifrons fed on H. armigera ($F = 0.891$; $df = 19, 40$; $P < 0.05$, $F = 1.899$; $df = 22, 37$; $P < 0.05$ and $F = 1.221$; $df = 16, 11$; $P < 0.05$ for H. armigera, D. cingulatus and A. gossypii, respectively). The post-oviposition of the R. longifrons were significantly shorter ($F = 2.757$; $df = 8, 19$; $P < 0.05$ and $F = 2.807$; $df = 8, 19$; $P < 0.05$ for D. cingulatus and P. solenopsis, respectively).

Fecundity and hatchability

Fecundity and hatchability of R. longifrons reared with different preys are presented in table 1. The fecundity

of R. longifrons was higher when reared with *H. armigera* (F= 1.698; df =13, 46; P>0.05) but it is not significant when compared with *D. cingulatus* (F= 0.627; df=15, 44; P>0.05), *P. solenopsis* (F= 0.871; df=10, 20; P<0.05) and A. gossypii (F= 0.431; df=10, 17; P<0.05). The hatchability was also significantly higher in *R. longifrons* when fed with *H. armigera* (F=2.668; df= 19, 40; P< 0.05) when compared with other preys like D. cingulatus (F=1.738; df= 20, 40; P> 0.05), A. gossypii (F=0.960; df= 9, 18; P> 0.05) and P. solenopsis (F=1.811; df= 9, 21; P> 0.05).

Life table

The life table statistics of *R. longifrons* on four cotton pests reveal that the net reproductive rate (Ro) was lower than of that the gross reproductive rate for all the pests. For instance, the net reproductive rate (Ro) on H. armigera was higher (115.0) than on P. solenopsis (76.7), D. *cingulatus* (68.3) and *A. gossypii* (43.8) (*Table* 2). Similarly, the rate of natural increase on H. armigera was higher than D. cingulatus, *A. gossypii and P. solenopsis*. The finite rate of increase (λ) and weekly multiplication rate (WM) were higher (1.09 days with their doubling time of 7.28 days for three generations) in *H. armigera* fed predator. The intrinsic rate of increase (rm) of the *R. longifrons* has highest value (0.090 progeny/female/ day) when predator was provided with H. armigera and then decreases to 0.062 when predatory reduviid provided with *A. gossypii*. The finite rate of increase erm, means the population multiplication of predatory bug in a unit of time. The present results show that erm ranges between 1.06 to 1.09, according to the number of preys offered to the R. longifrons (Table 2).

Macro molecular profile of cotton pests

Among the four cotton pests analysed, the larvae of H. armigera (13.1 mg/100 mg) contain more amount of total body protein followed by nymphs of *D. cingulatus* (10.8 mg/100 mg) and *P. solenopsis* (4.50 mg/100 mg) adult (Fig 2). The total body carbohydrates content was higher in the adult of *P. solenopsis* (22.9 mg/100 mg) followed by larvae of *H. armigera* (16.5 mg/100 mg) and nymphs of *D. cingulatus* (13.5 mg/100 mg). The lipid content was also higher amount in H. armigera (25.1 mg/100 mg).

DISCUSSION

Reduviids are generalist predators, but some species are known to exhibit preferences for particular prey when they are simultaneously offered a number of different species. For example, in the presence of mixed pests, *R. marginatus* attacks termites first, then grasshoppers and finally ants (says Ambrose et al., 1990). Similarly *R. longifrons* shows preference against Spodoptera litura larvae than its factitious host, *C. cephalonica* (Ravi, 2004). In the present study *R. longifrons* shows higher growth when the predator is reared with H. armigera rather than other cotton pests tested and also with a factitious host C. cephalonica.

There are several factors that determine the prey suitability for insect predators, which can be divided into (i) nutritional factors (quality and quantity of micro and macro molecules) and (ii) non-nutritional factors (prey texture, movement, agility, type of mouth- parts, cuticular nature, presence and absence of hairs over the biology, flying capacity etc.). For a prey species to be suitable, it must provide all nutritionally important factors as proteins, carbohydrates, lipids, vitamins and minerals in a balanced proportion and concentration to meet a predator's metabolic requirements (House, 1966, 1977; Sahayaraj et al. 2004). Analysis of macromolecular profile of preys reveals that H. armigera has more amounts of total body protein and lipid reflecting higher growth and reproduction of the predator.

The influence of different prey on the biology of reduviids such as Acanthaspis pedestris (Ambrose and Subburasu, 1988), Neohaematorrhophus therasii (Sahayaraj and Ambrose, 1994a), Rhynocoris kumarii (Ambrose and Rani, 1991), Rhynocoris marginatus (Ambrose et al. 1990; George *et al.*, 2002; Sahayaraj and Sathiamoorthi, 2002; Sahayaraj *et al.* 2004), Cydnocoris gilvus (Venkatesan et al.,1997), *Rhynocoris longifrons* (Kumar, 1993; Ambrose et al., 2003; Ravi, 2004) were available in literature. It is still assumed by some authors (DeBach and Rosan 1991; Hoy 1994) that an effective biocontrol agent should be highly prey or host specific in order to be considered near as good/viable biological control agent. *Rhynocoris longifrons* has been recorded from various verities of cotton (SVPR II, SVPR IV and MCU 5) agro-ecosystem where there are lot of possibilities to feed wide range of prey which belongs to various insects order. Such informations are imperative to utilize this predator in cotton pest management. This study is an initial step to generate such information. To find out the host preference, we offered three hemipteran and one lepidopteran insect which are commonly distributed in the cotton agro-ecosystem. All these preys were provided to R. longifrons and its various biology traits were recorded.

The longest nymphal developmental period, lower fecundity and hatchability observed in *R. longifrons* reared on *A. gossypii and P. solenopsis* might be due to very smaller size and the presence of a white coating in the latter prey respectively. Ambrose et al. (2003) for the first time studied the biology of this predator using a C. cephalonica, H. armigera (the stage not mentioned) offered, Odentotermes obesus, and Clavigralla gibbosa (Spinola). They recorded a shorter nymphal developmental period of 51 days in H. armigera (Ravi, 2004) or 53 days in C. cephalonica in contrast to our observation (36, 41 and 42 days for first, second and third generation, respectively) for C. cephalonica. This indicates that provision of predator stage dependant prey offered is crucial to minimize the predator nymphal stadial period. The prey also increased/ enhanced the survival rate (60%). Maximum nymphal survival was recorded while the predator was reared with A. gossypii, as against *H. armigera* recorded by Ambrose et al. (2003). The presence study reveals that *H. armigera* was found to be the highly suitable prey for *R. longifrons* due to the following reasons: i) faster nymphal development, ii) higher nymphal survival, iii) higher fecundity and hatchability, iv) higher net and gross reproductivity and intrinsic rate and v) shorter population doubling time.

Abnormal hatching and more mortality resulted in nymphal mortality. Earlier, it was reported that sex ratio was female-biased (Ambrose et al., 2003) for R. longifrons. However, sex ratio was altering in F2 genera-

tion. The male and female adult longevity of *R. longifrons* fed with H. armigera was shorter than that with other pests. *Corcyra cephalonica* fed male and female lived 75 to 85 and 75 to 90 days respectively (Ambrose *et al.*, 2007b). Similar observation was recorded by Ambrose *et al.* (2003) while the predator was fed by different hosts. Further, they reported that the longer adult longevity was reported in H. armigera reared predator followed by *C. gibbosa and O. obesus*. Sahayaraj *et al.* (2004) reported that the higher fecundity of R. marginatus was influenced by the provision of *H. armigera* due to the higher macromolecular content as observed here. However, the mean egg production (43 eggs/ female) was lower than that observed by Ambrose *et al.* (2003).

The life table statistics of *R. longifrons* on three pests reveals that the net reproductive rate was lesser than that the gross reproductive rate. This is in accordance with the results of George *et al.* (1998a). They attribute such finding to the sharp decline in the survivorship value of parent females. The net reproductive rate observed in R. longifrons is comparatively higher (96.54) than that of other harpactorine reduviids like Sycanus collaris Fabricius (Ro = 30.46) (George et al. 1998a), *Rhynocoris marginatus* (Ro = 27.90) (George, 2000) and Sphedanolestes minusculus Bergroth (Ro = 79.35) (Ambrose et al. 2006) and also Rhynocoris longifrons (Ro = 46.606 and 115.0) (Ambrose et al., 2007b; GaneshKumar, 2011). However, an opposite trend was recorded for Rhynocoris marginatus (Ro = 181.979) (Sahayaraj *et al.* 2004) and R. fuscipes (Ro = 55.01) (Sahayaraj and Selvaraj, 2003). The value of true intrinsic rate of increase was slightly higher than the capacity for increase in number, as expected for insects having overlapping generations (Southwood, 1978). The value of true intrinsic rate of increase in the present study was higher (0.11) than those reported for the reduviids such as R. longifrons (0.042) (GaneshKumar, 2011), Cydnocoris gilvus (0.060) (Venkatesan *et al.*, 1997), *Acanthaspis siva* (0.063) (George et al., 1998b), *Rhynocoris fuscipes* (0.041 and 0.053) (George, 2000; Sahayaraj and Selvaraj, 2003) and *Rhynocoris marginatus* (0.070) (Sahayaraj *et al.*, 2004). The daily finite rate of increase (λ) ranges from 1.06/ female/ day (A. gossypii) to 1.09/ female/day *(C. cephalonica and H. armigera)*. At this rate the population of *R. longifrons* is expected to double every 7.71 (for C. cephalonica) to 11.4 days (for A. gossypii). These statistical values are high when compared to the data of Ambrose et al. (2007b) for the same reduviid. This might be due to differences in the rate of fecundity and the proportion of survival of female insects. Doubling time for populations of *R. longifrons* was lower for *D. cingulatus* than for *P. solenopsis* and *A. gossypii* as observed in another reduviid, *Rhynocoris fuscipes* (George, 2000).

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). The reproductive potential such as fecundity and percent hatchability of insect predators are determined by the nutrient composition of the prey species (Fuller, 1988). The egg laying potential, hatching success and longevity of adults were maximum on H. armigera than on other pests due to higher content of primary nutrients. Reduced level of fecundity and longevity of *Cydnocoris* gilvus Burm was observed on *Odentotermes obesus* than on *Spodoptera litura* (Vekatesan *et al.*, 1997). Shortest nymphal developmental period, maximum fecundity, hatchability, male and female adult longevity, gross and net reproductive rate, doubling time and intrinsic rate of increase might be due to the presence of high total protein and lipid recorded in H. armigera larvae. This shows that the reduviids appear to require animal protein for development (Taylor and Schmidt, 1996).

The only nymphal of *R. longifrons* did not developed during third generation due to the failure of moulting in first instar. Though, other cotton pests are in favour for the life traits of this predator, A. gossypii does not proceeds to its third generation. This might be due to i) the small body size which needs to consume enormous number of preys by spending more energy ii) low amount of nutrition (carbohydrate, protein and lipid), iii) feeding stress of aphids for previous two generations and iv) toxic alleochemicals produced by the aphis (Francis *et al.*, 2001). Edwards (1962a) found that the reduviid required at least one meal of prey for moulting to occur. Moreover, R. longifrons nearly enclosed third generation nymphs that must be sustaining themselves for the first few days after emergence. This may be due to lack of nutrients results persisting from the egg stage. Similar idea was also expressed by Taylor and Schmidt (1996). Furthermore though A. gossypii were available in plenty R. longifrons did not begin until four days old. This leads to a cannibalistic tendency among the *R. longifrons*.

CONCLUSION

In this study among the four cotton preys reared *R. longifrons* showed maximum survival and fecundity, hatchability of H. armigera. From these results we concluded *H. armigera* is a suitable prey for laboratory rearing of *R. longifrons* and it can be used as a biological control agent against *H. armigera* followed by other cotton pests. Macromolecule profile (Protein, Carbohydrate and Lipid) of pests revealed that lepidopteran pest *H. armigera* has higher quantity of protein and lipids. This too may be responsible for enhancing the reproduction of *R. longifrons*. However, carbohydrate was high in hemipteran pest *P. solenopsis*.

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Table 1. Nymphal survival (%) and reproductive parameters of R. longifrons on C. cephalonica, D. cingulatus, H. armigera, P. solenopsis and A. gossypii (mean value ± SE)

Parameter	*C. cephalonica*	*D. cingulatus*	*H. armigera*	*P. solenopsis**	*A. gossypii**
Nymphal survival (%)	60.4±3.3^{abc}	45.3±4.1^{ab}	54.4±4.0^{ac}	27.8±3.9^{abcd}	61.4±2.7^{abcde}
Male longevity (days)	67.6±0.50^{a}	60.4±0.64^{ab}	65.4±0.34^{bc}	57.6±0.98^{bc}	55.1±0.82^{acd}
Female longevity (days)	68.0±0.30^{a}	67.9±0.54^{ab}	71.3±0.68^{abc}	65.4±0.45^{abcd}	63.0±0.42^{abcde}
Sex ratio (Male: Female)	1:0.48	1:0.59	1:0.54	1:0.56	1:0.37
Pre-oviposition days	10.4±0.16^{a}	10.6±0.20^{ab}	9.5±0.18^{ac}	10.6±0.30^{ab}	10.8±0.43^{abc}
Oviposition days	43.6±0.62^{a}	37.6±0.52^{ab}	47.2±0.80^{abc}	38.2±0.73^{abc}	34.0±0.73^{abcde}
Minimum number of eggs/batch	5.54±0.23^{a}	6.0±0.28^{ab}	6.7±0.34^{ac}	4.8±0.39^{acd}	4.6±0.50^{abcde}
Maximum number of eggs/batch	16.1±0.31^{a}	12.0±0.45^{a}	18.3±0.41^{ac}	10.2±0.46^{acd}	9.39±0.37^{abcde}
Oviposition index	0.050±0.001	0.052±0.001	0.054±0.002	0.047±0.002	0.045±0.002
Post-oviposition days	14.8±0.40^{a}	15.7±0.41^{ab}	14.4±0.37^{abc}	15.9±0.58^{abcd}	18.3±0.43^{abcde}
Fecundity	39.3±0.42^{a}	32.6±0.63^{a}	43.5±0.55^{cb}	30.6±0.64^{bcd}	29.7±0.89^{acde}

*Mean value for two generations

Table 2. Life table parameters of R. longifrons provided with C. cephalonica, H. armigera, D. cingulatus, P. solenopsis and A. gossypii

Parameters	*C. cephalonica*	*H. armigera*	*D. cingulatus*	*P. solenopsis*	*A. gossypii*
Gross reproductive rate (GRR)	133.8±2.21	149.4±3.0	115.9±8.6	131.0±2.7	135.1±3.2
Net reproductive rate (NRR) Ro	86.8±24.7	115.0±31.1	68.3±12.9	76.7±19.8	43.8±15.39
Length of generation (Tc)	46.3±0.87	45.9±1.47	56.7±1.20	62.6±25.6	60.6±1.75
innate capacity for increase (r_c)	0.094±0.007	0.09±0.008	0.073±0.001	0.068±0.002	0.060±0.004
Intrinsic rate of increases Corrected (r_m)	0.090±0.006	0.09±0.007	0.073±0.002	0.71±0.002	0.062±0.005
Finite rate of increase (λ)	1.09±0.005	1.09±0.001	1.08±0.003	1.07±0.002	1.06±0.006
Weekly multiplication of population	1.86±0.06	1.69±0.31	1.67±0.03	1.63±0.02	1.54±0.55
Doubling time (in days)	7.71±0.53	7.28±0.70	9.42±0.31	9.7±0.30	11.4±1.27
Hypothetical female in F2 generation	8765.3±48.33	15220.3±75.53	5007±19.29	6276.5±30.43	2162±13.51

Figure 1. The total nymphal developmental period (day) of R. longifrons reared on CC- Corcyra cephalonica (CC), Dysdercus cingulatus (DC), Helicoverpa armigera (HA), Aphis gossypii (AG) and P. solenopsis (PS)

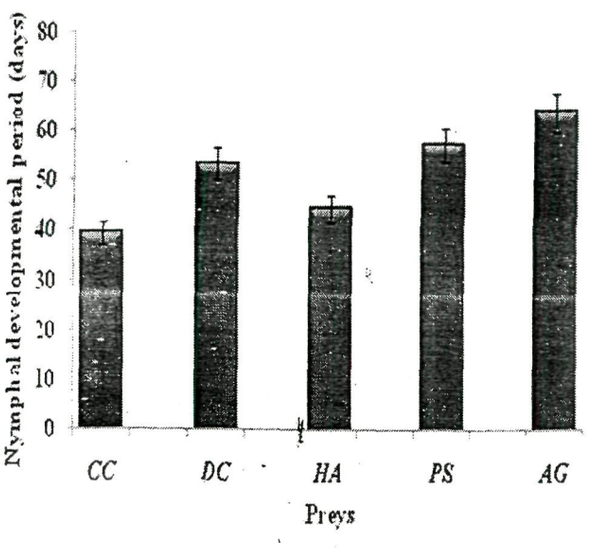

Figure 2. Biochemical analysis of total body protein, lipid and carbohydrate in selected cotton pests (mg/100mg)