429

INTEGRATION OF SELECTED REDUVIIDS AND BOTANICALS IN GROUNDNUT PEST MANAGEMENT

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By

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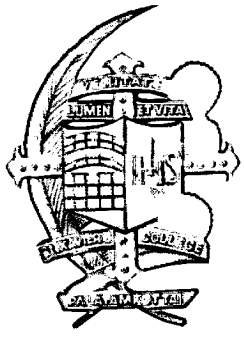

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CERTIFICATE

The thesis entitled **"INTEGRATION OF SELECTED REDUVIIDS AND BOTANICALS IN GROUNDNUT PEST MANAGEMENT"** submitted by **C. RAVI (Reg. No. 1333)** for the award of Degree of Doctor of Philosophy in Zoology of Manonmaniam Sundaranar University is a record of bonafide research work done by him and it has not been submitted for the award of any degree, diploma, associateship, fellowship of any University/Institution.

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DECLARATION

I here by declare that the thesis entitled **"INTEGRATION OF SELECTED REDUVIIDS AND BOTANICALS IN GROUNDNUT PEST MANAGEMENT"** submitted by me for the Degree of Doctor of Philosophy in Zoology is the result of my original and independent research work carried out under the guidance of **Dr. K. Sahayaraj,** Director, Crop Protection Research Centre, Department of Zoology, St. Xavier's College (Autonomous), Palayamkottai - 627 002, and it has not been submitted for the award of any degree, diploma, associateship, fellowship of any University/Institution.

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ABBREVIATIONS USED

AM	–	*Ante meridiem*
ANOVA	–	Analysis Of Variance
BE	–	Benzene Extract
C	–	Celsius
CBB	–	Coommassie Brilliant Blue
CBR	–	Cost Benefit Ratio
CC	–	*Corcyra cephalonica*
cc	–	cubic centimeter
CE	–	Chloroform Extract
cm	–	centimeter
CNS	–	Central Nervous System
Fig.	–	Figure
g	–	gravity
GC - MS	–	Gas Chromatography and Mass Spectra
gm	–	gram
HCH	–	Hydro Chloro Hexane
hrs	–	hours
IC	–	*Ipomea carnea*
IPM	–	Integrated Pest Management
$Kgha^{-1}$	–	Kilogram per hectare
L	–	Litre
LD_{50}	–	Median Lethal Dose
LSD	–	Least Significant Difference

Ltd.	–	Limited
m	–	metre
ml	–	milliliter
mm	–	millimeter
NPV	–	Nuclear Polyhedrosis Virus
NRR	–	Net Reproductive Rate
PM	–	*Post Meridiem*
PTU	–	Phenyl Thio Urea
Pvt.	–	Private
SDS-PAGE	–	Sodium Dodecyl Sulphate - Poly Acrylamide Gel Electrophoresis
sec	–	seconds
sq.	–	square
TMV	–	Tamilnadu Mutan Variety
VN	–	*Vitex negundo*
WE	–	Water Extract

CONTENTS

ABSTRACT

An effort was undertaken to mass rear two harpactorine reduviids, namely, *Rhynocoris longifrons* Stal and *Rhynocoris kumarii* Ambrose and Livingstone at different densities (1, 5, 10, 15 and 20 predators/1 L container for *R. longifrons* and 1, 5, 10 and 20 predators/1 L container for *R. kumarii* respectively) in the laboratory in small scale by using different densities (1 and 2) of alive *Corcyra cephalonica* Stainton (Pyralidae: Lepidoptera) larvae. Experimental results suggested that 10 and 20 predators/1 L container with 2 alive *C. cephalonica* larvae for *R. longifrons* and *R. kumarii,* respectively was the ideal predator and prey proportion for small scale rearing. Heat killed *C. cephalonica* and alive *Spodoptera litura* Fabricius (Noctuidae: Lepidoptera) larvae further enhanced the growth, reduced the developmental period and increased the fecundity and longevity of both the reduviids. Life table studies also confirmed the same. Both the reduviids (IV, V instars and adults) consumed maximum *S. litura* than *Helicoverpa armigera* Hubner (Noctuidae: Lepidoptera) in the laboratory pot study. The extracts of *Vitex negundo* Linn. (*Verbanaceae*) and *Ipomea carnea* Jacq. (*Convolvulaceae*) showed the presence of phenols, carbohydrates, tannins, lignins, triterpenoids and flavonoids. Major compounds present in *V. negundo* are 1H-Indene, Cyclododecanol, Patchoulane, 1, 2-Dihexylcyclopropene-3-carboxylic acid, 2-Heptenoic acid, (+)-Aromadendrene, Trans-caryophyllene, 7-Oxabicyclo (4.1.0) heptane, Cyclohexane, Farnesol, Pentadecane and 1-Octanol. The extacts of *I.*

carnea yielded the compounds such as Neophyadiene, 1-Decanol, Tetradecanoic acid, Pentadecane, 1-Iodo-2-methylundecane, Trans-caryophyllene, Eicosane, 2-Butenoic acid and Cholestan-3-one. Benzene, chloroform and water extracts of *V. negundo* and *I. carnea* significantly caused larval and pupal mortality, increased the larval period and decreased the pupal period, pupal weight and adult longevity and caused many deformities and abnormalities in *S. litura*. They also teared and weared the mandibles of the mouthparts of the larvae. The chloroform extract of both the botanicals had superiority than the other two (benzene and water) extracts. The field release of the laboratory colonized *R. kumarii* life stages and application of the crude water extract of both the botanicals in the groundnut field greatly reduced the incidence and infestations of the groundnut pests and increased the yield and cost benefit ratio. Maximum yield (1655.50 $Kgha^{-1}$) and cost benefit ratio (1 : 2.09) was achieved in the *R. kumarii* released field. From the results, it is concluded that these components could be used in the groundnut pest management.

Review of Literature

I. REVIEW OF LITERATURE

I. 1. Pest and pesticides

The term pest is anthropocentric and is defined differently by diverse segments of the human population. There are no pests in ecological sense; in the absence of humans, all organisms are just part of an ecosystem (Norris *et al.*, 2002). Use of synthetic pesticides is still considered to be the most reliable and economic way to control the pests. Farmers and agricultural workers are exposed directly when they mix and spray pesticides. The soil, water and air are also polluted and there by cause severe health hazards to human beings, domestic animals and also various beneficial organisms such as microbes, arthropods etc. (Sladecek *et al.*, 1982; Jeyarathnam, 1990; David, 1992 and Rao, 1995). The chronic effects are particularly alarming in the light of new studies linking certain pesticides to cancer and decrease in fertility (Soto, 1993; Hileman, 1994 and Pandey, 2003), including a fall in the sperm quantity of human beings (Danish EPA, 1995), general immune system suppression has also been noted (Repetto and Baliga, 1996). In India, nearly 179 pesticides are being used to control various pests (Statistical Information on Plant Protection, 2001) and a recent study revealed that twelve soft drinks manufacturing in India contain pesticide residues (CSE, 2003).

I. 2. Integrated Pest Management (IPM)

IPM was initially considered as a strategy to manage a single pest, combining all the available IPM components in a compatible manner and

using pesticides as a last resort (Jayaraj *et al.*, 1994). IPM has been practicing for the past few decades in most of the developed and developing countries. Over the past decade, IPM has developed into a more holistic approach, in which the pest complex in a crop is considered as a whole, with ecological processes that already exist in the agro-ecosystem being encouraged to do most of the work (Fliert, 1998). The major components of IPM are predators, parasitoids, botanicals, pathogens, microbes, intercrops and pesticides.

I. 3. Biological control and biological control agents

Biological control is the use of parasitoid, predator, pathogen or competitor populations to suppress a pest population, making the pest less abundant and less damaging than it would be in the absence of the biological control agents. One should also ignore that biological control does not cause immediate reduction in the target pest population (Norris *et al.*, 2002).

I. 3. 1. Biopesticides

Living organisms or its product used to control the pests are called as "biopesticides". Biological control agents such as *Stethorus panperculus* (Puttasamy, 1969); *Bacillus thuringiensis* Var. galleria spicturin (Basha, 1970; Babu, 1972; Govindarajan, 1973 and Balasubramanian *et al.*, 2002); *Verticillium lecanii* (Easwaramoorthy and Jayaraj, 1978); *Trichogramma* sp., *Chelonus blackburni* (Natarajan and Jayaraj, 1979); *Metarhizium anisopliae* (Babu, 1980); *Fusarium semitectum* (Nagalingam, 1983); Granulosis virus (Easwaramoorthy, 1984); Nuclear Polyhedrosis Virus (NPV) (Jayaraj, 1985;

Jayaraj *et al.*, 1985 and Rabindra and Balasubramanian, 1985) were studied and analysed against various crop pests.

I. 3. 2. Hemipteran bugs as biological control agents

The important hemipteran bugs used as biological control agents are *Anthocoris* spp. (Anderson, 1962; Beiyer and Chawin, 1992); *Platymeris laevicollis* Distant (Antony *et al.*, 1979); *Nabis* spp. (Anonymous, 1987); *Geocoris puntipes* Say (Cohen, 1984 and Cohen and Byrne, 1992); *Zelus renardii* Kolenati (Cohen, 1993); Stink bug (Hough-Gold stein and Whalen, 1993); *Podisus maculiventris* (Say) (Mukerji and Leroux, 1964) and *P. maculiventris* and *Podisus sagitta* (Fab.) (Declercq and Degheele, 1994); *Eocanthecona furcellata* (Wolff) (Rani and Wakamura, 1993); *Cyrtopeltis tenius* (Torreno, 1994); *Perillus biculatus* (Cloutier and Baudin, 1995); *Cardiastethus exigus* Poppius and *Buchananiella sodalis* (Sujatha and Singh, 1999); *Rhynocoris marginatus* Fabricius (Sahayaraj, 1995, 1998a, 1999b, 2002b; Ambrose and Claver, 1999b and Sahayaraj and Martin, 2003); *Rhynocoris kumarii* Ambrose and Livingstone (Claver, 1998; and Ambrose Claver, 2001a and Claver and Ambrose, 2001b, c) and *Pristhisancus plagipennis* Walker (Grundy and Maelzer, 2000 and Grundy *et al.*, 2000). Navarajanpaul (2002) reported seven hemipteran predators as biological control agents. Recently, Ballal *et al.* (2003) included *Blaptostethus pappescens* Poppius as a biological control agent.

I. 3. 3. Plant biopesticide

Various plant products are being used to control crop and storage pests, vectors and pathogens since the beginning of civilization. The plant kingdom is the rich storehouse of biologically active compounds and various plant products are in use for a long time in India to minimize losses in crops and grain storage. The use of botanicals for protecting crops from insect pests had assumed greater significance in recent years (Krishnarajah and Ganesalingam, 1981; Banerjee *et al.*, 1985; Dakshinamurthy, 1988; Chari *et al.*, 1990; Morallo-Rejesus *et al.*, 1992; Parmar, 1993; Pandian *et al.*, 1994; Baskaran and Naryanasamy, 1995; Pandey and Misra, 1996; Kulkarni and Joshi, 1997 and Ignacimuthu, 1998) because of the growing awareness on the harmful effects of synthetic pesticides. Sundararajan and Kumuthakalavalli (2000b) stressed the main advantages of plant derived pesticides to ecosystem are that they are eco-friendly, easily biodegradable, do not accumulate and biomagnify in the ecosystem.

Grainge *et al.* (1985) and Bhatnagar and Sharma (1994) have reported that worldwide, there are 1005 species of plants with insecticidal properties, 384 species with antifeedant properties, 297 species with repellent properties, 27 species with attractant properties and 31 species with growth inhibition properties. Role of neem (*Azadirachta indica* A. Juss) and pongamia (*Pongamia pinnata* Pierre) were investigated thoroughly (Rout, 1986; Schmutterer, 1990; Mathur and Nigam, 1993; Mordue and Blackwell, 1993; Saradamma *et al.*, 1993; Rao *et al.*, 1995; Shi-Cheng-Ien *et al.*, 1995 and Tamilsolai *et al.*, 1997). Azadirachtin, a potent bioactive compound from

neem was identified and explored for the manufacture of various biopesticides (Schumutterer, 1995) and its insecticidal properties (Tewari, 1992; Ley *et al.*, 1993; Bonford and Isman, 1996; Govindachari *et al.*, 1996 and Mani *et al.*, 1996) were carried out. Even though several other plants such as *Nerium indicum* Mills, *Thevitia peruviam* Mrr. (Dhanapackiam and Shanazbegum, 1995); *Piper nigrum* L., *Annona reticulata* L., *Dillenia retusa* L. and *Ocimum sanctum* L. (Rajapaske, 1996); sweet flag (Behra and Satapathy, 1997); *Adhatoda vasica* Nees, *Annona squamosa*, L. *N. indicum*, *Cleodendrum inerme* L., *P. pinnata*, *Prosopis chinensis*, *Vitex negundo* Linn., *A. indica*, *Aegle marmelos* L. and *Madhuca indica* Gmel. (Hiremath *et al.*, 1997); *Aglaia* spp. (Koul *et al.*, 1997); neem, custard apple and madar leaf (Singh *et al.*, 1998); *Gnidia glauca* Gilg., *Leucas aspera* Link and *Toddalia esicatia* Lam. (Sundararajan and Kumuthakalavalli, 2000a); *Calotropis gigantea* Linn., *Tridax procumbens* L., *V. negundo*, *P. pinnata* (Sahayaraj and Paulraj, 1998a, b, c and 2000 and Paulraj, 2001); *Cassia* sp., *Tridax* sp., Parthenium, Dalbergia, Lawsonia, Eucalyptus and Aegle (Dwivedi and Bajaj, 2002); *Balanites aegyptica*, *Caragona sukiensis*, *Lyonia villosa* (Goel *et al.*, 2002); *Eclipta alba* L. and *Ocimum sanctum* L. (Paulraj and Sahayaraj, 2002); *Christella parasitica* (L.) H. Lev., *Heminiotis arifolia* (Brun) and *Pteridium aquilinum* (L.) Khun (Selvaraj, 2002); *C. parasitica* and *Ipomea carnea* Jacq. (Sahayaraj *et al.*, 2003) are reported to posses pesticidal properties. However, all the above said plants have not explored fully or partially except *A. indica*.

I. 3. 4. *Vitex negundo* Linn.

V. negundo (Family : *Verbenaceae*) is a shrub, commonly found in moist areas, open waste lands and near deciduous forests, usually not browsed by cattle and can be propagated easily by vegetative cutting (Chandha, 1976). It has been reported to possess broad spectrum pesticidal activity (Abraham *et al.*, 1972; Jacobson, 1975; Chandha, 1976; Bai and Kandasamy, 1985; Campos and Quilantang, 1985; Grainge *et al.*, 1985; Tripathi and Rizvi, 1985; Kandasamy *et al.*, 1987; Kalavathi *et al.*, 1991; Manalo, 1992; Rajesus *et al.*, 1993; Sahayaraj and Sekar, 1996; Sahayaraj and Paulraj, 1998c; Amancharla *et al.*, 1999; Raja *et al.*, 2000 and Paulraj, 2001), ovicidal property (Doureessamy *et al.*, 1990 and Rajesus *et al.*, 1993); repellent property (Hebbalkar *et al.*, 1992) against various pests and its leaves certainly act as grain protectants against many stored pests (Krishnarajah *et al.*, 1985; Mia *et al.*, 1985 and Prakash and Rao, 1989) and depressant activity in the Central Nervous System (CNS) of mice (Gupta *et al.*, 1999).

I. 3. 4. a. Chemical constituents of *V. negundo*

The chemical constituents of *V. negundo* were analysed (Anzaldo, 1980; Rao and Jena, 1996). Riaz and Ashraf (1990) discussed about the chemical constituents and their therapeutic use. The identified compounds in *V. negundo* are 4 - 4 - dimethoxy - trans - stilbene (Banerji *et al.*, 1988); flavonoids, casticin, chrysoplenol D, lutcolin, isoorientin, P-hydroxy benzoic acid (Dayrit *et al.*, 1987); lignan (Chawla *et al.*, 1992); iridoids (Dayrit and Lagurin, 1992, 1994); sabinene, P-cymene, beta phelladune, gamma - terpinene, terpinen - 4 - ol, beta-caryophyllene, globul and viridifloral

(Mallavarpu *et al.*, 1994); mono and sesquiterpenes (Jirovetz *et al.*, 1998); viridiflorol, beta-eudesmol and beta-caryophyllene (Dayal and Singh, 1999, 2000). On the whole, the volatile constituents of the *V. negundo* leaves possess sixty six compounds (Dayal and Singh, 1999 and Singh *et al.*, 1999).

I. 3. 5. *Ipomea carnea* Jacq.

Ipomea carnea Jacq. (Family: *Convolvulaceae*) is a weed plant present throughout India, having insecticidal property against many economically important stored product pests (Pandey *et al.*, 1986 and Panigrahi and Sahu, 2000) and crop pests (Kulat *et al.*, 1997; Ramamurthy and Venugopal, 1997; Ramamurthy and Rajaram, 2001 and Thakre *et al.*, 2003) and repellent property against *Tribolium castanaem* Herbst. (Sahayaraj and Ravi, 2003). The water extract of the whole plant of *I. carnea* is used for anti-rheumatic remedy and muscle pain relaxant in Bolivia (Frey, 1995). Very recently, Sahayaraj *et al.* (2003) reported the pesticidal property of *I. carnea* on *Achaea janata* Linn. So far, no phytochemical studies were carried out in this plant except the work of Nair and Shukla (2003). According to them, flavonoid and saponin compounds are present in *I. carnea* and are used in the manufacturing of paper.

I. 4. Groundnut

Groundnut (*Arachis hypogea* Linn.) belongs to the Family *Papilionaceae,* oil extracted from the seeds and having very much economic importance. The groundnut oil cake is a fodder for the cattle and the leaves are also given as supplementary food for certain domestic animals. In India,

groundnut is being cultivated in a vast area (8.6 million hectares) both in rain-fed and irrigated conditions. In area wise, India is first among the world countries cultivating groundnut but the average production is just 945 $Kgha^{-1}$ where the developed countries could achieve 2500 to 4000 $Kgha^{-1}$ (Ghewande *et al.*, 1996; Dharne and Patel, 2000 and Paulraj, 2001).

The farmers cultivated groundnut in India until 1968 need to worry about only four serious insect pests, namely, aphids, groundnut leaf miner, hairy caterpillars and termites. After that, the number of pests infesting groundnut crop has increased and now there are nearly 120 pests attack groundnut both at crop stage and storage (Ramaraju *et al.*, 1998). The pests are classified as defoliators, sucking pests, root and pod feeders and storage pests. The important defoliators are the larval forms of *Amsacta albistrigia* Walker, *Aproarema modicella* Dev., *Spodoptera litura* (Fab.), and *Helicoverpa armigera* Hubner and they cause severe damage to the crop (Amin, 1983; Panchabhavi and Nethradhaniraj, 1987; Wightman and Rao, 1993 and Brar *et al.*, 1995). The other major pests of groundnut are jassids (Singh *et al.*, 1993), thrips, white flies, bugs, beetles and grasshoppers (Jayanthi *et al.*, 2000 and Sridhar and Mahto, 2000). The irrigated groundnut in Tamil Nadu is severely attacked by *S. litura* and *H. armigera* (Peter and David, 1998).

The extent losses of groundnut feeding and transmitting virus disease of aphids are well reported (Brar and Sandhu, 1975 and Veeresh *et al.*, 1989). Biopesticides affect the growth and development of aphids (Vikaria and Patel, 2000). Grasshopper cause leaf damage and yield loss in

groundnut. Thomas *et al.* (1996) stressed the importance of biopesticide in grasshopper management and it is being controlled by both biological and chemical agents in groundnut field (Peveling *et al.*, 1999). Incorporation of biopesticides in the IPM of grasshoppers and locust (Lomen *et al.*, 1999) and other pests (Nandagopal, 1982; Ghewande and Misra, 1986 and Manjula and Sulochanamma, 2001) were reported.

1. 5. *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

S. litura is commonly called as Army worm. Initially it was the pest of tobacco alone, later, it attained the polyphagous status and feeds more than 112 cultivated crops all over the world (Moussa *et al.*, 1960) and 40 species of plants in India (Thobbi, 1961; Lefroy, 1980 and Singh *et al.*, 1998). It is distributed throughout Asia and the Pacific islands (Wightman and Rao, 1993 and Martinez and Emden, 1999). The larvae of *S. litura* defoliate the plants like groundnut, cotton, castor, tomato, onions, legumes etc. and the presence of three egg masses of an average size (230 eggs/batch) in 15 m^2 crop area may cause significant loss of groundnut pod (Panchabhavi and Nethradhaniraj, 1987). One *S. litura* larva per plant during the seedling stage reduced the pod yield of groundnut by 25.8 per cent (Dhir *et al.*, 1992).

The crop loss in oil seeds due to *S. litura* was studied by Jayarajan (1990); Gupta and Rao (1994); Mani and Rao (1998); Ramaraju *et al.* (1998) and Narendran *et al.* (1999). Severe out break of this pest on cotton, tobacco, chilli and groundnut were noted in Tamil Nadu (Santharam, 1986). Application of insecticides is unavoidable to control this pest and a few

insecticides have been recommended (Chari and Patel, 1972, 1983 and Manjula and Sulochanamma, 2001). *S. litura* became resistance to HCH in Rajasthan during 1965 (Srivastava and Joshi, 1965). The reports of Verma *et al.* (1971) and Ramakrishnan *et al.* (1984) revealed that *S. litura* became resistant to HCH, malathion, endosulfan and carbaryl in Haryana and Andhra Pradesh. Now it is likely resistant to most of the insecticides throughout India (Singh and Singh, 1998). Apart from the resistance of the pest to the pesticides, the pesticides also cause adverse effect in human and other animals (Brown, 1986). So an alternate remedy is essential to minimize the *S. litura* infestation without much harm to the environment.

I. 5. 1. Plant products' impact on *S. litura*

Various plant products have been tested against *S. litura* in the laboratory for its antifeedant property (Joshi and Ramaprasad, 1975; Koul, 1985; Ayyangar and Rao, 1989a, b; Gupta and Rao, 1994; Jayarajan *et al.*, 1990; Dhanapakiam and Shanazbegum, 1995; Mohapatra *et al.*, 1995; Chitra and Rao, 1996; Koul *et al.*, 1996; Senthilkumar *et al.*, 1997; Murugan *et al.*, 1998; Sahayaraj, 1998a; Yasui *et al.*, 1998; Narendran *et al.*, 1999 and Dwivedi and Mathur, 2000); repellent property (Sayed, 1983 and Ayyangar and Rao, 1989a); ovicidal property (Suryakala *et al.*, 1995) and mortality (Bai and Kandaswamy, 1985; Sahayaraj and Paulraj, 1998a, b, c; Martinez and Emden, 1999 and Sahayaraj *et al.*, 2001).

Botanicals such as *V. negundo* (Bai and Kandaswamy, 1985; Sahayaraj and Sekar, 1996 and Paulraj, 2001); garlic (Mukherjee and Sharma, 1990);

wild groundnut (Stevenson *et al.*, 1993); *Cymbogan citratus* Stapf. (Suryakala, 1995); pine species (Salam and Ahmad, 1997); neem, vitex, calotropis and pongamia (Sahayaraj and Paulraj, 1998a, b, c and Paulraj, 2001); *Artemisa vulgaris* (Singh and Rao, 1999); Annona (Boreddy and Chitra, 2001); *Allium sativum* L. (Lakshmanan, 2001); *Coleus aromaticus* Benth (Sahayaraj *et al.*, 2001); pteridophytes (Selvaraj, 2002) were evaluated in the laboratory on *S. litura.*

I. 6. Field application of botanicals

Various botanicals were applied in the field and their impact on pest infestation and incidence were studied (Mohamad, 1981; Dhir *et al.*, 1992; Kalyanasundaram *et al.*, 1994; Ramamurthy and Venugopal, 1997; Ramaraju *et al.*, 1998; Paulraj, 2001; Selvaraj, 2002 and Sahayaraj, 2002c). The impact of the botanicals on *S. litura* and *H. armigera* in the field were evaluated (Doureesamy *et al.*, 1990; Vinuela *et al.*, 1999; Obulapathi *et al.*, 2000; Paulraj, 2001; Selvaraj, 2002 and Sahayaraj, 2002c). The groundnut production and cost benefit ratio was also studied (Kalyanasundaram *et al.*, 1994; Paulraj, 2001 and Selvaraj, 2002). The extracts of *V. negundo* (Ramamurthy and Venugopal, 1997; Paulraj, 2001 and Sahayaraj, 2002c) and *I. carnea* (Ramamurthy and Venugopal, 1997 and Kulat *et al.*, 1997) are also applied in the field level.

I. 7. Reduviids

Predators are organisms that kill and feed on their prey, require several preys to complete their development and they account for much of the

reduction of pest insect populations in nature. Reduviids are generalist predators that mainly feed on lepidopteran, coleopteran, hemipteran and isopteran insects and considered as a potential biological control agent against various pests (Joseph, 1959; Schaefer and Ahmad, 1987; Schaefer, 1988; Zanucio *et al.*, 1992; James, 1994; Zanucio *et al.*, 1994; Ambrose, 1995, 1996, 1999, 2000; Sahayaraj, 1999b, 2002b and Sahayaraj and Martin 2003) and they can be utilized as a biological control agent where a variety of pests occur (Sahayaraj, 2002b and Ambrose, 2003). The incidence of reduviid predators in diverse cropping system and their biological control potential is documented (Werner and Butler, 1957; Whitcomb and Bell, 1964; Altieri and Whitcomb, 1980; Mc Pherson *et al.*, 1982; Ambrose, 1987, 1988, 1991, 1995, 1996, 2000; Sahayaraj, 1991, 1998a, 1999b, 2001, 2002b; Claver, 1998; Claver and Ambrose, 2001a, b and Sahayaraj, 2004).

I. 7. 1. Mass multiplication of reduviids

Rearing and mass multiplication of the reduviids in the laboratory is an important requirement for successful biological control programme (Manjunath, 1984; Schaefer, 1988; Cohen, 1993; Ambrose, 1995; Grundy *et al.*, 2000 and Sahayaraj, 2001, 2004). Attempts were made by several workers to mass multiply the reduviids in the laboratory (Lakkundi, 1989; Sahayaraj, 1991; Kumaraswami, 1991; Claver, 1998; Grundy and Maelzer, 2000; Sahayaraj and Paulraj, 2001 and Sahayaraj, 2002b). But it is being a major constraint because of its high labor cost, laborious process (Ambrose, 1988, 1999) and nymphal cannibolism (Ambrose, 1999; George and Ambrose, 2000 and George, 2000b). The reduviids are reared in the laboratory mainly on the

rice moth *Corcyra cephalonica* Stainton alive larvae (Lakkundi and Parshad, 1987; Sahayaraj, 1991, 2001, 2002b; Claver, 1998; George, 1999, 2000a; Ambrose, 1999 and Sahayaraj and Paulraj, 2001) and other prey such as *S. litura* (George *et al.*, 1998; George, 2000a and Sahayaraj and Paulraj, 2001); *Earias vitella* Fabricius (George *et al.*, 1998 and George, 2000a); *H. armigera* and *Nezara viridula* L. (Grundy and Maelzer, 2000); frozen larvae of *C. cephalonica* (Sahayaraj and Jayalakshmi, 2002) and *H. armigera* (Grundy and Maelzer, 2000) and oligidic diet (Sahayaraj, 2004) were also tried for rearing the reduviids. In India, a few reduviids were mass reared in the laboratory and a little success was achieved. They are, *P. laevicollis* (Antony *et al.*, 1979); *Acanthaspis quinquespinosa* Fabricius (Lakkundi, 1989 and Sahayaraj, 1991); *Brassivola hystrix* Distant, *Coranus* sp., *Endochus parvispinus* Distant, *Irantha armipes* Stal, *Isyndus heros* (Fabricius) (Lakkundi, 1989); *Acanthaspis pedestris* Stal (Sahayaraj, 1991); *Cyndocoris gilvus* Burm (Venkatesan *et al.*, 1997); *R. kumarii* (Claver, 1998); *R. marginatus* (George, 2000b and Sahayaraj, 2002c); *Neohaematorrophus therasii* Ambrose and Livingstone (Sahayaraj, 2001).

I. 7. 2. Life table studies in reduviids

Life table is an important tool in the insect population studies and prediction of the F_2 generation (Deevy, 1947; Birch, 1948; Watson, 1964 and Southwood, 1978). Life table studies on reduviids are scanty except *Eupeodes carollae* Fabricius (Sharma and Bhalla, 1995); *C. gilvus* (Venkatesan *et al.*, 1997); *R. kumarii* (Claver, 1998); *Acanthaspis siva* Distant (George *et al.*, 1998); *R. marginatus* (George, 1999, 2000a; Sahayaraj and Paulraj,

2000; Sahayaraj, 2002b and Sahayaraj and Jeyalakshmi, 2002); *P. plagipennis* (Grundy and Maelzer, 2000) and *Rhynocoris fuscipes* Fab. (George, 2002).

I. 7. 3. Nutritional quality of the prey

The quality and quantity of nutrients of the prey influence not only the growth rate and survival of the predator (Slansky and Scriber, 1985; Flower *et al.*, 1987; Ambrose and Subbarasu, 1988; Ambrose *et al.*, 1990; O'Neil and Widenman, 1990; Ambrose and Rani, 1991; Cohen, 1990, 1993 and George *et al.*, 2002) but also the fecundity and life table characteristics such as generation time and intrinsic rate of population increase (Awadallah *et al.*, 1986 and George, 1996, 1999, 2000a). The nutritional quality of the prey is having a high influence over the whole physiological process of a predator (Ananthakrishnan, 1996).

I. 7. 4. Frozen, heat killed and dead larvae in predators' biology

Rearing of the predators by using frozen, heat killed and dead larvae were attempted by several workers. Yasuda and Wakamura (1991) tested the suitability of *S. litura* larvae to *Eocanthecona furcellata* (Wolff). Attempts were made on feeding dead larvae of *Bombyx mori* L. and frozen larvae of *Psorocampa denticulate* to *Apiomerus* sp. and *Montina confusa* (Zanuncio *et al.*, 1992); hot water killed larvae of *Tenebris molitor* (L.) and *H. armigera* to *P. plagipennis* (Grundy *et al.*, 2000) and frozen larvae of *C. cephalonica* to *R. marginatus* (Sahayaraj and Jeyalakshmi, 2002) and evaluated the biological parameter of the predators.

I. 7. 5. *Rhynocoris kumarii* Ambrose and Livingstone (Heteropetra : Reduviidae)

R. kumarii (plate 1a and b), a harpactorine reduviid predator, described by Ambrose and Livingstone (1986), generally present in agroecosystems, semiarid zones, scrub jungles and tropical rain forests (Ambrose and Livingstone, 1986; Vennison and Ambrose, 1990; Sahayaraj, 1991; Kumar, 1993; Kumaraswami and Ambrose, 1994; Ambrose and Rajan, 1995 Edwin and Ambrose, 1996 and Rajan and Ambrose, 1996). Its biology (Ambrose and Livingstone, 1987a and Claver, 1998); mating behaviour (Ambrose and Livingstone, 1987b); parental age on development of off springs (Ambrose *et al.*, 1988); the saliva spitting behaviour (Vennison, 1988 and Vennision and Ambrose, 1990) and predatory behaviour (Ambrose and Claver, 1996); nymphal cannibolism (George, 2000b) were studied extensively. Ambrose and George (1996) reported the effect of flooding on the incubation and hatchability of *R. kumarii* eggs. Five types of haemocytes were observed in the haemolymph of this bug (Ambrose and George, 1996 and George, 1996). The insecticidal impact on the postembryonic development (George, 1996 and George and Ambrose, 1999a); biochemical modulations by insecticides (George and Ambrose, 1999b) were studied. Impact of antennectomy and eye blinding on the predatory behaviour (Claver and Ambrose, 2001d) were also studied.

It has been reported as a potential predator on various economical important agricultural pests such as *S. litura* (Kumaraswami, 1991; Sahayaraj, 1994; Ambrose and Claver, 1995, 2001a, b; Sahayaraj and Sivakumar, 1995; Claver and Ambrose, 2001a); *Papilio demoleus* L. (Kumaraswami, 1991);

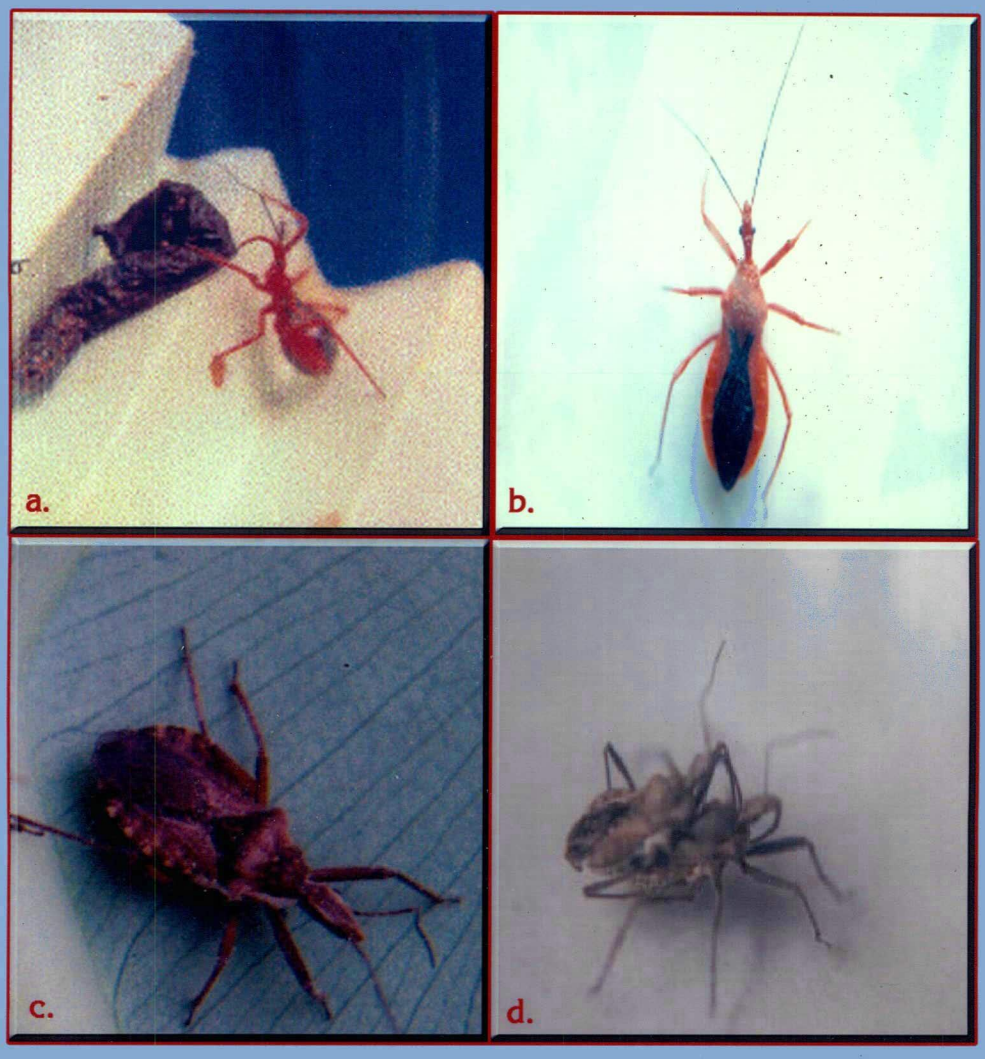


Plate. 1. Experimental reduviids, *Rhynocoris kumarii* Ambrose and Livingstone nymph feeding on *S. litura* larvae (a) and adult (b), *Rhynocoris longifrons* Stal adult (c) and mating behaviour (d)

Dysdercus cingulatus Fabricius (Kumaraswami, 1991 and Ambrose, 1995); *Earias vitella* Fabricius (Kumaraswami, 1991 and Ambrose, 1995); *Euproctis mollifera* Walker (Kumaraswami, 1991 and Ambrose, 1995); *Oxycarneus hyalipennis* Costa (Kumaraswami, 1991 and Ambrose, 1995); *Earias insulana* Boisdual (Ambrose, 1995); *Euproctis fraterna* Moore (Ambrose, 1995); *Achaea janata* Linn. (Ambrose and Claver, 1995 and Ambrose, 1996); *Ergolis merione* Costa (Ambrose, 1995 and Ambrose and Claver, 1995); *Patanga succinata* L. (Ambrose, 1995); *Anomis flava* Fabricius (Ambrose and Claver, 1995); *Pectinophora gossypiella* Saunders (Ambrose, 1995; Ambrose and Claver, 1995); *Calacoris angustate* Lethiery (Ambrose and Claver, 1995 and Ambrose, 1996); *Mylabris pustulata* Thunberg (Ambrose, 1995) and *H. armigera* (Ambrose, 1995, 1996, and Claver and Ambrose, 2001b). In the laboratory, it also feeds on the stored product pests such as *Tribolium confusum* Duv. (Ambrose, 1988) and larvae of *C. cephalonica* (Kumaraswami, 1991; Ambrose, 1996 and Claver, 1998).

Biological control potential related aspects like host preference (Kumaraswami, 1991); searching behaviour (Claver and Ambrose, 2001a); functional and numerical response (Ambrose and Claver, 1996); biological control potential in the laboratory (Kumaraswami, 1991; Sahayaraj, 1994 and Claver, 1998), field cages (Claver and Ambrose, 2001b) and field (Claver and Ambrose 2001c). Moreover, the development and life table on *S. litura, E. vitella* and *C. cephalonica* (George, 2002a) was reported. Mass rearing (Claver *et al.*, 1996) of this reduviid was also studied. Ambrose and Claver

(1999a) observed the impact of substrata impact on mass rearing of this reduviid.

I. 7. 6. *Rhynocoris longifrons* Stal (Heteroptera: Reduviidae)

R. longifrons (plate 1c and d), a potential biological control reduviid predator, generally found in agroecosystems, semi-arid zones and scrub jungles (Kumar, 1993 and Ambrose, 1999). The postembryonic development (Kumar, 1993), mating behaviour (Das, 1996) and oviposition (Ambrose, 1999) was studied. Ambrose (1980) and Kumar (1993) reared this bug with *Componotus compressus* Fabricius and *Odontotermes obesus* Rambur respectively.

It was reported to feed on *H. armigera* and *S. litura* (Ambrose, 2003). Kumar and Ambrose (1996) and Claver *et al.* (2002) studied the functional response of this bug on *Clavigrella gibbosa* Spinola. Very little information was available about the biological control potential of this bug.

I. 7. 7. Field release of reduviids

In India, an exotic reduviid predator *P. laevicollis* was colonized and the laboratory reared bugs were released in large numbers on the coconut palm in Kerala, Lakshadeep and Karnataka and they controlled the beetle *Oryctes rhinoceros* Linn. (Antony *et al.*, 1979). After a long time, Sahayaraj (1999b) mass reared *R. marginatus* in the laboratory and released in the groundnut field and reported that *R. marginatus* greatly suppressed the population of *S. litura*, *H. armigera* and *A. modicella*. The field release of *R. kumarii* (Ambrose and Claver, 1999b and Claver and Ambrose, 2001b, c)

and *R. marginatus* (Sahayaraj, 2002c and Sahayaraj and Martin, 2003) was also performed.

From the available literature, it is known that various pests are affecting the groundnut crop and production and *S. litura* is the predominant and destructive one. Since *V. negundo* and *I. carnea* have insecticidal property and not explored much, both these plants were chosen for the laboratory examination on *S. litura* and field evaluation against groundnut pests and its impact on groundnut production. *R. kumarii* is a potential biological control agent as evident from the reported works and *R. longifrons* is a little known but efficient predator. The above mentioned two reduviids were selected for the present study because the literature review revealed that there is a lacuna in the small-scale mass rearing/group culture with various prey types and their efficiency in controlling the groundnut pest and its impact on groundnut production. Moreover, no one has attempted the release of *R. kumarii* in the groundnut field and evaluated the impact on the groundnut pests' suppression and groundnut yield. Hence, an attempt has been made with the following objectives:

i. To find out the suitable prey (alive *C. cephalonica*) and predator (*R. kumarii* and *R. longifrons*) density for the small-scale mass rearing/group culture of the two reduviids.

ii. To check whether the frozen and/or heat killed *C. cephalonica* and alive *S. litura* have any impact on the biological and life table parameters of both *R. kumarii* and *R. longifrons*.

iii. To evaluate the predatory potential of *R. kumarii* and *R. longifrons* on *S. litura* and *H. armigera* in the laboratory by pot studies.

iv. To analyse the chemical constituents (both qualitative and quantitative) of both *V. negundo* and *I. carnea* leaves and identification of the compounds present in them using GC-MS.

v. To find out the impact of chloroform, benzene and water extracts of *V. negundo* and *I. carnea* on the development and ovipostion of *S. litura* third instar in the laboratory.

vi. To assess the efficacy of both the botanicals and *R. kumarii* life stages in the groundnut field and their impact on groundnut pest suppression and groundnut production.

"Integration of chosen reduviids and botanicals in groundnut pest management". Ph.D. Thesis submitted by C. Ravi to Manomaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

CHAPTER - 1

LABORATORY COLONIZATION AND BIO-EFFICACY OF TWO HARPACTORINE REDUVIIDS

1. 1. Introduction

One of the important component of the Integrated Pest Management is the natural enemies. Reduviids are the most important and unexplored natural enemy in the IPM programme. Reduviids play a major role in suppressing the pest population in nature (Sahayaraj, 1991; Ambrose, 1999, 2000 and Sahayaraj, 2002c) and they can be utilized as a biological control agent, where a variety of pests occur (Schaeffer, 1988; Lakkundi, 1989; Grundy and Maelzer, 2000; Sahayaraj, 2002c; Ambrose, 2003 and Sahayaraj and Martin, 2003). Mass multiplication of the predators in the laboratory is an important requirement for successful biocontrol programme (Schaefer, 1988; Manjunath, 1992; Cohen, 1993; Ambrose, 1995; Sahayaraj, 1998a and Grundy *et al.*, 2000). Attempts were made by several workers to mass multiply the reduviids in the laboratory (Lakkundi, 1989; Grundy and Maelzer, 2000 and Sahayaraj and Jeyalakshmi, 2002). In India, various reduviids such as *Platymeris laevicollis* Distant (Antony *et al.*, 1979); *Brassivola hystrix* Distant, *Coranus* sp., *Endochus parvispinus* Distant, *Iranths armipes* Stal, *Isyndus heros* (Fabricius) (Lakkundi, 1989); *Acanthaspis quinquespinosa* Fabricius (Lakkundi, 1989 and Sahayaraj, 1991); *Acanthaspis pedestris* Stal (Sahayaraj, 1991); *Cyndocoris gilvus* Burm (Venkatesan *et al.*, 1997); *Rhynocoris kumarii*

Ambrose and Livingstone (Claver, 1998); *Rhynocoris marginatus* Fabricius (George, 2000a; Sahayaraj, 2002b and Sahayaraj and Jeyalakshmi, 2002) and *Neohematorrophus therarii* Ambrose and Livingstone (Sahayaraj, 2001) were mass reared in the laboratory and a little success was achieved. But it is being a major constraint because of the non-availability of the techniques, suitable prey and high labor costs (Sahayaraj, 1998a and Ambrose, 1999). Cost effective mass rearing system for beneficial insects to be used in augmentative biological control should enable the production of large number of insects at the lowest possible price (Grenier *et al.*, 1994 and Lappla and King, 1997).

The quality and quantity of nutrients of the prey influences not only the growth rate and survival of the reduviid predator (Ambrose and Subbarasu, 1988; Ambrose *et al.*, 1990; Ambrose and Rani, 1991; Cohen, 1990, 1993 and George *et al.*, 2002) but also the fecundity and life table characteristics such as generation time and intrinsic rate of population increase (Awadallah *et al.*, 1986; George, 1999 and George *et al.*, 1998). The reduviids were reared in the laboratory mainly on the live larvae of the rice moth *C. cephalonica* and *S. litura* (Sahayaraj, 1991, 2002b; George, 1999, 2000a; Claver, 1998 and Ambrose, 1999) and frozen lepidopteran larvae (Grundy and Maelzer, 2000 and Sahayaraj and Jeyalakshmi, 2002).

Life table studies on the reduviids are scanty except the works on *C. gilvus* (Venkatesan *et al.*, 1997); *R. marginatus* (George, 1999, 2000a; Sahayaraj and Paulraj, 2001 and Sahayaraj and Jeyalakshmi, 2002); *R. kumarii*

(Claver, 1998) and *P. plagipennis* (Grundy and Maelzer, 2000). Although augmentation and conservation of reduviid predators have been attempted in a small scale, more studies are needed to achieve economically large scale mass rearing (Ambrose, 2000).

R. kumarii is a potential reduviid predator of various economical important agricultural pests (Kumaraswami, 1991; Sahayaraj, 1994; Ambrose and Claver, 1995, 2001a, b, c; Sahayaraj and Sivakumar, 1995; Ambrose, 1995, 1996, 2000; Ambrose and Claver, 2001a, b and Ambrose, 2003). Further more, in the laboratory, it also feeds on the factitious pest such as *Tribolium confusum* Duv. (Ambrose, 1988 and Claver, 1998). The substrata impact on mass rearing (Ambrose and Claver, 1999a); development and life table (George, 2000a); nymphal cannibolism (George, 2000b) and impact of antennectomy and eye blinding on the predatory behaviour (Claver and Ambrose, 2001d) were also studied.

R. longifrons, a harpactorine reduviid predator, generally found in agro ecosystems, semi arid zones and scrub jungles (Kumar, 1993 and Ambrose, 1999). The postembryonic development (Kumar, 1993); mating behaviour (Das, 1996); functional response (Kumar and Ambrose, 1996 and Claver *et al.*, 2002) and oviposition (Ambrose, 1999) were studied. Ambrose (2003) mentioned the biological control potential of this bug in his review paper. Ambrose (1980) and Kumar (1993) reared this bug on *C. compressus* and *O. obesus* respectively. However, the mass multiplication of *R. kumarii* and *R. longifrons* in the laboratory and the prey and predator density on its

development, survival and life table characteristics are lacking. The present study has the following objectives:

i. To find out a small-scale rearing technique for both *R. kumarii* and *R. longifrons* in the laboratory condition using alive, frozen and heat killed *C. cephalonica* and alive *S. litura* larvae

ii. To find out the impact of prey and predator density on the survival, development, reproduction and life table parameters of the two reduviids and

iii. To evaluate the predatory potential of *R. kumarii* and *R. longifrons* on *S. litura* and *H. armigera* larvae (pot study) as a preliminary test for field release

1. 2. Materials and Methods

1. 2. 1. Collection and maintenance of reduviids

Nymphs and adults of *R. kumarii* and *R. longifrons* were collected from the scrub jungles and bordering agroecosystems of Aralvaimozhi and Chunkankadai, Kanyakumari District, Tamil Nadu. They were maintained in the laboratory on the larvae of *C. cephalonica* at a temperature of 29 ± 2^{0}C; 70 - 80% RH and 13 L : 11 D hours photoperiod. The reduviids were reared in plastic boxes (1L capacity), filter paper was placed at the bottom of the box and chart paper folded into zig-zag pattern (10 × 15 cm) was kept inside the boxes for the resting of the predator. Newly hatched nymphs (>24 hrs) were used for the experimental purposes.

1. 2. 2. Rearing of the factitious host

The following ingredients were used as the culture medium for rearing *C. cephalonica*: Wheat flour (1 kg); Powdered Groundnut (200 g); Baker's yeast (5 g); Streptomycin (15 mg) (Sarabhai Piramal Pharmaceuticals Pvt. Ltd., Mumbai, India) and Multivitamin (500 mg) (Glaxo, India). The ingredients were mixed thoroughly and taken in a plastic trough (3 L capacity). Half cc of *C. cephalonica* egg was purchased from District Agriculture Office, Palayamkottai and sprinkled over the medium and the trough was covered with a white muslin cloth, tightly closed with rubber bands. The troughs were kept undisturbed in a wooden rack at ambient laboratory conditions (as mentioned earlier). After 25 days, the larvae (approximately 4.5 mm and 45 mg length and weight, respectively) were collected manually and their heads were crushed using a forceps to prevent the larvae from forming webs and provided to the reduviids.

1. 2. 3. Collection and maintenance of *Spodopetra litura* (Fabricius)

Egg mass and caterpillars of *S. litura* were collected from groundnut, castor and bhendi agroecosystems of Alankulam, Athioothu and Pavoorchatram, Tirunelveli District and Vallanad, Theivacheyalpuram and Chakkamalpuram, Tuticorin District, Tamil Nadu, India. They were transported to the laboratory and reared in wide mouth plastic containers (2 L capacity, 20 cm diameter) on groundnut leaves with the earlier mentioned laboratory conditions. Laboratory emerged third and fourth instar larvae were used for rearing the reduviids.

1. 2. 4. Collection and maintenance of *Helicoverpa armigera* Hubner

The caterpillars of *H. armigera* were collected from groundnut and bhendi agroecosystems of the same locality where *S. litura* caterpillars were collected. They were transported to the laboratory and reared individually in plastic containers (60 ml capacity) to avoid cannibolism in the earlier mentioned laboratory conditions on groundnut leaves and flowers. Laboratory emerged fourth instar larvae were used for the biological control potential evaluation studies.

1. 2. 5. Experimentation

Experiments were performed in four categories such as reduviids reared on alive (first), heat killed (second), frozen (third) larvae of *C. cephalonica* and alive larvae of *S. litura* (fourth category).

1. 2. 5. 1. Alive *C. cephalonica* larvae

R. longifrons were reared at five different densities viz., 1, 5, 10, 15 and 20 predators/container with 1 prey/predator (A1, A2, A3, A4 and A5) and 2 preys/predator (A6, A7, A8, A9 and A10). Similarly, *R. kumarii* was reared at four predator densities viz., 1, 5, 10 and 20 predators/container with 1 prey/predator (B1, B2, B3 and B4) and 2 preys/predator (B5, B6, B7 and B8). Sixty replications were made in each category.

1. 2. 5. 2. Heat killed *C. cephalonica* larvae

Water was heated at 60^0C in a glass beaker and the IV and V instar *C. cephalonica* larvae were allowed to immerse in it, after thirty seconds, they were taken out and the water was removed using blotting paper and then

provided as feed to the reduviids at 2 prey/predator/day (10 predators/container for *R. longifrons* and 20 predators/container for *R. kumarii,* respectively) with 60 replication in each category.

1. 2. 5. 3. Frozen *C. cephalonica* larvae

In another experiment, alive fourth and fifth instar larvae of *C. cephalonica* were collected and taken in a petridish. They were kept in the refrigerator (4^0C), undisturbed for four hours. Then they were taken out and kept at room temperature for five to ten minutes and provided as food to the reduviids (2 prey/predator) (10 predators/container for *R. longifrons* and 20 predators/container for *R. kumarii* respectively) with 60 replication in each category.

1. 2. 5. 4. Alive *S. litura* larvae

In the fourth category, *R. longifrons* (10 predators/container) and *R. kumarii* (20 predators/container) were provided with alive *S. litura* (equal number of III and IV instar larvae at 2 prey/predator/day) with 60 replications.

All the experiments were performed in 1L plastic containers and in all the experiments, the adults were maintained with the same feed till their death in the same container. Further more, equal number of male and female was maintained in all the experimental categories. The predators were fed once in three days and twice in a week during the nymphal instars and adults, respectively. The unfed if any and the exuvae were removed daily. The parameters such as individual nymphal developmental period, survival

rate, predatory rate (no. of prey consumed/predator/day) and sex ratio (♀/♀+♂) were observed. Further more, pre-oviposition period, number of eggs laid by a female and hatching percentage of eggs was recorded for all the experimental categories.

1. 2. 6. Life table

Life tables were constructed according to the methods recommended by Birch (1948) and elaborated by Howe (1953), Watson (1964), Laughlin (1965) and Southwood (1978). For each female, oviposition and mortality were checked at two or three days intervals. Daily oviposition was calculated by dividing the total number of eggs laid over an interval by its length (number of days). Time of death was the beginning of the age interval in which the animal died.

In life table statistics, the intrinsic rate of increase has been determined using the equation $\sum e^{-rm \times lxmx-l}$, where e is the base of natural logarithms, x is the age of the individuals in days, l_x is the number of individuals alive at age x as the proportion of 1 and m_x is the number of female offspring produced per female in the age interval x. The sum of products $l_x m_x$ is the net reproductive rate (R_0). The rate of multiplication of population for each generation has been measured in terms of females produced per generation. The precise value of cohort generation has been calculated as follows,

$$T_c = \frac{\sum l_x m_x}{R_0}$$

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

$$r_c = \frac{\log_e R_0}{T_c}$$

This is an appropriate rm value. The values of negative exponent of e^{-rmx} ascertained from this experiment often lay outside the range. For this reason both sides of the equation have been multiplied by a factor of $\Sigma\ e^{7\text{-}rm\ x\ lxmx}$ - 1096.6 (Birch, 1948 and Watson, 1964). The two values of $\Sigma\ e^{7\text{-}rm\ x\ lxmx}$ have been then plotted on the horizontal axis against their respective arbitrary rm on the vertical axis. Two points have been then joined to give a line, which is intersected by a vertical line drawn from the desired value of $e^{-rm\ x\ lxmx}$ (1096.6). The point of intersection gives the value of rm accurate to three decimal places. The precise generation time (T) has been then calculated from the equation

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

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

1. 2. 7. Bio-effficacy of reduviids on *S. litura* and *H. armigera*

Groundnut plant (TMV 7) was grown in the mud pot (30 cm diameter and 45 cm height). The pots were covered with top opened transparent plastic acrolic sheet (0.5 mm thickness). After 45 days of plant age, one and two *S. litura* (IV instar) larvae separately were introduced in to each pot, allowed to acclimatize for one hour and covered the top with nylon net (50 mesh/sq.cm). Weighed *R. longiforns* and *R. kumarii* (IV and V instars and adults) were introduced into the pots separately. After 24 hrs, the prey consumed by the predator was recorded and the weight of the predator was also measured. The weight gain of the predator was calculated by subtracting the final weight from the initial weight of the predator. Similar procedure was followed for *H. armigera* (IV instar) larvae also. Ten replications were maintained for each prey as well as predator stage.

1. 2. 8. Statistical analysis

Statistics such as ANOVA and post ANOVA, Least Significant Difference test (LSD), Correlation and Chi square test were worked out using SYSTAT and STATISTICA computer package and were interpreted.

1. 3. Results

1. 3. 1. Alive *Corcyra cephalonica* larvae

1. 3. 1. 1. Developmental period

In *R. longifrons*, among the one-prey category, the total developmental period was significantly shorter in A4 (41.50 ± 0.96 days), A3 (41.90 ± 2.27 days) and A5 (42.61 ± 1.12 days). These groups were statistically significant at 5% level when compared to A2 (49.42 ± 1.84 days) and A1 (52.90 ±

Table. 1. Biological parameters and predatory rate of *R. longifrons* fed with one alive *C. cephalonica* larvae (mean ± SD)

Parameters	A1	A2	A3	A4	A5
Total nymphal period (Days)	52.90 ± 1.20	49.42 ± 1.84	41.90 ± 2.27	41.50 ± 0.96	42.61 ± 1.12
Survival (%)	100.00 ± 0.00	80.00 ± 10.0	50.00 ± 5.00	62.22 ± 3.84	71.66 ± 6.96
Sex ratio	0.66	0.50	0.50	0.57	0.66
Pre-oviposition period (Days)	17.92 ± 2.46	16.46 ± 1.32	16.24 ± 3.12	15.54 ± 2.18	14.82 ± 1.96
Eggs/female (No.)	107.00 ± 5.48	102.50 ± 6.36	109.79 ± 9.76	116.00 ± 7.20	121.00 ± 4.50
Hatching percentage	79.43 ± 6.66	77.07 ± 8.48	74.70 ± 7.54	74.60 ± 5.48	76.70 ± 8.32
Predatory rate (Prey/Predator/Day)	0.98 ± 0.24	0.80 ± 0.42	0.92 ± 0.72	0.98 ± 0.66	0.85 ± 0.45

A1 - 1 prey/1 predator

A2 - 1 prey/5 predator

A3 - 1 prey/10 predator

A4 – 1 prey/15 predator

A5 - 1 prey/ 20 predator

Table 2. Biological parameters and predatory rate of *R. longifrons* fed with two alive *C. cephalonica* larvae (mean ± SD)

Parameters	A6	A7	A8	A9	A10
Total nymphal period (Days)	51.04 ± 1.16	49.70 ± 1.24	40.29 ± 1.24	43.65 ± 1.56	41.83 ± 1.06
Survival (%)	100.00 ± 0.00	80.0 ± 5.00	80.00 ± 4.50	73.33 ± 6.66	78.33 ± 5.99
Sex ratio	0.66	0.60	0.72	0.69	0.74
Pre-oviposition period (Days)	18.32 ± 2.84	16.18 ± 2.36	14.06 ± 1.92	20.94 ± 3.42	15.32 ± 1.96
Eggs/female (No.)	114.33 ± 5.42	116.50 ± 3.50	120.60 ± 4.54	127.00 ± 6.00	130.60 ± 4.50
Hatching Percentage	83.09 ± 3.66	78.34 ± 6.36	85.70 ± 8.13	77.80 ± 4.36	80.28 ± 1.18
Predatory rate (Prey/Predator/Day)	1.60 ± 0.20	1.80 ± 0.25	1.64 ± 0.18	1.58 ± 0.24	1.51 ± 0.18

A6 - 2 prey/1 predator

A7 - 2 prey/5 predator

A8 - 2 prey/10 predator

A9 - 2 prey/15 predator

A10 - 2 prey/20 predator

1.20 days) by LSD test. The total nymphal period was reduced but not statistically significant when the predators were provided with two-prey (see table 1 and 2, fig. 1). Among the two prey categories, the shortest and longest developmental period was observed in A8 (40.29 ± 1.24 days) and A6 (51.04 ± 1.16 days) respectively and is statistically significant at 5% level. Irrespective of the predator and prey densities, the second and fifth instar had the shortest and longest duration respectively. As observed in *R. longifrons*, in *R. kumarii* also, the total developmental period was shorter and longer in one and two prey categories respectively. The developmental period was shorter in B8 (50.08 ± 1.62 days), followed by B7 (53.42 ± 1.76 days), B3 (54.99 ± 1.24 days), B4 (54.99 ± 1.86), B6 (55.35 ± 1.48 days), B5 (56.92 ± 1.18 days), B1 (58.0 ± 2.00 days) and B2 (58.08 ± 1.62 days) respectively (fig. 2) and the comparison between B7 to B3 and B4, B6 with B3, B4 and B5 between B1 and B2 were not statistically significant ($P > 0.05$) by LSD test. The comparison between B3 and B8 showed a higher significance ($P < 0.05$). The comparison between the predatory rate and development days is having a higher significance ($t = -61.144$ and -32.491 for one and two prey category, respectively) (table 3 and 4).

1. 3. 1. 2. Survival rate and sex ratio

100% survival of *R. longifrons* nymphs was observed in both A1 and A6 and is highly significant ($P < 0.05$) than the rest of the categories by LSD test. Except one and five predator densities, the survival rate was the highest in two-prey density than one-prey density. The minimum survival of the predator was observed in A3 (50.0 %). The survival rate recorded in A8

Table 3. Biological parameters and predatory rate of *R. kumarii* fed with one alive *C. cephalonica* larvae (mean ±SD)

Parameters	B1	B2	B3	B4
Total nymphal period (Days)	58.00 ± 2.00	58.08 ± 1.62	54.99 ± 1.24	54.99 ± 1.86
Survival (%)	100.0 ± 0.00	100.0 ± 0.00	66.66 ± 5.33	78.33 ± 4.66
Sex ratio	0.66	0.58	0.54	0.65
Pre-oviposition period (Days)	26.34 ± 3.21	25.89 ± 2.63	25.68 ± 3.31	24.38 ± 3.24
Eggs/female (No.)	132.00 ± 6.50	134.70 ± 5.83	131.50 ± 5.49	150.40 ± 6.56
Hatching percentage	75.75 ± 7.25	85.80 ± 6.24	52.33 ± 4.66	86.6 ± 7.27
Predatory rate (Prey/Predator/Day)	0.80 ± 0.20	0.80 ± 0.15	0.80 ± 0.20	1.0 ± 0.00

B1 - 1 prey/1 predator

B2 - 1 prey/5 predator

B3 - 1 prey/10 predator

B4 - 1 prey/20 predator

Table 4. Biological parameters and predatory rate of *R. kumarii* fed with two alive *C. cephalonica* larvae (mean ± SD)

Parameters	B5	B6	B7	B8
Total nymphal period (Days)	56.92 ± 1.18	55.35 ± 1.48	53.42 ± 1.76	50.08 ± 1.62
Survival (%)	100.0 ± 0.00	80.00 ± 5.50	70.00 ± 3.00	73.33 ± 2.66
Sex ratio	0.66	0.66	0.66	0.74
Pre-oviposition period (Days)	25.38 ± 2.96	23.58 ± 2.54	23.12 ± 3.42	20.23 ± 2.81
Eggs/female (No.)	140.00 ± 5.50	141.30 ± 7.54	123.00 ± 7.50	169.33 ± 5.66
Hatching percentage	85.75 ± 3.81	87.50 ± 4.01	87.50 ± 3.50	88.50 ± 6.50
Predatory rate (Prey/Predator/Day)	1.00 ± 0.00	1.80 ± 0.02	1.54 ± 0.18	1.75 ± 0.21

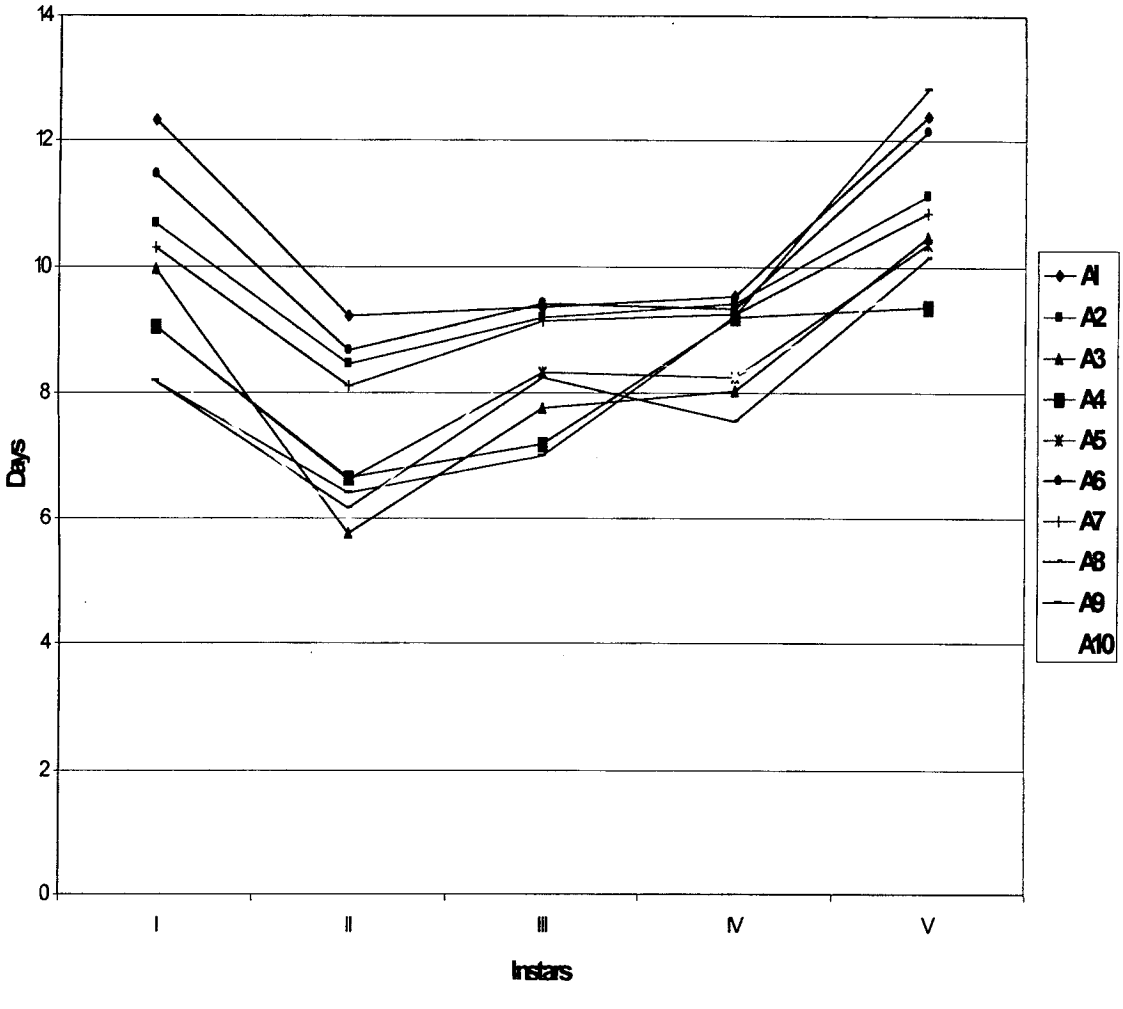
B5 - 2 prey/1 predator

B6 - 2 prey/5 predator

B7 - 2 prey/10 predator

B8 - 2 prey/20 predator

Fig.1. Nymphal developmental period (in days) of *R. longifrons* fed with one and two alive *C. cephalonica* larvae



A1 – 1 prey/ 1 predator

A2 – 1 prey/5 predator

A3 – 1 prey/10 predator

A4 – 1 prey/15 predator

A5 – 1 prey/20 predator

A6 – 2 prey/ 1 predator

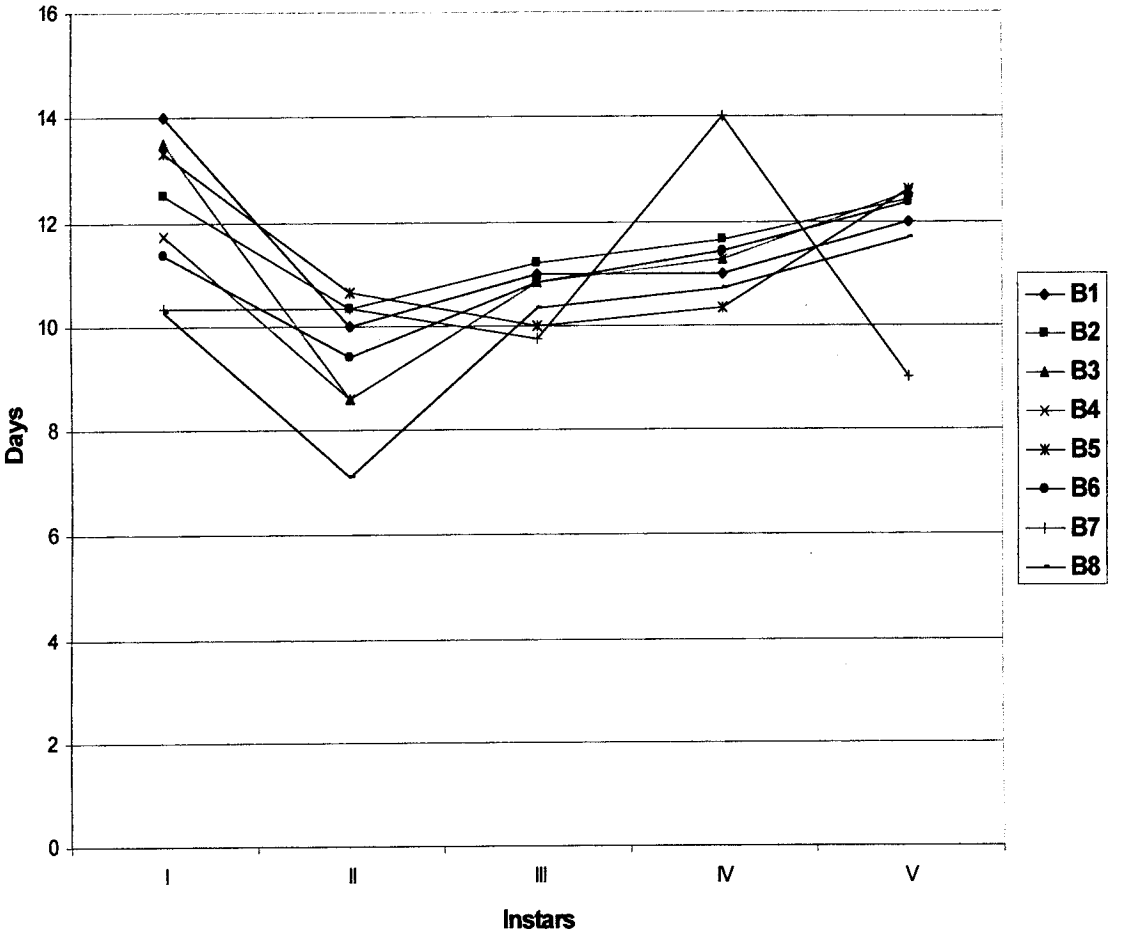
A7 – 2 prey/5 predator

A8 – 2 prey/10 predator

A9 – 2 prey/15 predator

A10 – 2 prey/20 predator

Fig. 2. Nymphal developmental period (in days) of *R. kumarii* fed with one and two alive *C. cephalonica* larvae



B1 – 1prey/ 1 predator

B2 – 1 prey/5 predator

B3 – 1 prey/10 predator

B4 – 1 prey/20 predator

B5 – 2prey/ 1 predator

B6 – 2 prey/5 predator

B7 – 2 prey/10 predator

B8 – 2 prey/20 predator

was 80.0 ± 4.50 %. Almost all the tested categories showed female biased sex ratio except A2 and A3 (0.5) and it was high in A10 (0.74) (table 1 and 2). Chi-square test showed a higher significance for the latter category. The correlation between predatory rate and sex ratio shows a higher significance ($P < 0.05$; $P = 0.0014$ and 0.00018 for 1 prey and 2 prey category, respectively). As observed in *R. longifrons*, in *R. kumarii* also, in general, two-prey category had more survival than one-prey category. Nymphs of B1 and B5 group had 100% survival. Least nymphal survival was noticed in B3 (66.66 ± 5.33%) and for B8 it was 73.33 ± 2.66% and is statistically significant ($P < 0.05$). The sex ratio was female biased in all the categories and was high and low in B8 (0.74) and B3 (0.54), respectively (table 3 and 4).

1. 3. 1. 3. Reproduction

In *R. longifrons*, the pre-oviposition period was shorter in A8 (14.06 ± 1.92 days) and longer in A9 (20.94 ± 3.42 days) and they are statistically significant ($P < 0.05$; $P = 0.009$). Mean number of eggs produced by *R. longifrons* on different predator and prey densities are presented in table 1 and 2. Maximum and minimum egg production was obtained in A10 (130.6 ± 4.5 eggs/female) and A1 (107.0 ± 5.48 eggs/female), respectively. The LSD analysis between the developmental days and egg production shows high significance ($t = -12.654$ for one prey category and $t = -15.606$ for two prey category) and a positive correlation exists between the prey density and eggs/female ($r = 0.85$). The hatching percentage was high in A8 (85.70 ± 8.13 %) and low (74.60 ± 5.48 %) in A4 (table 1 and 2).

In *R. kumarii*, the pre-oviposition period was shorter in B8 (20.23 ± 2.81 days) and longer in B1 (28.34 ± 3.21 days) and is significant at 5% level by LSD test. Two-prey category shortened the pre-oviposition period, but they were not statistically significant except the comparisons between the B4 and B8. A positive correlation (r = 0.49) exists between the 1 and 2 prey categories. The egg laid per female was high in two-prey group than one prey group in all predator densities (except B7) and maximum fecundity was recorded in B8 (169.33 ± 5.66 eggs/female) (table 3 and 4). The hatching percentage of the eggs was high in B8 (88.50 ± 6.50%) and low in B3 (52.33 ± 4.66%) and shows a higher significance ($P < 0.05$). In general, the two-prey category had a better hatching percentage than one prey category.

1. 3. 1. 4. Bio-efficacy of reduviids

Reduviids reared on high prey density ate more number of *C. cephalonica* than those reared on low prey density. In *R. longifrons*, it was maximum in A7 (1.8 prey/predator/day) and minimum in A2 (0.80 prey/predator/day) and are highly significant ($P < 0.05$) (table 1 and 2). Similarly, in *R. kumarii*, 2 prey fed group had the maximum predatory rate and was very least in B1, B2 and B3 (0.8 prey/predator/day). A predatory rate of 1.54 ± 0.18 prey/predator/day was recorded in B8 (table 3 and 4).

1. 3. 1. 5. Adult longevity

Irrespective of predator densities and prey categories, the females lived longer than the males. The male adult longevity of *R. longifrons* was longer

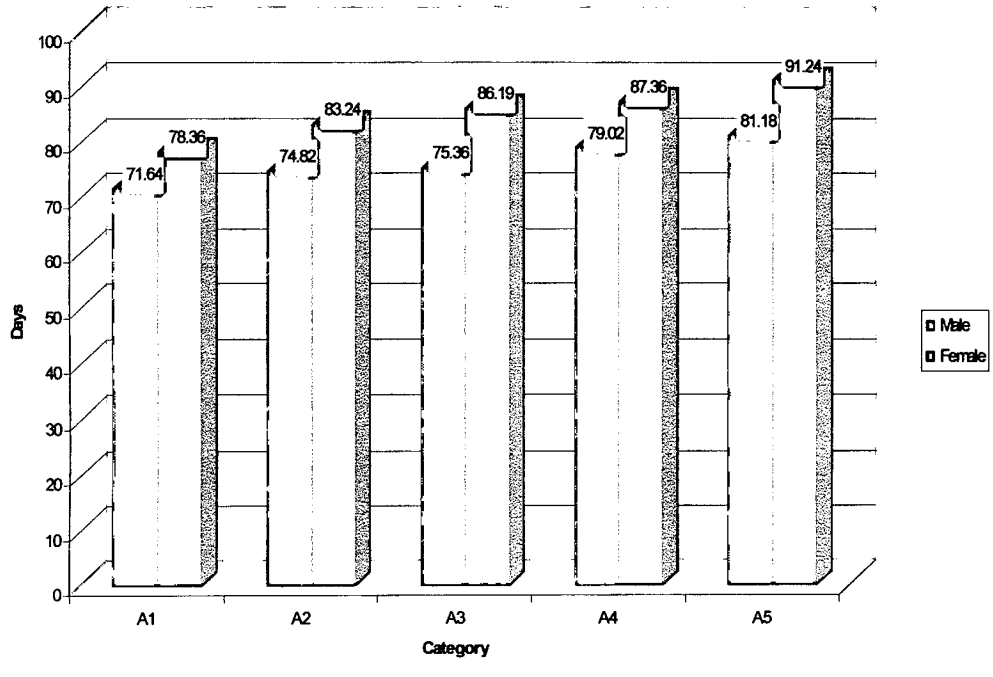
in A8 (101.66 ± 3.22 days) and shorter in A1 (71.64 ± 3.48 days) respectively and longest female longevity was observed in A8 (114.63 ± 5.84 days) and are highly significant ($P < 0.05$) (fig. 3 and 4). Male longevity of *R. kumarii* in descending order are B8 (104.56 days), B7 (95.82 days), B4 (93.48 days), B6 (91.42 days), B2 (89.4 days), B5 (89.35 days), B1 (88.50 days) and B3 (87.33 days) respectively and the comparison between B8 and to the rest of the categories shows a higher significance ($P < 0.05$). The female adult longevity was longer in B1 (109.50 days) (fig. 5 and 6).

1. 3. 1. 6. Life table statistics

The life table statistics of *R. longifrons* fed with one and two alive *C. cephalonica* are shown in table 5 and 6. The prey density had a great influence on the net reproductive rate (NRR) and was higher in A9 (54.76) and minimum in A7 (41.8). The NRR for A8 was 47.5. As the predator density increased, the mean length of generation (Tc) decreased and was very low in A10 (69.69). The calculated Tc and corrected rm for A8 were 75.48, and 0.051, respectively. The doubling time was very short in A10 (11.02) and similar doubling time was recorded for A3, A5 and A8 (12.33). The hypothetical F_2 female was maximum in A9 (2998.65) and minimum in A7 (1747.24) and moderate in A8 (2256.25).

The life table statistics of *R. kumarii* fed with one and two prey are shown in table 7 and 8. Very high and low NRR was recorded in B5 (70.5) and B7 (47.29), respectively. The mean length of generation was long in B1 (97.88) and short in B3 (83.8). The corrected rm for B1 to B8 are 0.075,

Fig.3. Adult longevity (in days) of *R. longifrons* fed with one alive *C. cephalonica* larvae



A1 – 1 prey/ 1 predator

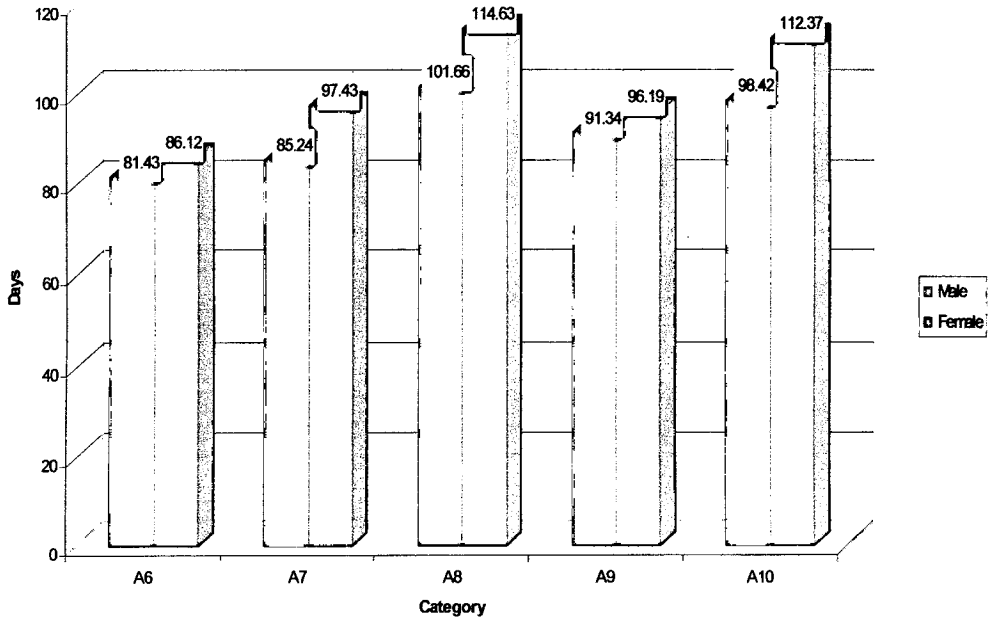
A2 – 1 prey/5 predator

A3 – 1 prey/10 predator

A4 – 1 prey/15 predator

A5 – 1 prey/20 predator

Fig.4. Adult longevity (in days) of *R. longifrons* fed with two alive *C. cephalonica* larvae



A6 – 2 prey/ 1 predator

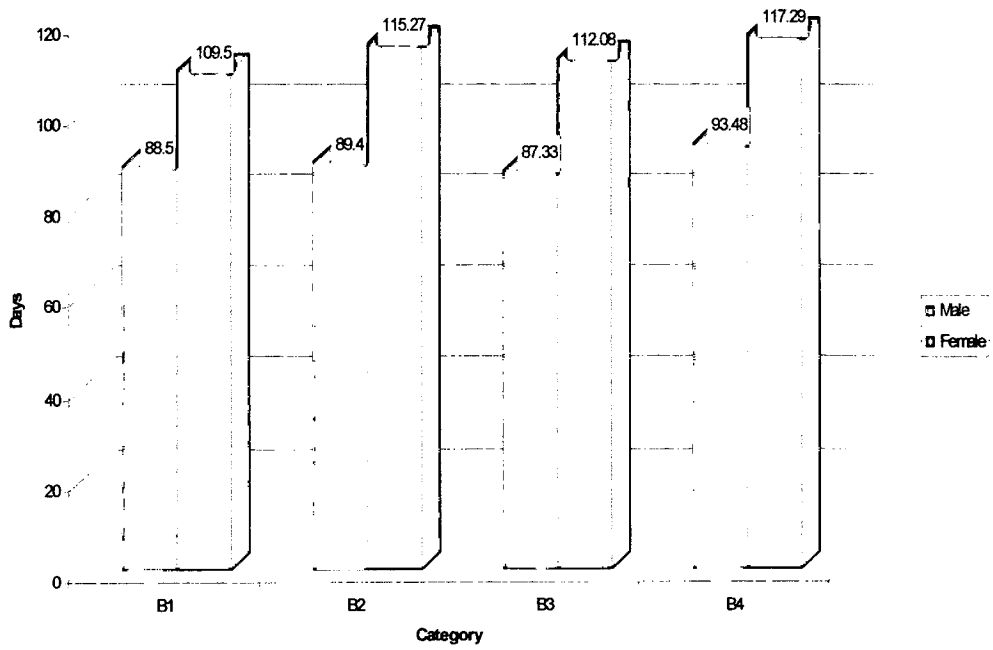
A7 – 2 prey/5 predator

A8 – 2 prey/10 predator

A9 – 2 prey/15 predator

A10 – 2 prey/20 predator

Fig.5. Adult longevity (in days) of *R. kumarii* fed with one alive *C. cephalonica* larvae



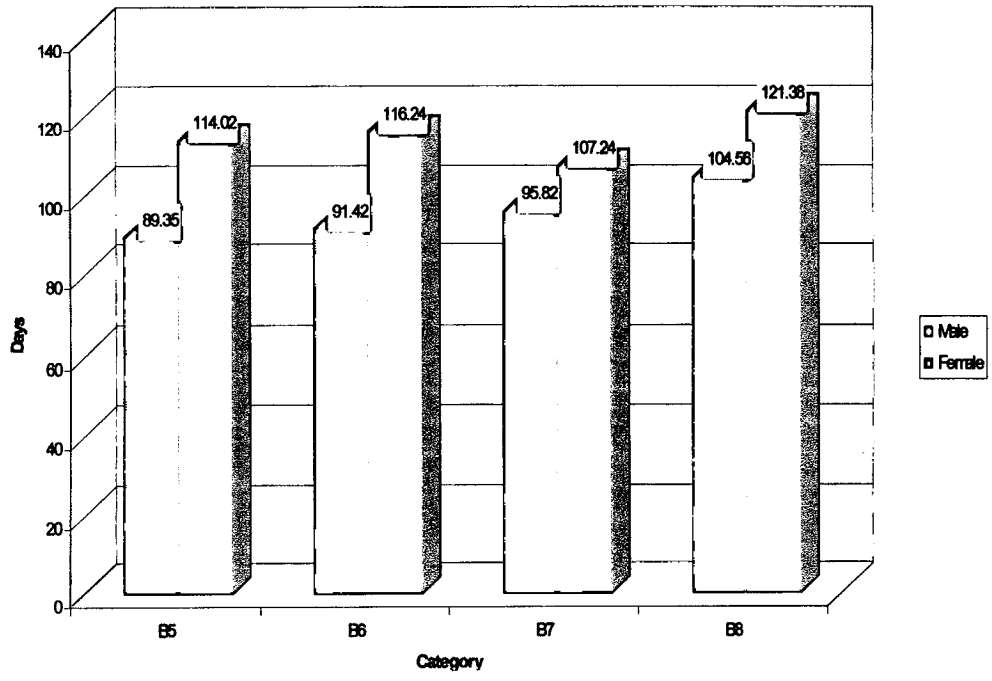
B1 – 1prey/ 1 predator

B2 – 1 prey/5 predator

B3 – 1 prey/10 predator

B4 – 1 prey/20 predator

Fig.6. Adult longevity (in days) of *R. kumarii* fed with two alive *C. cephalonica* larvae



B5 – 2prey/ 1 predator

B6 – 2 prey/5 predator

B7 – 2 prey/10 predator

B8 – 2 prey/20 predator

Table 5. Life table parameters of *R. longifrons* fed with one alive *C. cephalonica* larvae

Parameters	A1	A2	A3	A4	A5
Net reproductive rate (NRR)	50.25	52.75	44.65	43.44	50.33
Mean length of generation (Tc)	75.6	71.23	76.71	75.90	76.24
Innate capacity for natural increase (rc)	0.051	0.055	0.049	0.049	0.051
Corrected rm	0.055	0.059	0.057	0.056	0.057
Precise generation time (T)	71.21	67.21	66.64	67.34	68.74
Finite rate of increase (λ)	1.056	1.060	1.058	1.057	1.058
Doubling time (C)	12.75	12.04	12.33	12.54	12.33
Weekly multiplication time (erm^7)	1.469	1.540	1.490	1.479	1.058
Hypothetical female in F_2 generation	2525.06	2782.56	1993.62	1887.03	2533.1

A1 - 1 prey/1 predator

A2 - 1 prey/5 predator

A3 - 1 prey/10 predator

A4 – 1 prey/15 predator

A5 - 1 prey/ 20 predator

Table 6. Life table parameters of *R. longifrons* fed with two alive *C. cephalonica* larvae

Parameters	A6	A7	A8	A9	A10
Net reproductive rate (NRR)	54.0	41.8	47.5	54.76	48.43
Mean length of generation (Tc)	84.25	83.93	75.48	74.12	69.69
Innate capacity for natural increase (rc)	0.047	0.044	0.051	0.054	0.055
Corrected rm	0.054	0.050	0.057	0.061	0.063
Precise generation time (T)	73.87	74.65	67.73	65.62	61.58
Finite rate of increase (λ)	1.055	1.051	1.058	1.062	1.065
Doubling time (C)	12.97	13.93	12.33	11.53	11.02
Weekly multiplication time (e^{rm^7})	1.459	1.419	1.490	1.062	1.554
Hypothetical female in F_2 generation	2916.0	1747.24	2256.25	2998.65	2345.46

A6 - 2 prey/1 predator

A7 - 2 prey/5 predator

A8 - 2 prey/10 predator

A9 – 2 prey/15 predator

A10 - 2 prey/ 20 predator

Table 7. Life table parameters of *R. kumarii* fed with one alive *C. cephalonica* larvae

Parameters	B1	B2	B3	B4
Net reproductive rate (NRR)	58.0	51.16	46.5	63.53
Mean length of generation (Tc)	97.88	89.29	83.8	86.01
Innate capacity for natural increase (rc)	0.070	0.044	0.045	0.048
Corrected rm	0.075	0.049	0.048	0.055
Precise generation time (T)	54.13	80.30	79.98	75.45
Finite rate of increase (λ)	1.077	1.05	1.049	1.056
Doubling time (C)	13.43	14.26	14.54	12.75
Weekly multiplication time (e^{rm^7})	1.690	1.409	1.399	1.469
Hypothetical female in F_2 generation	3364.0	2617.34	2162.25	4036.06

B1 - 1 prey/1 predator

B2 - 1 prey/5 predator

B3 - 1 prey/10 predator

B4 - 1 prey/20 predator

Table 8. Life table parameters of *R. kumarii* fed with two alive *C. cephalonica* larvae

Parameters	B5	B6	B7	B8
Net reproductive rate (NRR)	70.5	58.84	47.29	67.78
Mean length of generation (Tc)	97.48	91.33	87.1	92.59
Innate capacity for natural increase (rc)	0.043	0.044	0.044	0.045
Corrected rm	0.055	0.050	0.050	0.052
Precise generation time (T)	77.37	81.49	77.12	81.05
Finite rate of increase (λ) .	1.056	1.051	1.051	1.050
Doubling time (C)	12.75	13.93	13.93	14.33
Weekly multiplication time (erm^7)	1.46	1.419	1.419	1.430
Hypothetical female in F_2 generation	4970.25	3462.14	2236.34	4580.58

B5 - 2 prey/1 predator

B6 - 2 prey/5 predator

B7 - 2 prey/10 predator

B8 - 2 prey/20 predator

0.049 0.048, 0.055, 0.055, 0.050, 0.050 and 0.052, respectively. The doubling time (C) ranged from 12.75 to 14.54. The hypothetical F_2 female was maximum in B5 (4970.25) and minimum in B3 (2162.25). B8 had moderate number of progeny (4580.58) in F_2 generation.

1. 3. 2. Biology of reduviids on HK and FL of *C. cephalonica* and ASL (alive *S. litura*) larvae

1. 3. 2. 1. Biological parameters

Based on the previous experiment, *R. longifrons* reared with 10 predators with two-prey, was moderately superior to the other categories. Similarly, 20 predators/container with two-prey was chosen as the better category fort *R. kumarii* and were tested with HK, ASL and FL. *R. longifrons* developed faster in ASL (35.31 ± 1.86 days) than HK (36.58 ± 2.42 days) and FL (45.62 ± 1.92 days) (table 9, fig. 7). The comparison between the ASL and HK were not statistically significant at 5% level. The sex ratio was female biased in all the three groups and were significant by chi-square test except FL. The fecundity was higher in ASL (149.72 ± 4.27 eggs/female), followed by HK (143.20 ± 5.81 eggs/female) and FL (109.34 ± 4.62 eggs/female) and HK and ASL are statistically significant than FL ($P < 0.05$). The pre-oviposition period was shorter in ASL (9.42 ± 0.96 days) and longer in FL (16.34 ± 2.62 days) and they are statistically significant ($P < 0.05$). The male and female longevity were also longer in ASL (114.36 ± 5.62 and 126.82 ± 4.16 days) for male and female respectively (fig. 9).

The developmental period of *R. kumarii* was significantly ($P < 0.05$) shorter in ASL (40.32 days), followed by HK (44.10 days) and FL (56.82

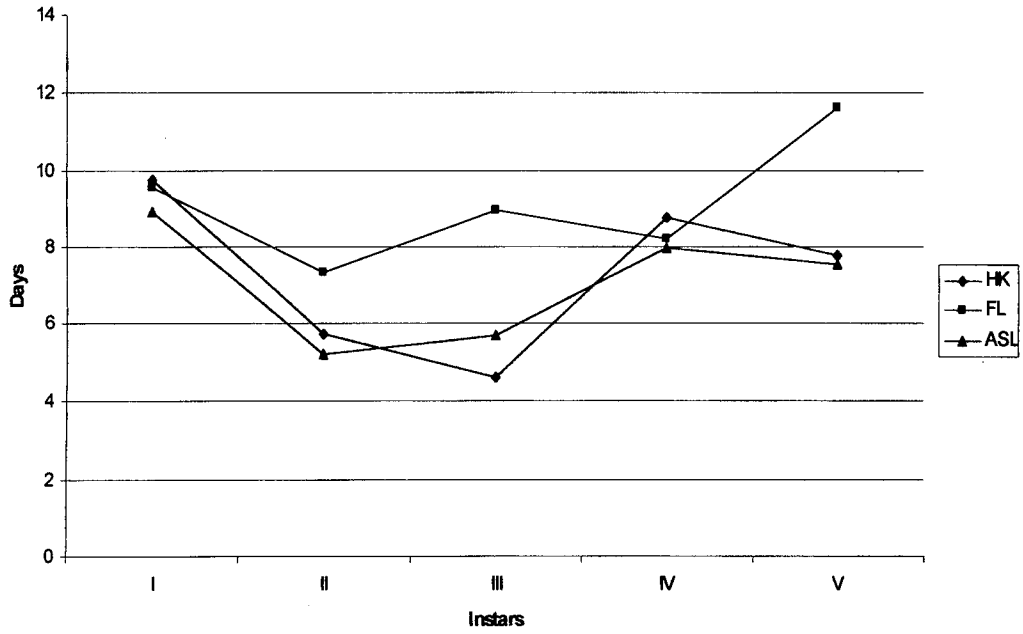
Table 9. Biological and life table parameters of *R. longifrons* fed with heat killed (HK) and frozen (FL) *C. cephalonica* and alive *S. litura* (ASL) larvae

Parameters	HK	FL	ASL
Total nymphal period (Days)	36.58 ± 2.42	45.62 ± 1.92	35.31 ± 1.86
Survival (%)	90.67 ± 5.63	55.0 ± 5.00	90.00 ± 10.00
Sex ratio	0.66	0.56	0.77
Pre-oviposition period (Days)	10.32 ± 1.74	16.34 ± 2.62	9.42 ± 0.96
Eggs/female (No.)	143.20 ± 5.81	109.34 ± 4.62	149.72 ± 4.27
Hatching percentage	85.49 ± 6.82	81.65 ± 2.34	93.95±4.03
Predatory rate (Prey/Predator/Day)	1.6	1.2	0.8
Net reproductive rate (NRR)	61.81	44.76	64.16
Mean length of generation (Tc)	77.80	76.75	77.69
Innate capacity for natural increase (rc)	0.053	0.049	0.0540
Corrected rm	0.060	0.055	0.060
Precise generation time (T)	68.73	69.10	69.35
Finite rate of increase (λ)	1.061	1.056	1.061
Doubling time (C)	11.71	12.75	11.71
Weekly multiplication time (e^{rm^7})	1.521	1.469	1.521
Hypothetical female in F_2 generation	3820.47	2003.45	4116.5

Table 10. Biological and life table parameters of *R. kumarii* fed with heat killed (HK) and frozen (FL) *C. cephalonica* and alive S. *litura* (ASL) larvae

Parameters	HK	FL	ASL
Total nymphal period (Days)	44.10 ± 0.98	56.82 ± 2.34	40.32 ± 1.64
Survival (%)	85.06 ± 3.86	68.42 ± 3.50	95.00 ± 5.00
Sex ratio	0.61	0.56	0.78
Pre-oviposition period (Days)	11.33 ± 1.66	25.19 ± 2.83	11.28 ± 7.18
Eggs/female (No.)	168.90 ± 8.64	127.20 ± 6.79	174.32 ± 6.49
Hatching percentage	89.66 ± 5.42	79.46 ± 3.52	94.82 ± 3.16
Predatory rate (Prey/Predator/Day)	1.8	1.4	0.9
Net reproductive rate (NRR)	63.48	50.72	72.58
Mean length of generation (Tc)	89.52	89.08	89.78
Innate capacity for natural increase (rc)	0.046	0.044	0.047
Corrected rm	0.053	0.050	0.054
Precise generation time (T)	78.31	78.4	79.33
Finite rate of increase (λ)	1.054	1.051	1.055
Doubling time (C)	13.20	13.93	12.86
Weekly multiplication time (e^{rm^7})	1.449	1.419	1.459
Hypothetical female in F_2 generation	4029.71	2572.51	5267.85

Fig. 7. Nymphal developmental period (in days) of *R. longifrons* fed with heat killed (HK) and frozen (FL) *C. cephalonica* and alive *S. litura* (ASL) larvae



days) (fig. 8). All the groups yielded female biased sex ratio and it was much pronounced in ASL (0.78) than HK (0.61) and FL (0.56). The pre-oviposition period was significantly shorter in ASL (11.28 days) and longer in FL (25.19 days). The hatching percentage was also high in ASL (94.82%) (table 10). The male adult longevity of *R. kumarii* fed with HK, ASL and FL were 106.24, 113.24 and 97.21 days, respectively and for female it was 127.36, 134.37 and 107.42 days, respectively (fig. 10). Both HK and ASL are superior to FL and alive *C. cephalonica.*

1. 3. 2. 2. Life table statistics

In *R. longifrons,* the weekly multiplication was the same for both HK and ASL (1.521). (table 9). Similar kind of observations was also recorded for ASL group that had high NRR (64.16), rm (0.054), precise generation time (69.39) and hypothetical F_2 generation (4116.5) (table 9). In *R. kumarii,* NRR was higher in ASL (72.58) and the corrected rm for that group was 0.054. The doubling time was less in ASL (12.86) and hypothetical F_2 female was also maximum is the same category (5267.85) (table 10).

1. 3. 3. Biological control potential

Pot studies revealed that at low prey density (one-prey), prey consumption given a uniform prey distribution was higher than that for contagious prey distribution ($P < 0.05$), but no similar prey consumption rates were observed at high prey density for the two prey spatial patterns ($P < 0.05$). *R. longifrons* preferred both *S. litura* and *H. armigera* and maximum consumption of 1.66 preys/predator/day for both pests. However, the

Fig. 8. Nymphal developmental period (in days) of *R. kumarii* fed with heat killed (HK) and frozen (FL) *C. cephalonica* and alive *S. litura* (ASL) larvae

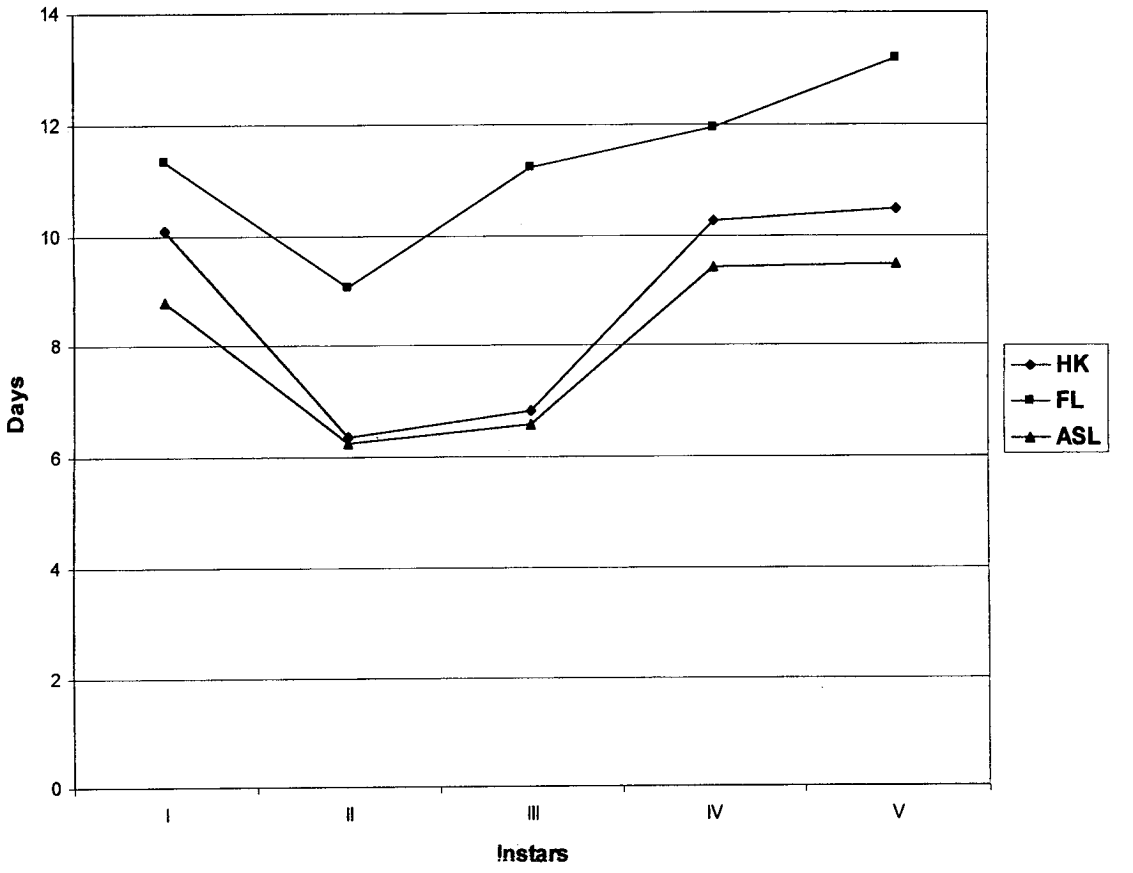


Fig.9. Adult longevity (in days) of *R. longifrons* fed with heat killed (HK) and frozen (FL) *C. cephalonica* and alive *S. litura* (ASL) larvae

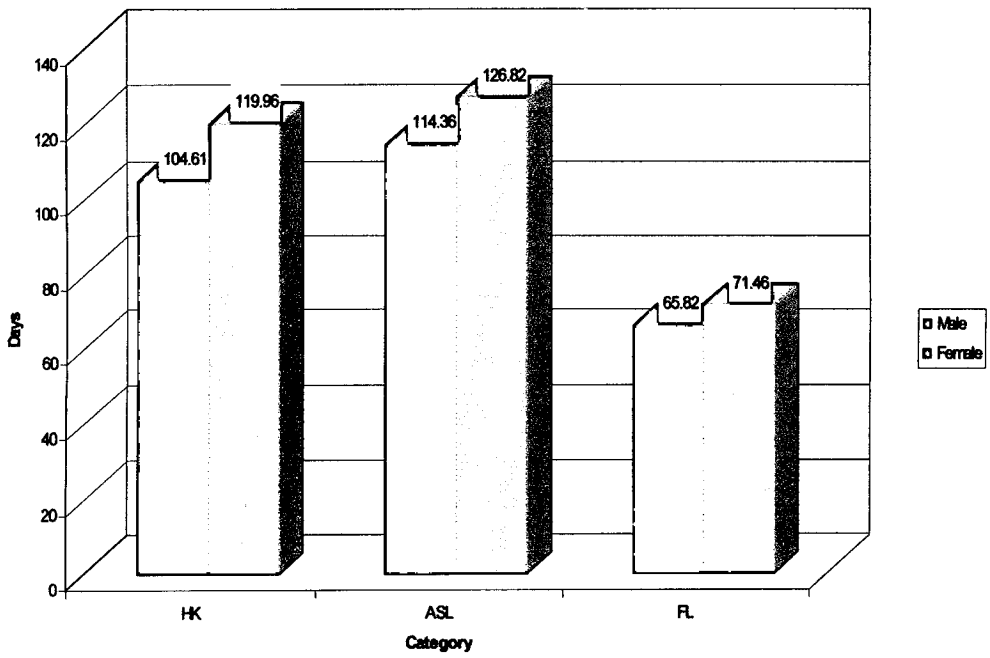
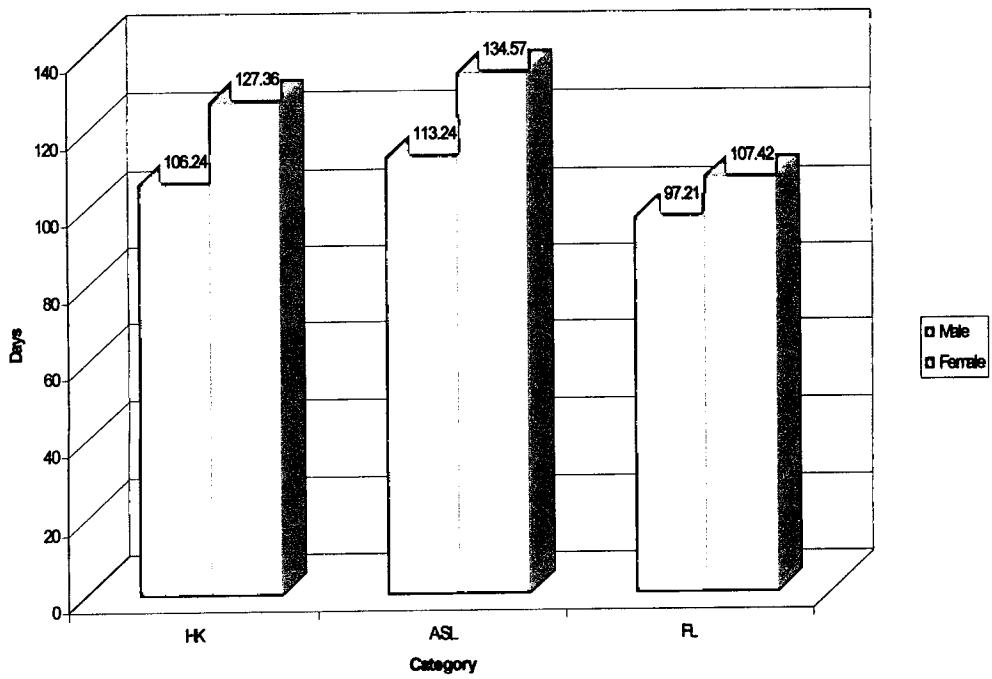


Fig.10. Adult longevity (in days) of *R. kumarii* fed with heat killed (HK) and frozen (FL) *C. cephalonica* and alive S. *litura* (ASL) larvae



maximum weight gain was observed in the adult that fed *S. lituna* (54.94 ± 5.00mg) (table 11). *R. kumarii* adults consumed more *S. litura* (1.83) than *H. armigera* (1.66) but not statistically significant at 5% level. The nymphal instars and the adult predators preferred *S. litura* slightly better than *H. armigera.* As observed in *R. longifrons,* in *R. kumarii* also the maximum weight gain was achieved by the adult fed on *S. litura* (62.13 ± 3.81 mg) (table 12). The life stages (IV, V instars and adult) consumed 1.32, 1.5 and 1.83 *S. litura* and 1.16, 1.32 and 1.66 *H. armigera* respectively when two-prey was provided.

1. 4. Discussion

1. 4. 1. Developmental period

The survival and growth of animals, their ability to pass through nymphal instars in life and the capacity to reproduce are all dependent on nutritional factors, but the actual nature of dietary demands in reduviids has not been analyzed, perhaps in *R. marginatus* (George *et al.*, 2002). In *N. therasii* and *A. pedestris*, studies of diet-based predator discrimination is the assumption that predator that have fed on a particular prey type should present a greater predation risk than predators that have fed on other prey (Sahayaraj, 2001).

The faster a predator completes its life cycle, the more generations it can produce in a specific period. Further more, the total developmental period of any insects, especially the predators mainly depend upon the prey species and its nutritional quality (Ambrose and Subbarasu, 1988; Ambrose

Table 11. Predatory rate (Prey/Predator/Day) and amount of food consumption (AFC) [in mg] of *R. longifrons* on *S. litura* and *H. armigera* larvae

Predator life stages	Prey	No. of prey		AFC
		Provided	Consumed	
IV	*S.litura*	1.0	1.00	34.85 ± 3.21
V			1.00	34.97 ± 4.55
Adult			1.00	39.58 ± 5.22
IV		2.0	1.16	39.59 ± 3.85
V			1.32	44.81 ± 2.96
Adult			1.66	54.94 ± 5.11
IV	*H.armigera*	1.0	1.00	29.15 ± 2.59
V			1.00	33.92 ± 3.28
Adult			1.00	37.61 ± 4.25
IV		2.0	1.16	31.23 ± 1.98
V			1.32	36.41 ± 3.56
Adult			1.66	47.47 ± 5.15

Table 12. Predatory rate (Prey/Predator/Day) and amount of food consumption (AFC) [in mg] of *R. kumarii* on *S. litura* and *H. armigera* larvae

Predator life stages	Prey	No. of prey		AFC
		Provided	Consumed	
IV	*S.litura*	1.0	1.00	41.24 ± 2.83
V			1.00	49.36 ± 3.24
Adult			1.00	57.72 ± 2.26
IV		2.0	1.32	44.56 ± 3.72
V			1.50	56.29 ± 4.24
Adult			1.83	62.13 ± 3.81
IV	*H.armigera*	1.0	1.00	40.37 ± 3.46
V			1.00	47.73 ± 2.26
Adult			1.00	54.43 ± 3.54
IV		2.0	1.16	42.36 ± 2.14
V			1.32	51.43 ± 3.56
Adult			1.66	57.24 ± 2.75

et al., 1990 and O'Neil and Widenmann, 1990). *C. cephalonica* serve as an important laboratory host for many predatory insects including reduviids (Lakkundi and Parshad, 1987 and Sahayaraj, 1998a, 2002b). It is a general concept that the amount of prey consumed is proportionate to the development, hence *R. longifrons* and *R. kumarii* developed faster in the two-prey category as reported by Ambrose (1999). Ten and twenty predators with two *C. cephalonica* fed *R. longifrons and R. kumarii* development was faster than the other groups. Rearing in groups also enhance the development of reduviids (Ambrose, 1999). Social behaviour of insects is of prime importance in the augmentation of predatory insects. In nature, some of the harpactorine reduviids like *R. kumarii, R. marginatus, R. fuscipes* and *R. longifrons* live in groups (Ambrose, 1999). However, it was well pronounced in *R. kumarii* than *R. longifrons* and reflected in the present study. It is a kind of adaptive measure of reduviids to promote higher fecundity, hatchability and adult longevity for better success.

Alive *S. litura* and heat killed *C. cephalonica* further reduced the developmental period in both the reduviids and this preferred prey accelerated the food consumption and development of the insects. In the present study, alive *S. litura* greatly reduced the developmental period of the reduviids. Ambrose (1999) pointed out that the most preferred and suitable prey for augmentation is based on the size and agility of the prey and the subsequent predatory stress developed by the predator to capture and satiate itself. George *et al.* (1998); George (1999, 2000a) and Sahayaraj and Paulraj (2001)

also reported faster development of reduviids when they reared the reduviids on *S. litura*. George *et al.* (2002) reported that the biochemical constituents (total carbohydrate, protein and lipids) of *S. litura* were much greater than *C. cephalonica* and that might be the reason for the faster development of these reduviids when reared on *S. litura*. The heat-killed larvae also reduced the developmental period of *R. longifrons* and *R. kumarii* than those fed with frozen and alive *C. cephalonica*. Similar findings were reported by Sahayaraj and Jeyalakshmi (2002) in *R. marginatus* where frozen *C. cephalonica* larvae postponed the developmental days. So the frozen larvae of *C. cephalonica* may be used to maintain the reduviids instead of using it for rapid multiplication purpose. It is ideal to group rear both the reduviids in the laboratory on alive *S. litura* larvae.

1. 4. 2. Survival rate and sex ratio

Cannibolism is a major constraint in group rearing of reduviids (Ambrose and Livingstone, 1989; Ambrose, 1999 and George, 2000b) and that would cause an enormous amount of mortality and reduce the survival rate of the predators. Natural death of the predators may also occur due to diseases, scarcity of food and shelter (Cohen, 1993 and Ambrose, 1999). But in the present study, no diseases were observed and the shelter was given in the form of a zig-zag chart and still mortality occurred and the reason for that may be natural death and cannibolism. Cannibolism might be a stronger reason for the mortality as reported by George (2000b) because 100% survival of *R. longifrons* and *R. kumarii* was observed when they were reared individually. Cannibolism occurs in predators mainly due to the scarcity of

the prey. But the present study reveals that, irrespective of the predator and prey densities, some preys remained in the rearing arena. Hence, the mortality of the predators was not due to the non-availability of the preys.

In both the reduviids, a female biased sex ratio was observed in all the experimental categories except A2 and A3. Sahayaraj (1991); Lakkundi (1989); Kumaraswami, (1991); Claver (1998) and Ambrose (1980, 1999) reported that the harpactorine reduviids are generally female biased in optimal conditions. In the laboratory, good condition was maintained like cleanliness and physical parameters for the culture of both the reduviids and that might be the reason for the female based sex ratio. Production of more number of females is a desirable characteristic of a best biological control agent.

1. 4. 3. Reproduction

Information on the fecundity is a very important criterion in selecting a predator as a biological control agent. The predator should have a reproductive potential close to that of its potential prey, and it should be able to increase its population with minimum time lag, when the prey population increase or decrease in the field. The relation between nutrition and reproduction has not been extensively studied among reduviids as in other insects. As observed for the development, the reproduction also depends upon the quality and quantity of the food provided (O'Neil and Widenmann, 1990). For *R. longifrons*, A10 had the maximum fecundity (130.6 ± 4.5 eggs/female) in the prey predator experiment and in general *S. litura* category recorded the maximum fecundity (149.72 ± 4.27 eggs/female). Similarly in *R. kumarii* also

the predators reared on *S. litura* had the maximum fecundity (174.32 ± 6.49 eggs/female). George (2000a) reported 132.78 eggs for a related harpactorine species (*R. marginatus*) reared on *C. cephalonica.* General hypothesis is that number of prey ingested (amount) also is of high value in improving fecundity and the total egg production being dependent on the quantity of nutrients taken in by the insects. Further more, enhanced quantity will promote maturation of more eggs. This hypothesis is not true in the present study. For instance, the fecundity (number of eggs laid/female) of A5, A8, A9 and A10 of *R. longifrons* and B8 of *R. kumarii* has not correlated with the predatory rate. Claver (1998) reported 133.7 eggs/female for *R. kumarii* on *C. cephalonica.* But in the present study 174.32 ± 6.49 eggs/female was recored in *R. kumarii* reared on *S. litura*, which might be due to the high nutrient content of *S. litura* (George *et al.*, 2002). Moreover, a close relationship between food supply and reproduction of the female has been recorded here.

In the present study, pre-oviposition period was shorter when the reduviids were reared on alive *S. litura* (9.42 ± 0.96 and 11.22 ± 2.71 days for *R. longifrons* and *R. kumarii,* respectively). The difference in pre-oviposition period occurs with varied prey type (Parajulee and Philips, 1993; Venkatesan *et al.*, 1997). The pre-oviposition of the predators also depends upon the prey and rearing condition. Group rearing shortened the pre-oviposition period (Ambrose, 1999).

Hatching is basically a temperature and humidity dependent event and here all the eggs were placed in the same environment but difference in the hatching percentage was observed suggests that the prey type also influence the hatching rate apart from the physical factors as reported by Fuller (1988). Maximum hatching was observed in *S. litura* fed category (93.95 ± 4.03 and 94.82 ± 3.16 % for *R. longifrons* and *R. kumarii*, respectively). Similar increase in hatching percentage for *R. marginatus* eggs was reported by George (2000c) and Sahayaraj and Paulraj (2001) when it was reared on *S. litura*.

1. 4. 4. Predatory rate

Predation is an important character of the carnivorous insects thereby meets their nutritional requirements by exploiting the prey's body content. The predatory rate of both the reduviids was high in the two-prey groups than the one-prey group. The predatory rate increased as the prey number increased is a general concept in the prey predator interaction. The present study coincides with the conclusions of Sahayaraj (1991, 2001) and Kumaraswami (1991) and Ambrose (1999). Claver (1998) also reported positive correlation between the number of prey and the predatory rate of *R. kumarii*.

1. 4. 5. Adult longevity

The longevity of any individual is a genetic factor that can be influenced by the biotic and abiotic factors. The longer a predator can survive in the field, the more effective it could be a biological control agent.

Longevity of female is of particular importance since it has a significant role in maintaining a high density of predator population in the field. Ambrose (1999) stated that eventhough longevity is a pre-determined one; the prey type and the amount of the prey can alter the adult longevity. He further reported that, in harpactorine reduviids, usually the ovipositing female live longer than the males. A starved animal cannot survive long, whatever be the genetical fitness the organisms have, it has to feed for long life. In the present study, the reduviids provided with two-prey lived longer than one-prey group. And the longevity was further increased in HK and ASL groups, in particular, the females in all the groups. In harpactorine reduviids, generally females live longer than males (Ambrose, 1999; Sahayaraj, 1991 and George *et al.*, 1998) and this might be due to the higher consumption of the food by the females to meet its extra energy requirement for oviposition. According to Parajulee and Phillips (1993) and Venkatesan *et al.*, (1997), the longevity of ovipositing females vary with species.

1. 4. 6. Life table

The life table is a useful method to study the life expectancy and net reproductive rate (Southwood, 1978). Life table of predatory reduviids are available only for few species (Venkatesan *et al.*, 1997; George, 1999, 2000 a; George *et al.*, 1998; Claver, 1998; Sahayaraj and Paulraj, 2001 and Sahayaraj and Jeyalakshmi, 2002). Life table studies give a clear-cut idea about the precise generation time, doubling time and hypothetical F_2 females. So the availability of predators for the future for a stipulated period could be easily predicted. *R. longifrons* and *R. kumarii* reared on *S. litura* had the

maximum NRR (64.16 and 72.58 respectively) and the doubling time also was shorter in this category (11.71 and 12.86 for *R. longifrons* and *R. kumarii,* respectively). In the prey-predator experiment, in *R. longifrons* A9 had the maximum NRR and minimum doubling time (11.53) with 2998.65 hypothtical female. In *R. kumarii* B5 recorded maximum NRR (70.5) and hypothetical F_2 female (4970.25) but not significantly differed from B8 (67.78, 11.33, 4580.58 for NRR, doubling time and hypothetical F_2 female, respectively). In both the reduviids, the heat killed larvae group had a better NRR, doubling time and hypothetical F_2 female than the alive and frozen *C. cephalonica* group. High NRR and intrinsic rate of increase have been reported for temporate bugs such as *Oncopeltus faxiatus* (Klausner *et al.*, 1980); *Pilegodorus guildnii* (Panizzi and Slansky, 1985) and *Clavigralla tomentosicollis* (Iheagwam, 1982) when reared on their preferred prey. In reduviids also, the *S. litura* larvae increased the NRR, life span and decreased the doubling time when compared to other prey species (George *et al.*, 1998). A maximum of 2256.25 and 4580.58 *R. longifrons* and *R. kumarii,* respectively (females) can be expected in the F_2 generation when they were reared with 10 and 20 predators per container, respectively with two alive *C. cephalonica.* The heat-killed larvae increased the NRR of *R. marginatus* and decreased the doubling time (Sahayaraj and Jeyalakshmi, 2002). *S. litura* had a great superiority than the other prey species for reduviids (George *et al.*, 1998; George, 1999; 2000a; Claver, 1998; Venkatesan *et al.*, 1997 and Sahayaraj and Paulraj, 2001) and the present findings also confirmed the same.

1. 4. 7. Biological control potential

R. longifrons and *R. kumarii* nymphs and adults feeds both *S. litura* and *H. armigua* larvae and the predatory potential remained the same for both the prey. *R. longifrons* and *R. kumarii* preferred *S. litura* than *H. armigera* larvae. In both the case, the adults consumed more quantity than the nymphs. Ambrose (1999) reported that larger predator predates more and the weight gain would be more than the smaller predators. Sahayaraj (1999a) reported that the predatory efficiency of *R. marginatus* on three major groundnut pests and he reported that the reduviid preferred the soft bodied prey than the prey with hard cuticle. Similar finding was observed in *R. kumarii* as it preferred the soft-bodied *S. litura* larvae. Grundy and Maelzer (2000) also reported the predation of *P. plagipennis* on *H. amigera* in the laboratory.

1. 5. Conclusion

It is concluded from this study that the quality and quantity of the food have been found to have influence on reduviids. *R. longifrons* can be reared in the laboratory with 10 individuals/1L container with 1 alive *S. litura* larvae/predator/day. Similarly *R. kumarii* can be reared at 20 predators/1L container with 1 alive *S. litura* larvae per day. Heat killed larvae of *C. cephalonica* can also be used during the non-availability of *S. litura.* Frozen *C. cephalonica* larvae can be used to maintain the predator culture during hard and critical times. Moreover, in the pot study, *R. longifrons* and *R. kumarii* life stages (IV, V instars and adults) consumed more *S. litura* larvae than *H. armigera* larvae and the weight gain were also high in that

category. The predator reared on a particular prey type with higher reproductive potential and adult longevity and low developmental time is indirectly considered the most suited prey for augmentation of reduviids.

"Integration of chosen reduviids and botanicals in groundnut pest management". Ph.D. Thesis submitted by C. Ravi to Manomaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

CHAPTER - 2

PHYTOCHEMISTRY OF *IPOMEA CARNEA* JACQ. AND *VITEX NEGUNDO* LINN.

2. 1. Introduction

New plant protection chemicals are needed for modern pest control management due to insect resistance and ecological disorders associated with numerous currently used pesticides. The plants harbors various phytochemicals which can act as toxicants to various insects. Worldwide, there are 1005, 384, 297, 27 and 31 species of plants with insecticidal, antifeedant, repellent, attractant and growth inhibition properties, respectively (Grainge *et al.*, 1985 and Bhatnagar and Sharma, 1994). The phytochemistry of *Vitex strickeri* L., *Vitex medinensis* and *V. negundo* Linn. (Subramanian and Misra, 1979; Anzaldo, 1980; Dayrit and Lagurin 1994; Dayrit *et al.*, 1987; Banerjee *et al.*, 1988; Manalo, 1992; Chawla *et al.*, 1992; Mallavarpu *et al.*, 1994; Jirovetz *et al.*, 1998 and Singh *et al.*, 2002); *Vitex* spp. (Riaz and Ashraf, 1990) *Vitex trifoliate* Linn. (Nair and Subramanian, 1975) and *Vitex lucens* (Horowitz and Gentilli, 1966) were studied.

Compounds such as flavonoids, casticin, chrysoplenol D, lutcolin, isoorientin, P-hydroxy benzoic acid (Dayrit *et al.*, 1987); 4 - 4 - dimethoxy - trans - stilbene (Banerji *et al.*, 1988); lignan (Chawla *et al.*, 1992); iridoids (Dayrit and Lagurin, 1994); sabinene, P-cymene, beta-phelladune, gammo-terpinene, terpinen-4-ol, beta-caryophyllene, globul and viridifloral (Mallavarpu

et al., 1994); mono and sesquiterpenes (Jirovetz *et al.*, 1998); viridiflorol, beta-eudesmol and beta-caryophyllene (Dayal and Singh, 1999, 2000) were identified so far in *V. negundo* (VN) (plate 2a). Published works are not available for *Ipomea carnea* Jacq. (IC) (plate 2b). Amino-oxy-B-Phenyl propionicacid was identified in a related species *Ipomea tricolor* (Amrhein and Hollander, 1979 and Singh *et al.*, 2002). Hence the present study was undertaken to know the phytochemicals (both qualitatively and qualitatively) present in various solvent extracts of VN and IC leaves and identification of compounds using GC-MS.

2. 2. Materials and Methods

2. 2. 1. Collection of plant material

V. negundo leaves were collected from St. Xavier's College Campus and home gardens in and around Palayamkottai, Tirunelveli District, Tamil Nadu, India. *I. carnea* leaves were collected from an eutrophicated lake (Elanthakulam), Palayamkottai, Tirunelveli District, Tamil Nadu, India. They were brought to the laboratory and washed well with tap water (2 to 3 times) and shade dried. After three weeks, they were powdered using a domestic grinder and stored in an airtight plastic container for further use.

2. 2. 2. Extraction of the plant material

A known quantity of the plant material (100 g) was taken and extracted using benzene, chloroform and water separately with a soxhlet apparatus. Then they were distilled and the final crude material was air dried and stored in the refrigerator for further use.

a. *Vitex negundo* Linn.

b. *Ipomea carnea* Jacq.

Plate. 2. Experimental plants *Vitex negundo* Linn. (*Verbenaceae*) (a) and *Ipomea carnea* Jacq. (*Convolvulaceae*) (b)

2. 2. 3. Qualitative estimation of the phytochemicals

The qualitative analysis of the benzene, chloroform and water extracts of both VN and IC were done according to the procedure of Brindha *et al.* (1981). The procedure is given in table 13.

2. 2. 4. Quantitative estimation of the phytochemicals

2. 2. 4. 1. Tannin

Weighed 0.5 g of the powdered material and transferred to a 250 ml conical flask. Added 75 ml water, heated the flask gently and boiled for 30 min. Centrifuged at 2000 g for 20 min and collected the supernatant in 100 ml volumetric flask and made up to the volume. Transferred 1 ml of the sample extract to a 100 ml volumetric flask contained 75 ml water. Added 5 ml of Folin-Denis reagent, 10 ml Sodium carbonate (35%) and diluted to 100 ml with water. Shook well, read the absorbance at 700 nm after 30 min. If the absorbance was greater than 0.7, then taken one ml of the sample and four ml of distilled water. Prepared a blank with water instead of sample. Prepared a standard graph by using 0 - 100 µg tannic acid (Schanderl, 1970).

2. 2. 4. 1. a. Calculation

Calculated the tannin content of the samples as tannic acid equivalents from the standard graph.

2. 2. 4. 1. b. Preparation of reagents

i. Folin-Denis reagent

Dissolved 100 gm sodium tungstate and 20 gm phosphomolybdic acid in 750 ml distilled water in a suitable flask and added 50 ml phosphoric

Table 13. Procedure for prelimnary phytochemical analysis tests

No.	Test	Observation	Inference
1.	Libermann Burchard test: Test solution + minimum amount of chloroform + 3 drops of acetic anhydride + 2 drops of conc. H_2SO_4	Purple colour changing to blue or green	Presence of steroids
2.	Test solution + piece of tin + 3 drops of thinoyl chloride	Violet (or) purple colour	Presence of triterpenoids
3.	Test solution + equal volume of Fehling A and Fehling B and heated in a water bath	Red Cu_2O precipitate	Presence of reducing sugars
4.	Test solution + Tollen's reagent	Silver mirror	Presence of reducing sugars
5.	Test solution + Molisch's reagent	Purple colour	Presence of reducing sugars
6.	Test solution + 10% NaOH solution and heated	Solution turned brown on heating	Presence of carbohydrates
7.	Test solution shaken with 2N HCl. Aqueous layer formed decanted to which one or two drops of Mayer's reagent is added	White turbidity or precipitate	Presence of alkaloids
8.	Alcoholic solution of test solution + one drop of ferric chloride	Intense colour	Presence of phenolic compounds
9.	Test solution + water, shaken well	Foamy lather	Presence of saponins
10.	Test solution + conc. HNO_3 + excess ammonia	Reddish orange precipitate	Presence of xanthoproteins
11.	Water soluble portion of the extracts treated with basic lead acetate solution	White precipitate	Presence of tannins
12.	Test solution + magnesium powder and treated with conc. HCl and heated. Cool the test tube under the running water	Orange colour	Presence of flavonoids

acid. Refluxed the mixture for 2 h and make up to one litre with water. Protected the regent from exposure to light.

ii. Sodium carbonate solution

Dissolved 350 gm sodium carbonate in one litre of water at 70 - 80^{0}C. Filtered through glass wool after allowing it to stand overnight.

iii. Standard tannic acid solution

Dissolved 100 mg tannic acid in 100 ml of distilled water.

iv. Working standard solution

Diluted 5 ml of the stock solution to 100 ml with distilled water. One ml contained 50 µg tannic acid.

2. 2. 4. 2. Phenols

2. 2. 4. 2. a. Procedure

Weighed exactly 0.5 to 1.0 gm of the sample and ground it with a pestle and mortar in 10 times volume of 80% ethanol. Centrifuged the homogenate at 10000 g for 20 min and saved the supernatant. Re extracted the residue with five times the volume of 80% ethanol, centrifuged and pooled the supernatants. Evaporated the supernatant to dryness. Dissolved the residue in a known volume of distilled water (5ml). Pipetted out different aliquots (0.2 to 2 ml) into test tubes. Made up to the volume in each tube to 3 ml with water. Added 0.5 ml Folin-Ciocalteau reagent. After 3 min, added 2 ml of 20% Na_2CO_3 solution to each tube. Mixed thoroughly, placed the tubes in boiling water for exactly one minute, cooled and measured

the absorbance at 650 nm against a reagent blank. Prepared a standard curve using different concentrations of catechol (Malick and Singh, 1980).

2. 2. 4. 2. b. Calculation

From the standard curve, found out the concentration of phenols in the test sample and expressed as mg phenols/100 gm material.

2. 2. 4. 2. c. Reagants

80% Ethanol, Folin-Ciocalteau reagent, Sodium carbonate (20%) and Standard (100 mg catechol in 100 ml water), diluted 10 times for a working standard.

2. 2. 4. 3. Flavonoids

2. 2. 4. 3. a. Methodology

Placed 1 ml of the alcohol extract contained not more than 0.1 ml of ethanol or methanol in 25 ml conical flasks and diluted to 2 ml with distilled water. Added 4 ml of vanillin reagent from a burette rapidly (with in 10 -15 sec) to flask A and 4 ml of sulphuric acid to flask B. Prepared a blank in flask C contained 4 ml of vanillin reagent and 2 ml of water. Shook the contents of flask A and B and kept in a water bath to keep its temperature below 35^0 C. Kept the flasks at room temperature for exactly 15 min. Measured the absorbance of the contents of flasks A, B and C in 1 cm cells at 500 nm against 47 percent sulphuric acid (flask D). Subtracted the absorbencies of the contents of flasks B and C from that of flask A. Alternatively, arranged the cells in the holder and read the absorbance of the contents from flask A + D against B + C. Calculated the flavonol content

using a standard curve prepared with phloroglucinol or keempferol or expressed the results as optical densities (A_{500}) (Swain and Hills, 1959).

2. 2. 4. 3. b. Comment

Upto 100 µg of flavonoids in the aliquots may be estimated. Measured the absorbance of the solution at a fixed time after the addition of the reagent, since the color fades slowly.

2. 2. 4. 3. c. Preparation of reagents required

i. Vanillin reagent: Dissolved 1 gm of recrystallised vanillin in 100 ml of 70% conc. H_2SO_4, prepared fresh.

ii. 70% H_2SO_4.

iii. 47% H_2SO_4 (Added 4 ml of 70% conc. H_2SO_4 to 2 ml of distilled water).

2. 2. 5. Identification of compounds

The benzene and chloroform extracts of IC and VN were subjected to the compound identification using GC-MS (model GC-MS - QP 500, make Shimadzu, Singapore) at a flow rate of 31.6 ml/minute and a split ratio of 33. The initial temperature was 70^0 C for five minutes and for every 5 minutes thereafter, 10^0 C was increased up to 260^0 C and allowed it to stand for 20 minutes. The peaks of the compounds obtained were compared with the already available compounds catalogue (class - 5000 software, Wiley 139. Library) and then predicted and interpreted.

2. 3. Results

2. 3. 1. Qualitative phytochemical analysis

The preliminary qualitative phytochemical analyses of IC and VN benzene extract (BE), chloroform extract (CE) and water extract (WE) are shown in table 14. WE of both the plants showed the presence of carbohydrates, phenolic compounds, saponins, xanthoproteins, tannins and flavonoids. The CE of VN had compounds like steroids, phenolic compounds, triterpenoids, saponins and tannins. The BE showed only steroids, carbohydrates, saponins and flavanoids. Carbohydrates, phenolic compounds, saponins and tannins are present in the BE of IC. CE showed the presence of steroids, carbohydrates, alkaloids, phenolic compounds, saponins, xanthoproteins and flavonoids.

2. 3. 2. Quantitative estimation of phytochemicals

In the quantitative estimation, VN possess 0.175 mg/gm, 33.8 µg/mg and 50.00 µg/mg of total phenols, tannins and flavonoids, respectively. IC contain more amount of total phenol, tannins and flavonoids (0.285 mg/gm, 37.5 µg/mg and 82 µg/mg, for phenol, tannins and flavonoids, respectively).

2. 3. 3. Compound identification through GC-MS

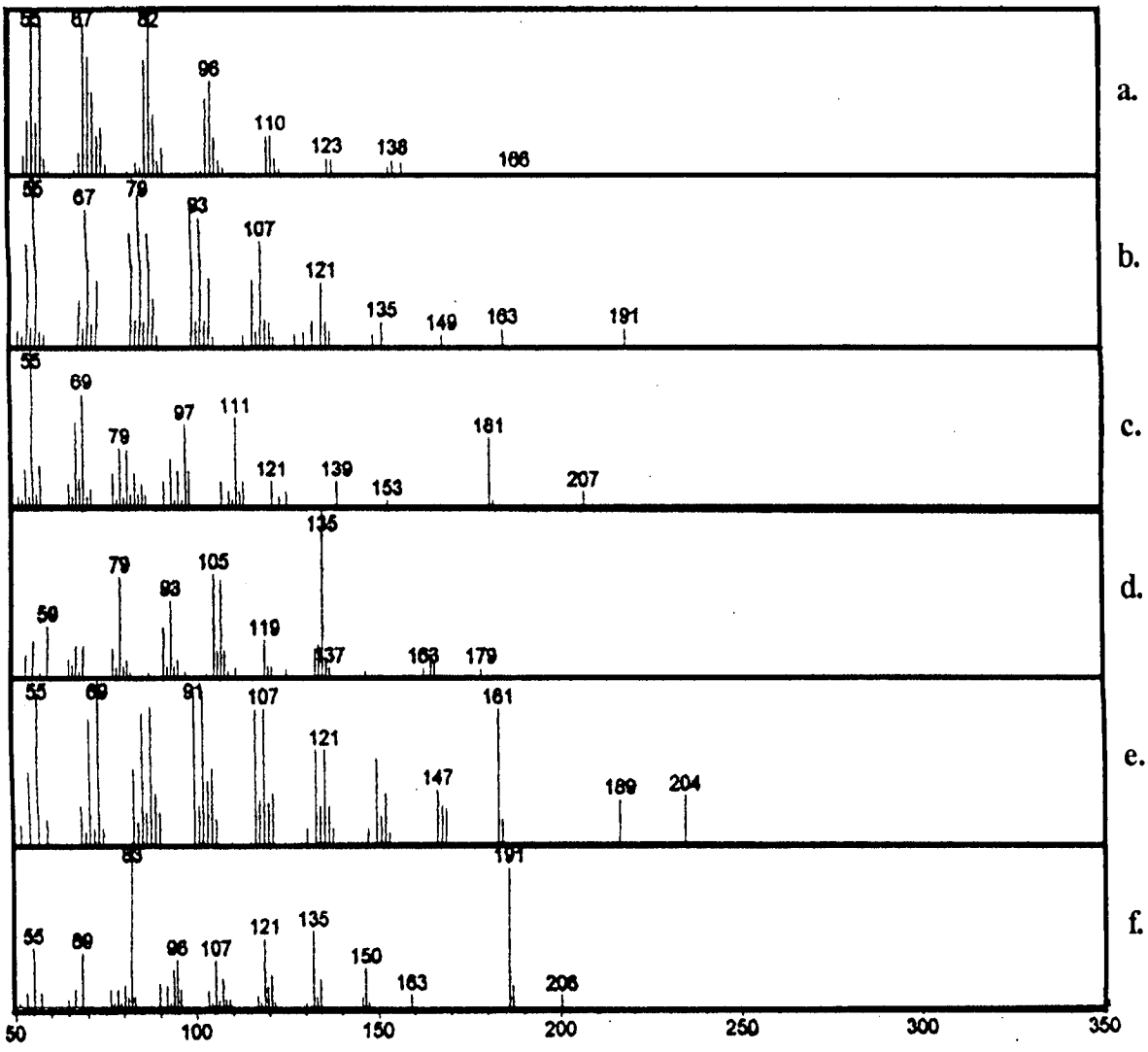
The peaks obtained in the GC-MS analysis for the extracts of both the plants are given in fig. 11-14. The major compounds present in the benzene and chloroform extracts of VN are 1H-Indene (fig. 15a), Cyclododecanol (fig. 15b), Patchoulane (fig. 15c), 1,2-Dihexylcyclopropene-3-carboxylic acid (fig. 15d), 2-Heptenoic acid (fig. 15e), (+) – Aromadendrene (fig. 15f), Trans-caryophyllene (fig. 15g), 7 - Oxabicyclo (4.1.0) heptane, Cyclohexane,

Table 14. Prelimnary phytochemical analysis of various extracts of *I. carnea* and *V. negundo*

Phytochemicals	*I. carnea*			*V. negundo*		
	Chloroform	Benzene	Water	Chloroform	Benzene	Water
Steroids	+	-	-	+	+	-
Triterpenoids	+	+	-	+	+	-
Reducing sugars (Fehling's test)	+	-	+	-	-	-
Reducing sugars (Tollens test)	+	-	+	-	-	-
Reducing sugars (Molisch test)	+	-	+	-	-	-
Carbohydrates	+	+	+	+	+	+
Alkaloids	+	-	-	-	-	+
Phenolic compounds	+	+	+	+	-	+
Saponins	-	+	+	+	+	+
Xantho proteins	-	-	+	-	-	+
Tannins	+	+	+	+	-	+
Flavonoids	+	-	+	+	+	+

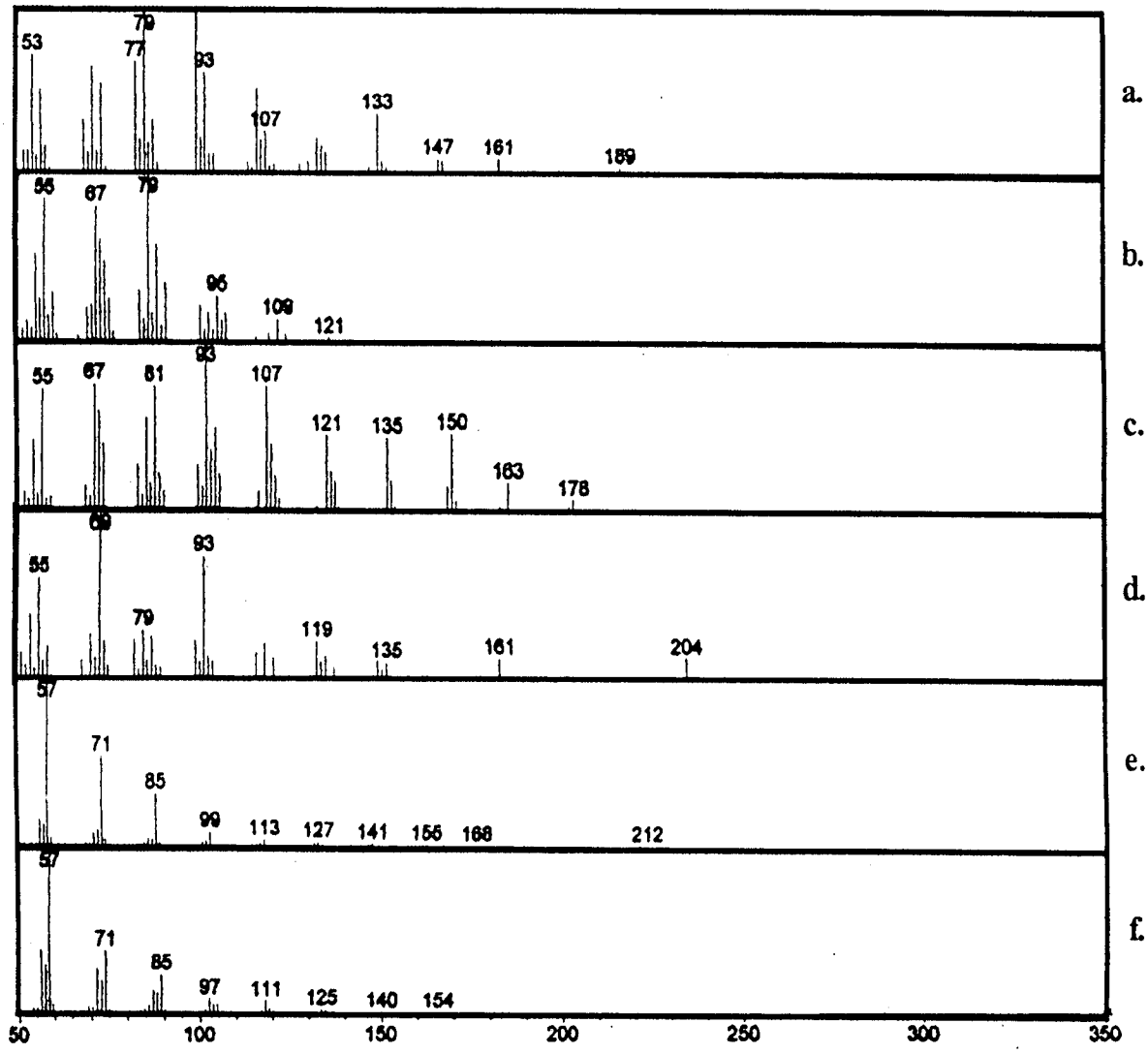
+ : present - : absent

Fig. 11. Peaks obtained from *V. negundo* benzene extract in GC-MS analysis.



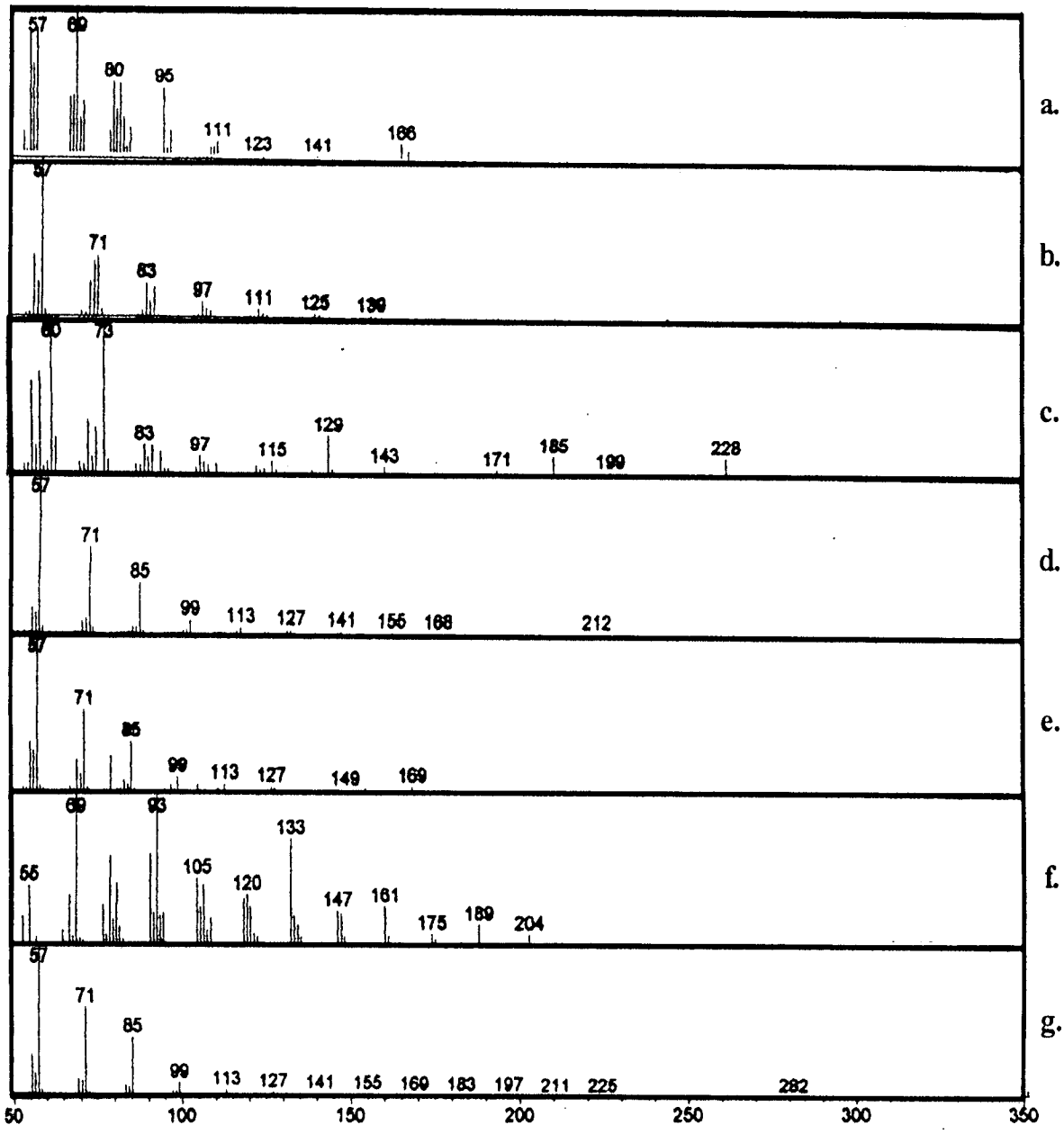
a. 1H-Indene
b. Cyclododecanol
c. Patchoulane
d. 1,2-dihexylcyclopropene-3-carboxylic acid
e. 2-Heptenoic acid
f. (+)-aromadendrene

Fig. 12. Peaks obtained from *V. negundo* chloroform extract in GC-MS analysis.



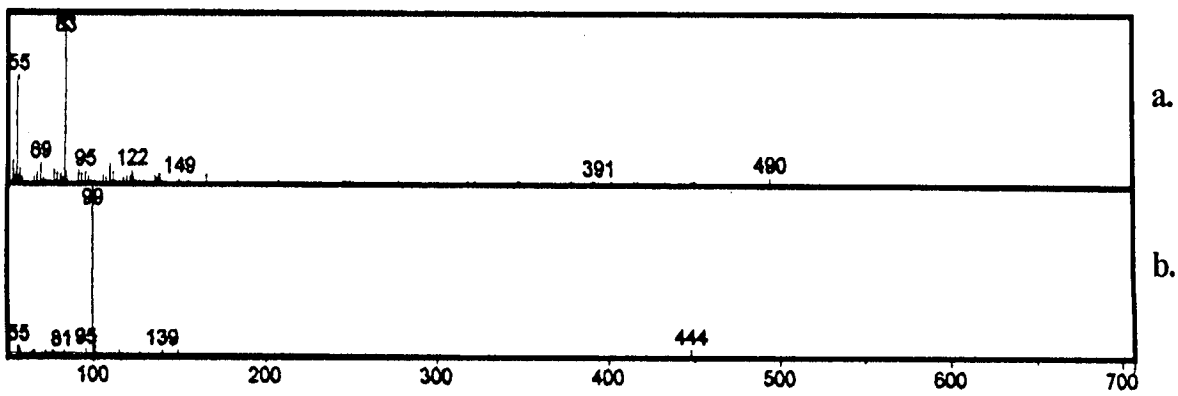
a. Trans-caryophyllene
b. 7-Oxabicyclo (4.1.0) Heptane
c. Cyclohexane
d. Farnesol
e. Pentadecane
f. 1-Octanol

Fig. 13. Peaks obtained from *I. carnea* benzene extract in GC-MS analysis.



a. Neophyadiene
b. 1-Decanol
c. Tetradecanoic acid
d. Pentadecane
e. 1-Iodo-2-Methylundecane
f. Trans-caryophyllene
g. Eicosane

Fig. 14. Peaks obtained from *I. carnea* chloroform extract in GC-MS analysis.

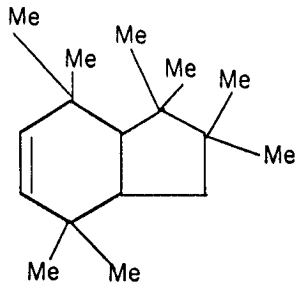


a. 2-Butenoic acid b. Cholestan-3-one

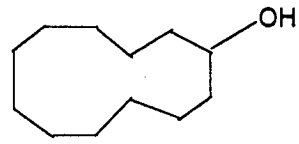
Table 15. Major compounds present in *V. negundo* and *I. carnea* by GC- MS analysis

Plants	Solvents	Name of the compound	Retention Time	Area	Molecular weight
V. negundo	Benzene	1-H-Indene	13.888	659290	206
		Cyclododecanol	16.509	3070370	184
		Patchoulane	16.897	523637	206
		1,2-Dihexylcyclopropene-3-carboxylic acid	18.039	912959	252
		2-Heptenoic acid	20.211	2176431	194
		(+) - Aromadendrene	20.295	1564021	204
	Chloroform	Trans-caryophyllene	10.817	958382	204
		7-Oxabicyclo (4.1.0) heptane	18.053	415325	140
		Cyclohexane	20.228	596939	178
		Farnesol	28.250	245578	222
		Pentadecane	24.055	1922006	212
		1-Octanol	25.756	2087347	186
I. carnea	Benzene	Neophyadiene	14.084	2471308	326
		1-Decanol	15.298	1116206	186
		Tetradecanoic acid	16.517	1570979	228
		Pentadecane	22.718	3743168	212
		1-Iodo-2-methylundecane	24.057	7096486	296
		Trans-caryophyllene	25.472	1695068	204
		Eicosane	25.742	2585936	282
	Chloroform	2-Butenoic acid	22.702	2082610	532
		Cholestan-3-one	24.035	3204781	444

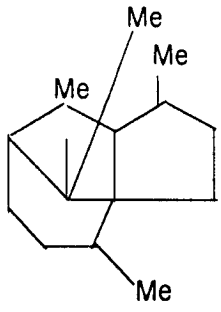
Fig. 15. Structure of few compounds identified through GC - MS from the extracts (benzene and chloroform) of *V. negundo*



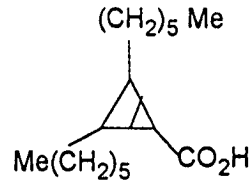
a. 1-H-Indene



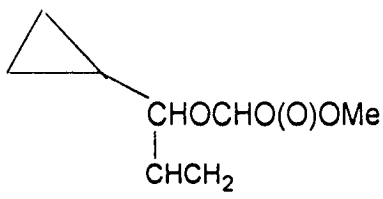
b. Cyclododecanol



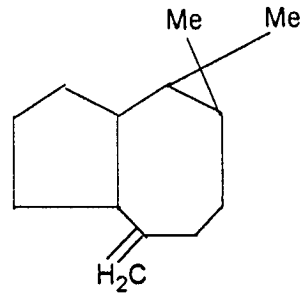
c. Patchoulane



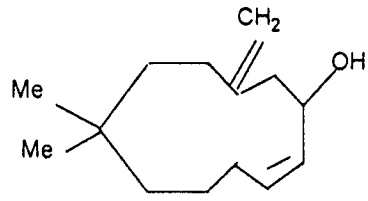
d. 1,2-Dihexylcyclopropene-3-Carboxylic acid



e. 2-Heptenoic acid



f. (+)- Aromadendrene



g. Trans-caryophyllene

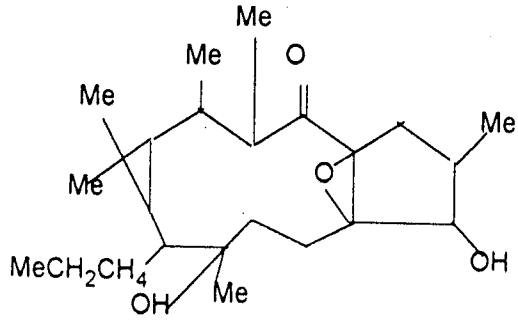
Farnesol, Pentadecane and 1-Octanol (table 15). The IC benzene and chloroform extracts yielded the compounds such as Neophyadiene, 1-Decanol, Tetradecanoic acid, Pentadecane, 1-Iodo-2-methylundecane, Trans-caryophyllene, Eicosane, 2-Butenoic acid (fig. 16a) and Cholestan-3-one (fig. 16b, table 15).

2. 4. Discussion

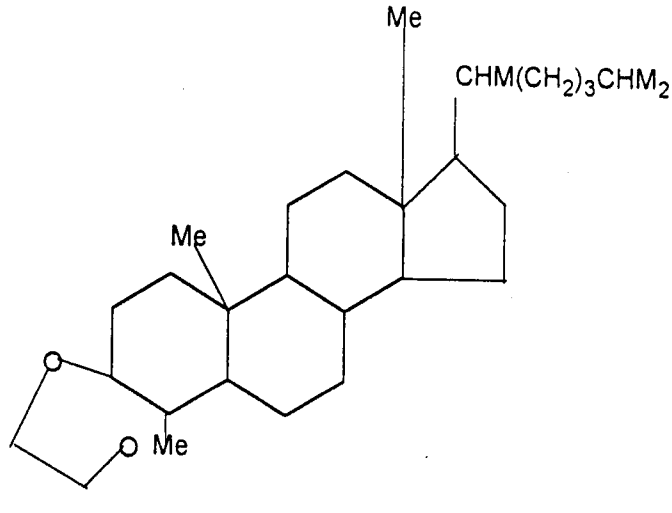
Plants are a rich source of chemical compounds, pigments, steroids etc. (Bernbaum, 1986). Various compounds were identified in different species of plants and they might be responsible for the insecticidal property or toxicity of those plants (Grainge *et al.*, 1985 and Bhatnagar and Sharma, 1994). A bioactive compound azadirachtin was identified and isolated from the neem tree (Schmutterer, 1990) and this azadirachtin plays a major role in IPM programme throughout the world. Enormous literature is available about the role of this product.

Plants are able to synthesize a variety of chemical substances such as mon-protein amino acids, alkaloids, terpenes, flavonoids and their chemical diversity has increased greatly during the course of evolution along the periodical changes in insect feeding pressure. The preliminary phytochemical analysis of both IC and VN showed the presence of various compounds such as flavonoids, lignins, tannins etc. The quantitative estimation showed that flavonoids are the predominant compound in both the botanicals. Dayrit *et al.* (1987) reported the high amount of flavonoids in VN. The bioactive substance present in the extract may be a flavonoid and that would be the reason for the pesticidal property. One of the features of secondary

Fig. 16. Structure of few compounds identified through GC - MS from the extracts (benzene and chloroform) of *I. carnea*



a. 2-Butenoic acid



b. Cholestan-3-one

metabolism is to cope with and adapt to a continually changing environment relates to chemical diversification, with intra-population variation being inherent. Furthermore, tannins are also an important secondary metabolite which has antibacterial, antifungal and antiviral activity. In addition, the present study showed that it also has insecticidal activity against the tested pest. Many published works also evidenced the occurrence of tannins in large proportions of genera in more ancient angiosperms than in recent taxa.

Major compounds present in the VN extracts are 1H-Indene, Cyclododecanol, Patchoulane, 1,2-Dihexylcyclopropene-3-carboxylic acid, 2-Heptenoic acid, (+) - Aromadendrene, Trans-caryophyllene, 7-Oxabicyclo (4.1.0) heptane, Cyclohexane, Farnesol, Pentadecane and 1-Octanol and the earlier reports on the compounds present in VN are casticin, chrysoplenol D, lutcolin, isooxientin, P-hydroxy benzoic acid (Dayrit *et* al., 1987); 4-4-dimethoxy trans-stilbene (Banerji *et al.*, 1988); iridoids (Dayrit and Lauguin, 1994); sabinene, p-cymene, beta phelladune, gamma-terpinene, terpinen 4-ol, beta caryophyllene, global and viridiflorol (Mallavarpu *et al.*, 1994); mono and sesquiterpenes (Jirovetz *et al.*, 1998); viridiflorol, betaeudesmol and betacaryophyllene (Dayal and Singh, 1999, 2000). Flavonoid copounds such as tetramethoxyflavone, trimethoxyflavone, ascerosin and 5-Glucosylrhamnoside (Subramanian and Misra, 1979) in VN were reported. Bonanzin and Artemetin, (Nair and Subramanian, 1975) in *V. trifoltiata* and vitexin (Horowitz and Gentilli, 1966) in *V. lucens* are the flavone compounds identified in the related species. Trans-caryophyllene is a terpene compound

and was reported in *Cannabis sativa* L. (Mediavilla and Steinemann, 1997) and *Protium unifoliolatum* (Mariadass *et al.*, 1993). Cyclododecanol a sesquiterpene compound is a fungi toxin (Burden, 1986).

The extracts of IC yielded a steroid, cholestan-3-one and this may be responsible for the insecticidal property. Cholestan-3-one is a steroidal compound and it has a high insecticidal property (Takatsuto *et al.*, 1999). Literatures are scarce regarding the chemical constituents of IC and the related species *Ipomea tricolor* had α-Aimo-oxy-p-phenyl propionic acid (Amrhein and Hollaender, 1979). Further analysis and identification of the compounds present in *V. negundo* and *I. carnea* are necessary to find out the exact compound responsible for the pesticidal property.

2. 5. Conclusion

The result of this study reveals that the uptake and transformation of plant natural products and volatiles constitute an important array of substances governing the sensitivity of the herbivore in food selection. Hence, an understanding of the variability of secondary metabolites and their role in insect repellence need attention in near future. The preliminary phytochemical analysis of IC and VN showed the presence of compounds such as phenols, saponins, xathoproteins, triterpenoids, tannins and flavonoids. The major compounds identified through GC-MS in VN are 1H-Indene, Cyclododecanol, Patchoulane, 1,2-Dihexylcyclopropene-3-carboxylic acid, 2-Heptenoic acid, (+) - Aromadendrene, Trans-caryophyllene, 7-Oxabicyclo (4.1.0) heptane, Cyclohexane, Farnesol, Pentadecane and 1-Octanol. Neophyadiene, 1-Decanol,

Tetradecanoic acid, Pentadecane, 1-Iodo-2-methylundecane, Trans-caryophyllene, Eicosane, 2-Butenoic acid and Cholestan-3-one are the major secondary metabolites in IC. Further identification of the compounds through IR, NMR etc. is necessary to find out the exact compound responsible for insecticidal property.

"Integration of chosen reduviids and botanicals in groundnut pest management". Ph.D. Thesis submitted by C. Ravi to Manomaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

CHAPTER - 3

IMPACT OF THE TWO BOTANICALS ON *SPODOPTERA LITURA* (FAB.)

3. 1. Introduction

Plants and their products play a major role in IPM and they are species specific, safe to the natural enemies and easily biodegradable. They possess the toxic substance that is sufficient to suppress the growth of the pest and/or cause mortality (Sahayaraj and Paulraj; 1998a, b and c and Singh *et al.*, 1998; Sundararajan and Kumuthakallavalli, 2000a; Paulraj, 2001 and Sahayaraj *et al.*, 2003). Impact of various botanicals on *S. litura* especially azadirachtin (Saxena, 1989; Ayyangar and Rao, 1989a, b and Senthilkumar *et al.*, 1997) and other plants (Stevenson *et al.*, 1993; Narendran *et al.*, 1999; Sundararajan and Kumuthakalavalli, 2000b and Selvaraj, 2002) were studied extensively.

Various plant products have been tested against *S. litura* in the laboratory for its antifeedant property (Joshi and Ramaprasad, 1975; Ayyangar and Rao, 1989a; Jayarajan *et al.*, 1990; Gupta and Rao, 1994; Dhanapakiam and Shanazbegum, 1995; Mohapatra *et al.*, 1995; Chitra and Rao, 1996; Koul *et al.*, 1996; Senthilkumar *et al.*, 1997; Sahayaraj, 1998b; Yasui *et al.*, 1998; Narendran *et al.*, 1999; Murugan *et al.*, 1999; and Dwivedi and Mathur, 2000); repellent property (Sayed, 1983 and Ayyangar and Rao, 1989a) and

mortality (Bai and Kandaswamy, 1985; Rao *et al.*, 1995; Sahayaraj and Paulraj, 1998a, b, c; Martinez and Emden, 1999 and Sahayaraj *et al.*, 2001).

The insecticidal property of *V. negundo* was evaluated by several workers in the laboratory (Abraham *et al.*, 1972; Bai and Kandasamy, 1985; Campos and Quilantang, 1985; Grainge *et al.*, 1985; Tripathi and Rizvi, 1985; Kandasamy *et al.*, 1987; Rabindra *et al.*, 1991; Manalo, 1992; Rajesus *et al.*, 1993; Sahayaraj and Sekar, 1996; Sahayaraj and Paulraj, 1998c and Paulraj, 2001) on many polyphagous pests. *I. carnea* is a weed having insecticidal properties (Pandey *et al.*, 1986; Kulat *et al.*, 1997; Ramamurthy and Venugopal, 1997; Panigrahi and Sahu, 2000; Ramamurthy and Rajaram, 2001 and Sahayaraj *et al.*, 2003).

The present study was undertaken to evaluate the impact of benzene, chloroform and water extracts of *I. carnea* and *V. negundo* on the development, morphology, haemolymph protein profile and mouth parts of the polyphagous pest *S. litura* third instar larvae.

3. 2. Materials and Methods

3. 2. 1. Collection and extraction of the plant materials

The details are furnished in the materials and methods part of Chapter II (see page 47).

3. 2. 2. Collection and maintenance of *S. litura*

It is explained in the materials and methods part of Chapter I (see page 24).

3. 2. 3. Acute toxicity study

Different concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5%) of the extract were prepared using the respective solvents. Ten gm of the groundnut leaves (TMV 7) were taken and soaked well in the known concentration of the benzene extract of VN for five minutes, then air-dried for five minutes. Plant extract treated groundnut leaves were placed in plastic container (1L capacity). Six hours starved six *S. litura* (third instar larvae) were taken from the laboratory culture and introduced into the container containing groundnut leaves, and allowed them to feed the leaves for 24 hrs. After 24 hrs, new plant biopesticide treated groundnut leaves were introduced and similar procedure was followed for 96 hrs continuously. All the experiments were carried out at a temperature of 29 ± 1^0C, light regime of 11 h light: 13 h dark and relative humidity of 65 ± 5%. Every day, unfed larvae and fecal pellets were removed. Surviving larvae were recorded until pupation since the start of the experiment. Similar procedure was followed for the other extracts of VN (chloroform and water) and also all the extracts of IC (benzene, chloroform and water). From the recorded data, the LD_{50} value was found out using Finney (1971). Ten replicates were maintained for each concentration.

3. 2. 4. Chronic toxicity study

From the calculated LD_{50} value, four sub-lethal concentrations, common for all the extracts of both the plants (0.05, 0.1, 0.15 and 0.2%) were chosen for each extract of both VN and IC and the earlier mentioned procedure was followed to feed the *S. litura* third instar larvae and the treatment was given

till the pupation. Fine sand was kept at the bottom of the container during the last instar to facilitate pupation. All the experiments were carried out in 1L plastic containers and ten animals were used in each category and replicated six times. The pupation took place in the earthen cocoon and they were carefully removed from the containers and placed in a petridish with moist cotton swab (to provide humidity) and kept inside a nylon mesh cage (30 × 30 × 90 cm) for the emergence of the adults. The emerged adults were provided with 10% honey solution till their death. Total larval developmental period, survival rate, pupal weight, pupation rate and adult longevity and morphological changes were observed.

3. 2. 5. Collection of haemolymph

Haemolymph was collected by amputating and draining the haemolymph from the fore leg of the V instar *S. litura* larvae (> 6 hrs old) (from control, benzene and chloroform extract treatment of VN and IC at 0.1%) using a fine, sharp and sterilized scissors in the morning hours (9.00 to 10.00 A.M) in an eppendorf tube (graduated) with a sufficient quantity of phenylthiourea (PTU) as an anticoagulant. Haemolymph samples were diluted with Tris-buffer (pH 6.8). This mixture was centrifuged at 5000 g for 10 minutes at 4^0C in a refrigerated centrifuge (Universal 16 R Hettich, Zentrifugen, Germany) to remove the haemocytes and other debris (Charles *et al.*, 1992). Supernatant was mixed with equal volume of sample buffer.

3. 2. 5. 1. SDS-PAGE

The electrophoretic protein profile of haemolymph was determined by one-dimensional Sodium Dodecyl Sulphate - Poly Acrylamide Gel Electrophoresis (SDS - PAGE) carried out according to Laemmli (1970) under discontinuous and dissociating buffer system. The haemolymph protein samples were mixed with sample buffer (50 mM Tris-Hcl, pH 6.8, 2% (w/v) SDS, 10% (v/v) Glycerol, 0.1% bromophenol blue, 5% 2-mercaptoethanol) such that the protein concentration of each sample is 100 µg per well. The gel was photographed and documented using a gel documentation system (Wilber Lourmat, made France and Biogene software).

3. 2. 6. Mouthparts of *S. litura*

The V instar larva of S. litura (> 4 days) was taken from all the experimental category and control. The head was ablated using a fine scissors and was placed in 15% potassium hydroxide solution taken in a test tube. It was gently heated for two to three minutes under Bunsen burner. The mandibles were separated gently from the mouthparts and then placed in a cavity slide and mounted using a cover glass with DPX mixture. The mandibles were examined under a light compound microscope and microphotographs were taken using a camera attached Nikon microscope (Model Eclipse E400, made Japan).

3. 2. 7. Statistical analysis

ANOVA and correlation were performed using SYSTAT and STATISTICA computer package and the results are interpreted.

3. 3. Results

3. 3. 1. Acute toxicity study

The LD_{50} concentrations of the IC benzene (BE), chloroform (CE) and water (WE) extracts on third instar *S. litura* larvae are given in table 16. The mortality was concentration dependant and at the end of 96 hrs, the CE recorded the lower dose (0.643%) followed by BE (0.830%) and WE (1.236%) respectively. In VN, the LD_{50} concentrations were slightly higher than the IC extracts. (1.066, 1.235 and 1.193% for CE, BE and WE, respectively). The required dose to cause 50% mortality of *S. litura* larvae decreased from the 24 hrs to 96 hrs in all the extracts of both IC and VN (table 16).

3. 3. 2. Chronic toxicity study

3. 3. 2. 1. Larval period

The larval period of *S. litura* and the concentration of the IC CE extracts are positively correlated ($r = + 0.9818$). The larval period for WE category was 15.50 ± 0.52 days and it was increased to CE (15.90 ± 0.52 days) and BE (15.80 ± 0.44 days) treated categories and are not statistically significant at 5% level by LSD test (table 17). The larvae treated with 0.20% CE of IC required more days to complete its larval period (17.82 ± 0.86 days). In both IC and VN, the CE prolonged the larval period than the BE and WE. The larval period of the water treated *S. litura* was 15.25 days. It was increased to 15. 90 days when treated with BE and further increase was observed in CE treated category (16.30 days) (table 17 and 19).

Table 16. LD_{50} concentrations (in %) of different extracts of *I. carnea* and *V. negundo* on third instar *S. litura* larvae

Time (in hrs)	CE	BE	WE
I. carnea			
24	1.383	2.122	2.705
48	1.069	1.604	2.034
72	0.816	1.150	1.464
96	0.643	0.830	1.193
V. negundo			
24	2.773	2.288	3.160
48	1.684	1.716	2.542
72	1.378	1.430	1.948
96	1.066	1.235	1.236

CE - Chloroform Extract

BE - Benzene Extract

WE - Water Extract

Table 17. Influence of *I. carnea* extracts (in %) on the developmental period (days) of *S. litura*

Extracts	Parameters	Control		Concentrations			
		Water	Solvent	0.05	0.10	0.15	0.20
CE	Larval period	15.50 ± 0.52	15.90 ± 0.52	16.34 ± 0.28	17.00 ± 1.00	17.60 ± 0.35	17.82 ± 0.86
	Pupal period	5.25 ± 0.45	6.30 ± 0.84	7.45 ± 0.82	7.90 ± 0.45	8.64 ± 0.48	9.25 ±0.75
	Adult longevity	6.90 ± 0.36	6.48 ± 0.62	5.96 ± 0.48	5.24 ± 0.52	4.82 ± 0.46	3.45 ± 0.36
BE	Larval period	15.50 ± 0.52	15.80 ± 0.44	16.25 ± 0.64	16.76 ± 0.53	17.28 ± 0.72	17.66 ± 0.32
	Pupal period	5.25 ± 0.45	5.90 ± 0.36	6.24 ± 0.48	6.92 ± 0.56	8.44 ± 0.38	8.86 ± 0.44
	Adult longevity	6.90 ± 0.36	6.75 ± 0.55	5.96 ± 0.84	5.58 ± 0.37	5.05 ± 0.15	4.82 ± 0.34
WE	Larval period	15.50 ± 0.52	15.50 ± 0.52	16.21 ± 0.56	16.66 ± .049	17.14 ± 0.74	17.54 ± 0.65
	Pupal period	5.25 ± 0.45	5.25 ± 0.45	5.78 ± 0.36	6.32 ± 0.52	6.79 ± 0.47	8.45 ± 0.54
	Adult longevity	6.90 ± 0.36	6.90 ± 0.36	6.50 ± 0.50	6.14 ± 0.46	5.75 ± 0.28	5.20 ± 0.69

3. 3. 2. 2. Pupation

The pupation was 100% in the control (water). As the concentration of the extract increased, the pupation rate decreased and a negative correlation (r = - 0.9696) between the concentration and pupation was observed (IC CE). Low pupation was observed in IC CE at 0.2% (36.67 ± 5.16%) and VN CE at 0.2% (56.66 ± 9.42%) and maximum pupation in 0.05% WE of both IC and VN (90.0 ± 0.0%) and is statistically significant at 5% level. In both the botanicals the CE treatment highly reduced the pupation rate than the BE and WE (table 18 and 20).

3. 3. 2. 3. Pupal weight

Irrespective of the plants and solvents, the pupal weight decreased as the concentration increased. For water control, the pupal weights were 231.67 ± 5.82 mg and significantly ($P < 0.05$) lower in chloroform (229.76 ± 2.34mg) and benzene control (227.46 ± 3.89 mg). In the treatments, very low pupal weight was recorded in 0.20% IC (157.36 ± 15.86 mg) and 0.2% VN (172.68 ± 13.28mg) CE. A significant reduction in pupal weight ($P < 0.05$) was observed with in the treatment (table 18 and 20).

3. 3. 2. 4. Pupal period

As noticed in the larval period, the pupal period was also increased as the concentration of the plant extracts increased. IC CE (0.2%) highly increased the pupal period (9.25 ± 0.75 days) of *S. litura* than control (5.25 ± 0.45 days) and is highly significant ($P < 0.05$) than VN CE at the same concentration (9.0 days) (table 17 and 19).

Table 18. Influence of *I. carnea* extracts (in %) on the pupation (%), pupal weight (mg) and adult emergence (%) of *S. litura*

Extracts	Parameters	Control		Concentrations			
		Water	Solvent	0.05	0.10	0.15	0.20
CE	Pupation	100.00 ± 0.00	95.00 ± 5.00	83.32 ± 5.16	73.50 ± 4.08	66.66 ± 4.71	36.67 ± 5.16
	Pupal weight	231.67 ± 5.82	229.76 ± 2.34	218.46 ± 13.42	200.24 ± 15.36	184.62 ± 11.73	157.36 ± 15.86
	Adult emergence	100.00 ± 0.00	92.54 ± 4.07	78.52 ± 5.20	52.64 ± 3.37	41.83 ± 6.55	23.58 ± 14.26
BE	Pupation	100.00 ± 0.00	93.33 ± 4.71	85.00 ± 5.00	78.34 ± 3.72	71.70 ± 4.08	46.66 ± 8.16
	Pupal weight	231.67 ± 5.82	227.46 ± 3.89	220.34 ± 14.96	204.93 ± 12.84	191.84± 13.42	168.39 ± 14.89
	Adult emergence	100.00 ± 0.00	90.90 ± 4.07	81.36 ± 7.53	68.92 ± 5.82	57.32 ± 7.67	41.56 ± 11.94
WE	Pupation	100.00 ± 0.00	100.00 ± 0.00	90.00 ± 0.00	85.00 ± 7.63	78.33 ± 4.08	64.28 ± 7.86
	Pupal weight	231.67 ± 5.82	237.67 ± 5.82	223.86 ± 12.24	209.54 ± 12.96	196.23 ± 16.52	177.89 ± 14.36
	Adult emergence	100.00 ± 0.00	100.00 ± 0.00	85.28 ± 5.36	74.36 ± 4.83	69.54 ±7.26	57.26 ± 6.84

Table: 19. Influence of *V. negundo* extracts (in %) on the developmental period (days) of *S. litura*

Extracts	Parameters	Control		Concentrations			
		Water	Solvent	0.05	0.10	0.15	0.20
CE	Larval period	15.50 ± 0.52	15.90 ± 0.52	16.28 ± 0.31	16.72 ± 0.19	17.20 ± 1.40	17.50 ± 0.50
	Pupal period	5.25 ± 0.45	6.30 ± 0.84	6.94 ± 0.42	7.41 ± 0.52	8.54 ± 0.48	9.00 ± 0.50
	Adult longevity	6.90 ± 0.36	6.48 ± 0.62	6.16 ± 0.56	5.84 ± 0.64	4.44 ± 0.54	3.96 ± 0.28
BE	Larval period	15.50 ± 0.52	15.80 ± 0.44	16.18 ± 0.38	16.72 ± 0.58	17.0 ± 0.50	17.42 ± 0.28
	Pupal period	5.25 ± 0.45	5.90 ± 0.36	6.20 ± 0.40	6.76 ± 0.64	8.32 ± 0.26	8.54 ± 0.73
	Adult longevity	6.90 ± 0.36	6.75 ± 0.55	6.54 ± 0.42	6.12 ± 0.96	5.86 ± 0.54	5.20 ± 0.40
WE	Larval period	15.50 ± 0.52	15.50 ± 0.52	15.94 ± 0.16	16.32 ± 0.28	16.84 ± 0.22	17.20 ± 0.41
	Pupal period	5.25 ± 0.45	5.25 ± 0.45	5.63 ± 0.72	6.14 ± 0.56	6.65 ± 0.38	8.12 ± 0.57
	Adult longevity	6.90 ± 0.36	6.90 ± 0.36	6.47 ± 0.54	6.12 ± 0.96	5.94 ± 0.62	5.64 ± 0.35

Table 20. Influence of *V. negundo* extracts (in %) on the pupation (%), pupal weight (mg) and adult emergence (%) of *S. litura*

Extracts	Parameters	Control		Concentrations			
		Water	Solvent	0.05	0.10	0.15	0.20
CE	Pupation	100.00 ± 0.00	95.00 ± 5.00	86.16 ± 5.16	80.00 ± 0.00	73.33 ± 4.71	56.66 ± 9.42
	Pupal weight	231.67± 5.82	229.76 ± 2.34	220.46 ± 10.52	209.46 ± 9.82	193.00 ± 12.93	172.68± 13.38
	Adult emergence	100.00 ± 0.00	92.54 ± 4.07	84.64 ± 5.21	72.46 ± 7.89	61.34 ± 7.21	48.92 ± 15.71
BE	Pupation	100.00 ± 0.00	93.33 ± 4.71	88.33 ± 3.72	81.66 ± 4.08	78.33 ± 4.71	63.33 ± 5.16
	Pupal weight	231.67± 5.82	227.46 ± 3.89	221.92 ± 14.32	211.36 ± 5.93	195.48 ± 12.64	181.58± 11.46
	Adult emergence	100.00 ± 0.00	90.90 ± 4.07	86.58 ± 6.43	76.57 ± 5.36	67.32 ± 6.64	59.86 ± 12.36
WE	Pupation	100.00 ± 0.00	100.00 ± 0.00	90.00 ± 0.00	88.32 ± 5.72	81.66 ± 4.08	76.64 ± 4.72
	Pupal weight	231.67± 5.82	231.67 ± 5.82	227.68 ± 12.86	213.47 ± 11.92	200.24 ± 14.83	181.39± 10.92
	Adult emergence	100.00 ± 0.00	100.00 ± 0.00	89.32 ± 3.64	78.59 ± 4.63	71.46 ± 7.51	63.57 ± 9.23

3. 3. 2. 5. Adult emergence

In the control (water), 100% adults emerged from the pupae and in the experimental categories, it was significantly reduced depending on the concentration. Only 23.38 ± 14.26% of adults were emerged from the IC CE (0.20%) treated *S. litura* larvae and less than 50% adult emergence were noticed in 0.15% CE (41.83 ± 6.55%); 0.20% BE (46.66 ± 8.16) (table 18) and VN CE (0.20%) (48.92 ± 15.71%) treated categories and are highly significant when compared to their respective controls ($P < 0.05$). Irrespective of the botanicals, the CE suppressed the adult emergence to a greater extent than BE and WE (table 18 and 20).

3. 3. 2. 6. Adult longevity

The adult longevity of *S. litura* and the concentration of the extracts are inversely proportional to each other (i.e.) a reduction in the longevity was observed, as there was an increase in the concentration. Very short adult longevity was recorded in 0.20% CE of IC (3.45 ± 0.36 days). A reduction in adult longevity in BE (4.82 ± 0.34 days) and WE treatments (5.2 ± 0.69 days) were also observed than their respective control (6.75 ± 0.55 and 6.90 ± 0.36 days for BE and WE control respectively). The adult longevity in VN CE at 0.20%, the adult longevity was 3.96 ± 0.28 days. The CE was superior in both the botanicals in reducing the adult longevity (table 17 and 19).

3. 3. 2. 7. Deformities and Abnormalities

Deformities were observed both in the larval and pupal stages. Larval-pupal intermediate with larval thoracic segments with the abdomen (IC

Plate. 3

Control *S. litura* larvae (a) and its larval pupal intermediate with larval thoracic segments (IC CE 0.15%) (b), shrunken larvae (VN BE 0.1%) (c), deformed larva (IC WE 0.2%) (d), larval pupal intermediate with pupal thoracic shield and larval legs (VN CE 0.2 %) (e), severely damaged larva (IC BE 0.2%) (f), larval pupal intermediate retaining the larval legs (VN CE 0.15%) (g), larval pupal intermediate retaining larval legs and skin (IC CE 0.1%) (h) and larval head and larval skin retaining in the partially developed pupa (IC BE 0.2%) (i).

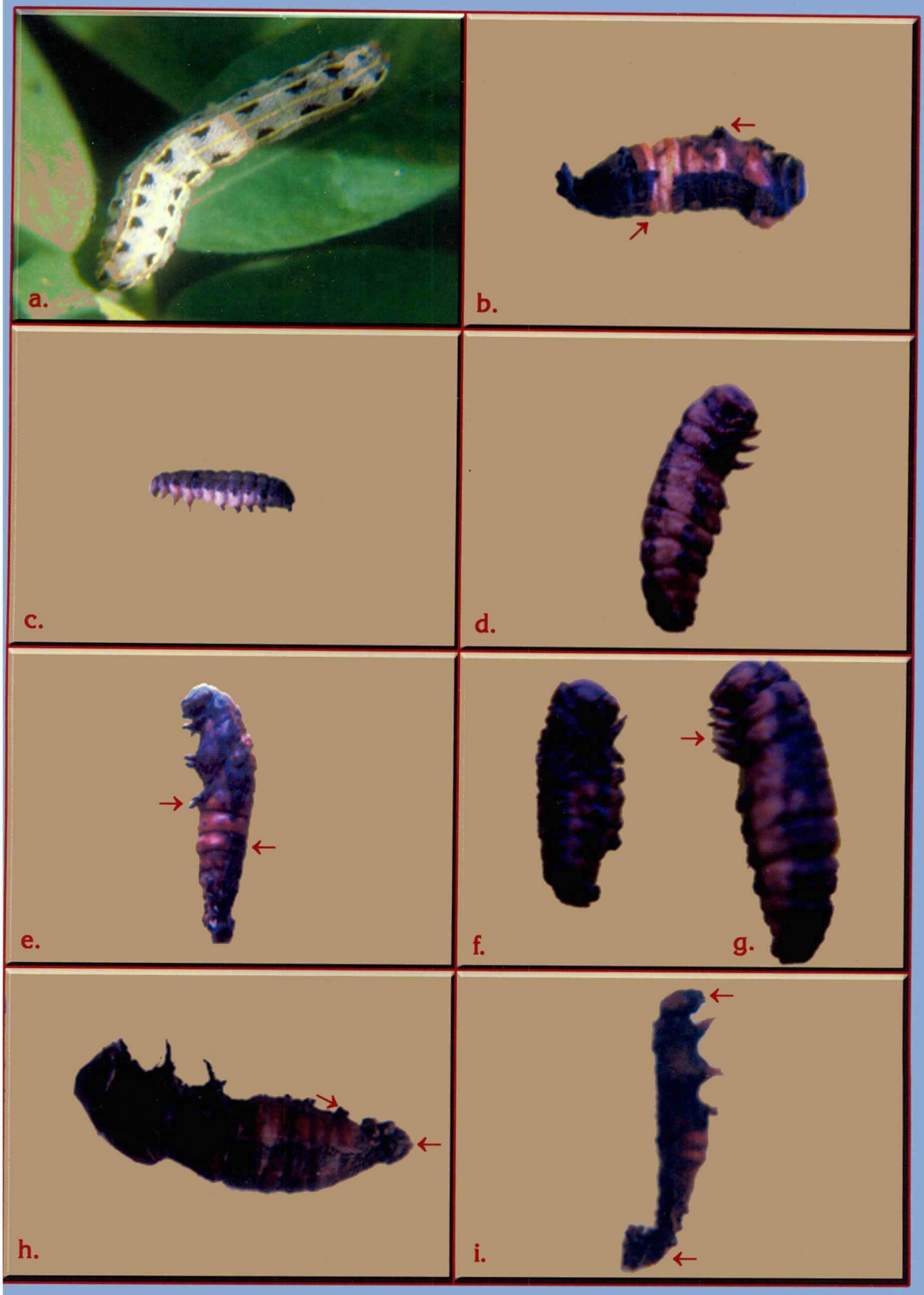


Plate. 3

Plate. 4

Control *S. litura* pupa (a) and its ruptured thoracic shield through which the hemocoel oozed out (IC CE 0.2%) (b), pupa retaining larval thoracic appendages (VN CE 0.2%) (c), pupa retaining moult skin and remnance of larval appendages, larval mouth parts on the ventral side (VN BE 0.15%) (d) and pupa retaining larval head, leg and thorax (IC BE 0.1%) (e).

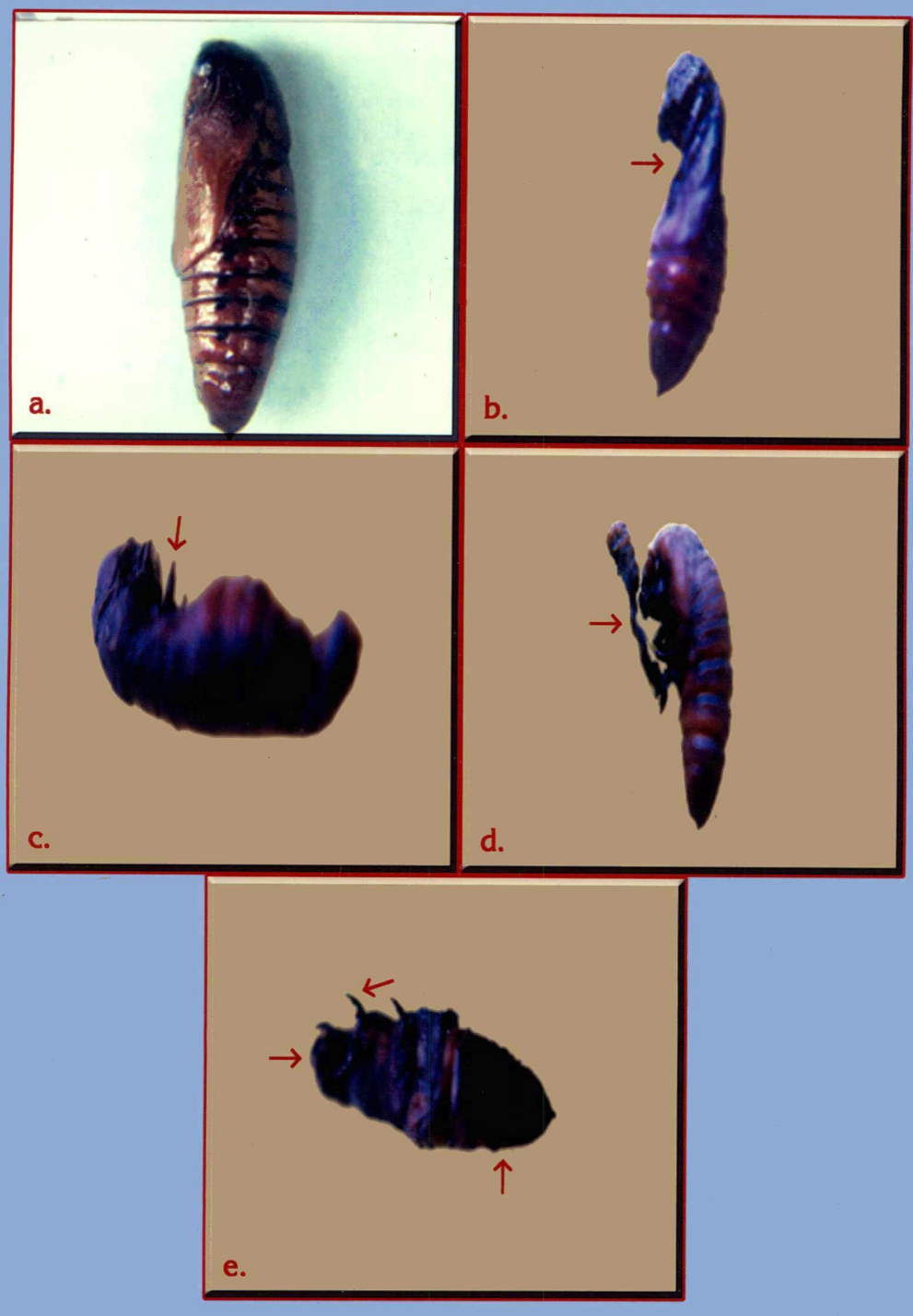


Plate. 4

CE, 0.15%), pupal thoracic shield and larval legs (VN CE, 0.2%), retaining larval legs and skin (IC CE, 0.1%), larval-pupal intermediate retaining the larval legs (VN CE, 0.15%), shrunken larvae (VN BE, 0.1%) and larval skin retaining in the partially developed pupa (IC BE, 0.2%) were observed (plate 3). Deformities such as ruptured shield of the pupa through which the hemocoel oozed out (IC CE, 0.2%), pupa retaining larval thoracic appendages (VN CE, 0.2%), molt skin and remnance of larval appendages and mouthparts on the ventral side (VN BE, 0.15%), pupa retaining larval head and thorax (IC BE, 0.1%) were observed (plate 4). However, the larval stages are more susceptible to the plant extracts and more deformities and abnormalities occurred in this stage only. Among the two plants, IC CE had caused maximum deformities and abnormalities in *S. litura*.

3. 3. 2. 8. Haemolymph protein profile

There are 14 bands observed in the water treated leaves fed *S. litura*. This was increased to 18 bands in the chloroform treated category. The plant extracts greatly altered the protein profile of the haemolymph in *S. litura* larvae. The amount and deposition of the storage protein increased in the BE treated category and in the CE category, two bands disappeared in the IC CE (plate 5 and table 21 and 22)

3. 3. 2. 9. Mouthparts of *S. litura*

S. litura mandible showed that it contain six highly pointed tooth. Changes were observed in the mandibles especially in the mandibular teeth of the larvae of *S. litura* treated with IC and VN extracts and are shown in

Table 21. Electrophorogram of *S. litura* larval heamolymph treated with benzene extract of *V. negundo* and *I. carnea*

Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
Marker	Water control	Benzene control	VN BE	IC BE
205000	485000	485000	489000	483000
97000	443000	451000	447000	441000
66000	415000	413000	397000	419000
43000	381000	383000	357000	359000
29000	319000	315000	315000	289000
20000	257000	261000	283000	249000
14300	229000	225000	200252	205000
6500	213000	202626	125510	149787
3000	183729	176728	97000	113107
	143416	131257	86652	89867
	121816	108274	779888	82881
	99584	98272	70641	71619
	95661	91942	51871	56403
	77556	76248	43868	46666
	68628	69138	38970	40834
	59948	61751	31979	38347
	50783	53539	23282	33920
	46666	48675	17566	25431
	42072	41763	3858	18285
	38034	38347		3858
	30666	34879		
	21750	31653		
	19969	22954		
	16674	16674		
	4792	4311		

Table 22. Electrophorogram of *S. litura* larval heamolymph treated with chloroform extract of *V. negundo* and *I. carnea*

Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
Marker	Water control	Chloroform control	VN CE	IC CE
205000	125605	202774	20274	197687
97000	72694	156353	15635	145672
66000	68296	119829	114319	113250
43000	48002	84105	80717	91669
29000	44531	71186	70201	81837
20000	375888	66000	65341	64024
14300	35034	46466	45861	52813
6500	32292	43250	42771	45658
3000	30376	39361	35034	42313
	26220	35658	32665	35241
	21262	32854	30874	24000
	17066	30376	29437	23247
	4572	27934	27661	17710
	2972	23837	23644	11274
		20262	18925	4882
		15319	12541	2985
		9353	6500	
		3365	3003	

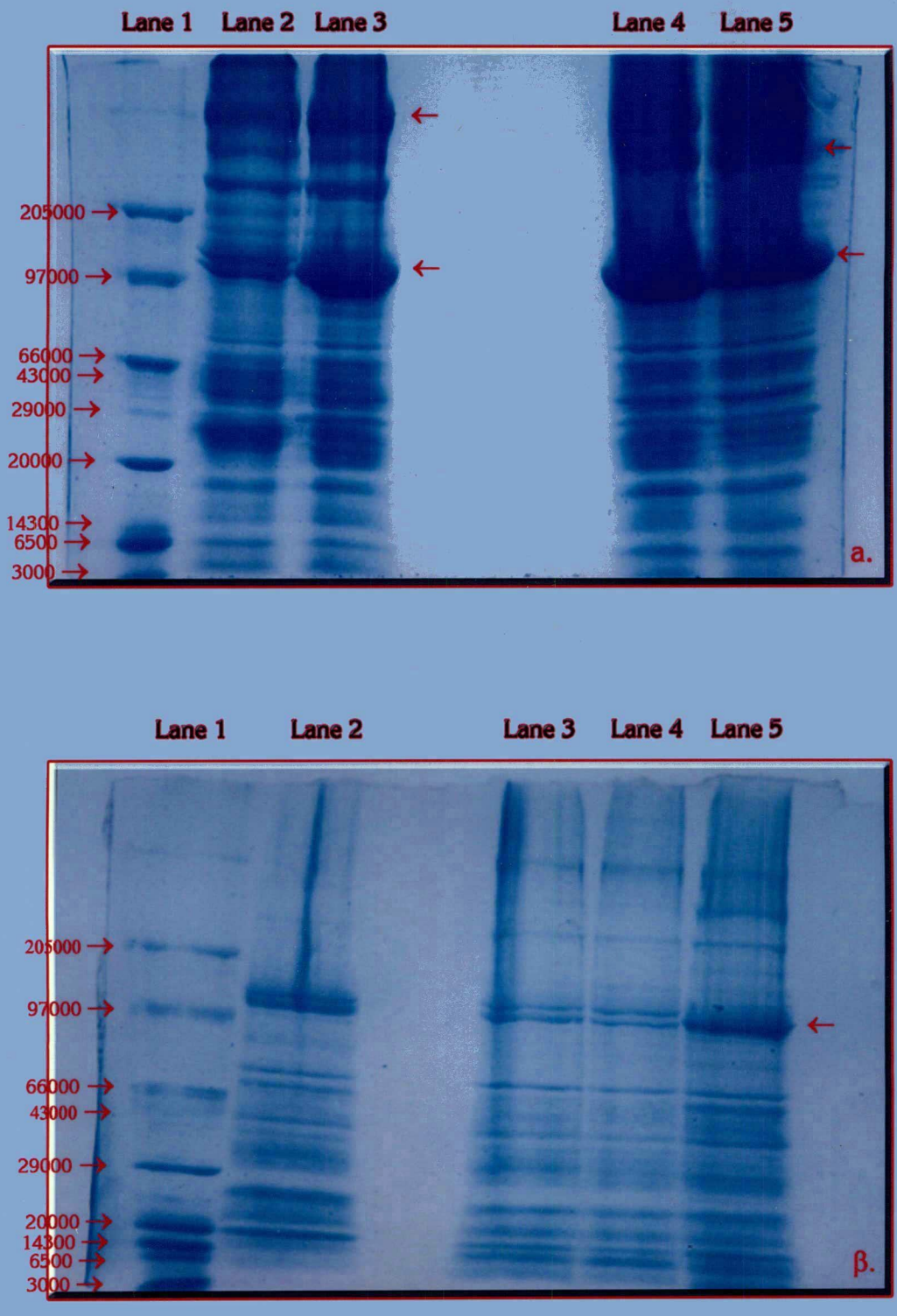


Plate. 5. Haemolymph protein profile of *S. litura* larvae treated with benzene (a) (Lane 3, Lane 4 and Lane 5) and chloroform (b) (Lane 3, Lane 4 and Lane 5) extractes of *V. negundo* and *I. carnea* (Lane 1 - marker, Lane 2 - water control)

plate 6 and 7. The mandibular teeth blunted at the tips partially (IC CE, 0.1% and 0.2% and VN CE, 0.15%) and/or completely (IC CE, 0.15%; VN CE, 0.2%), loss of the sharpness of the mandibular teeth (IC BE, 0.1%), erosion of the blades of the mandibular teeth (IC BE, 0.15%), degeneration of the mandibular teeth (IC BE, 0.15%), formation of new serration in mandibular teeth (VN BE, 0.15%) and wear and tear of sixth mandibular teeth (VN CE, 0.1%) are the significant changes observed in the botanicals treated *S. litura* larvae mandible when compared to the control larval mouthparts.

3. 4. Discussion

3. 4. 1. Acute toxicity

The dose that required to cause 50% mortality of *S. litura* third instar larvae in the laboratory by IC and VN CE, BE and WE showed that all the extracts caused significant mortality at 96 hrs and the IC CE recorded the minimum dose (0.643%) and VN WE had the highest LD_{50} value (1.236%). VN water extracts caused 100% (Bai and Kandasamy, 1985) and 83% (Sahayaraj and Sekar, 1996) mortality in fourth instar larvae of *S. litura*. Paulraj (2001) reported the LD_{50} value of VN water extract as 1.25% against the fourth instar *S. litura* larvae. In the present study, the mortality of *S. litura* third instar larvae occurred in slightly lower concentrations than they observed. The larval stage might be the reason because younger larvae are more susceptible to the plant toxicants than older larvae. Sahayaraj *et al.* (2003) recorded high mortality in *A. janata* larvae treated with *I. carnea* and *Christella parasitica* extracts. Similarly several workers reported the mortality

Plate. 6

Mandibular wear caused by *I. carnea* through mandibular teeth blunted at the tips in partial (0.1% CE) (5×10x) (a) or complete (0.15% CE) (5×10x) (b), fourth, fifth and sixth mandibular teeth blunted (0.15%0) (5×10x) (c), partially blunted mandibular teeth (0.2% BE) (5×10x) (d), the edges of the mandibular teeth lost its sharpness (0.1% BE) (5×5x) (e and f) and the sharp blades of mandibular teeth eroded (0.15% BE) (10×45x) (g) in *S. litura* larvae.

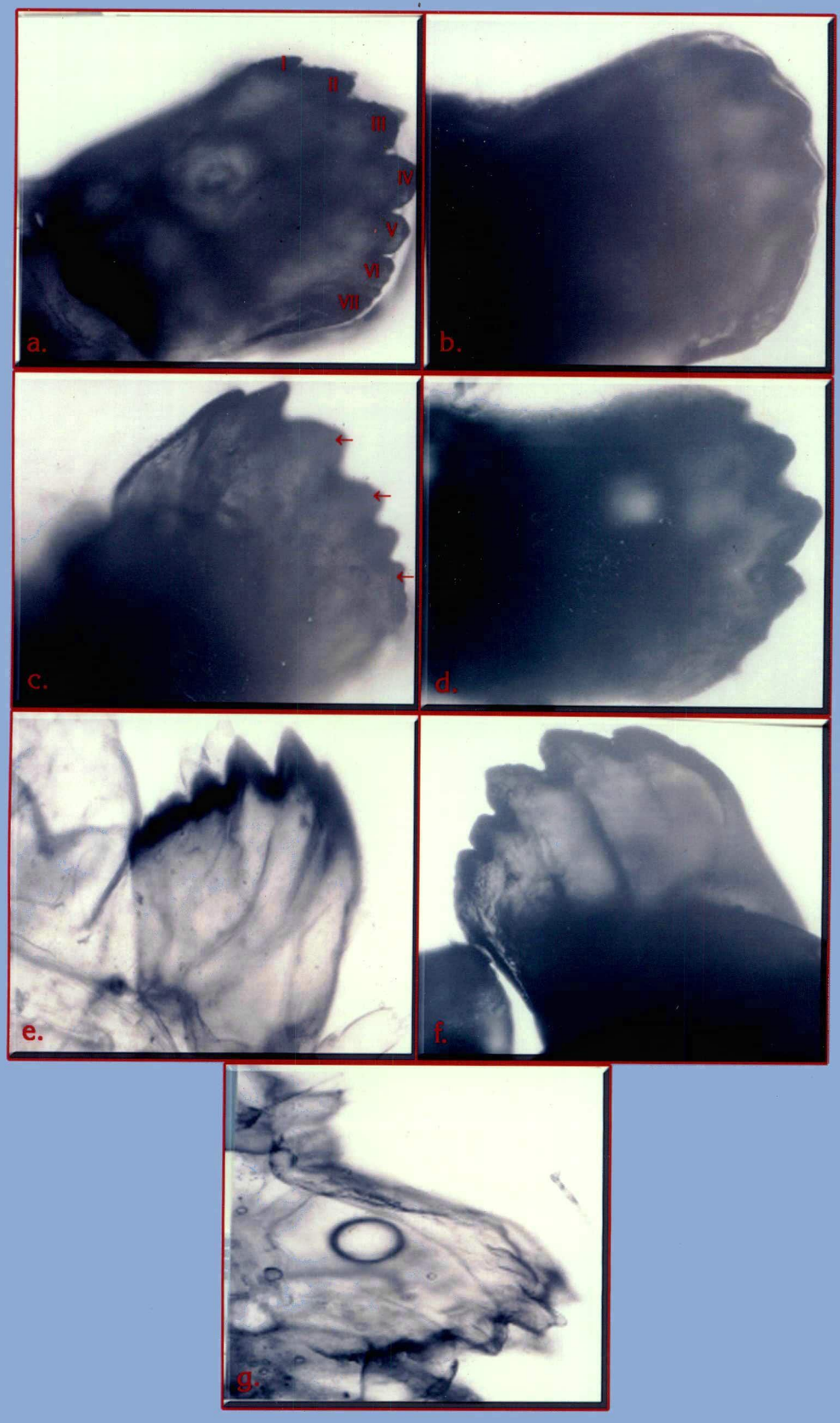


Plate. 6

Plate. 7

Mandibular wear caused by *V. negundo* by the formation of new serrations in the mandibular teeth (BE 0.15%) (5×15x) (a), fourth, fifth and sixth mandibular teeth blunting (CE 0.15%) (5×15x) (b), complete mandibular teeth blunting (CE 0.2%) (5×10x) (c), weared and teared sixth mandibular teeth (CE 0.1%) (5×10x) (d) and control mandible (2×10x) (e and f) of *S. litura* larvae.

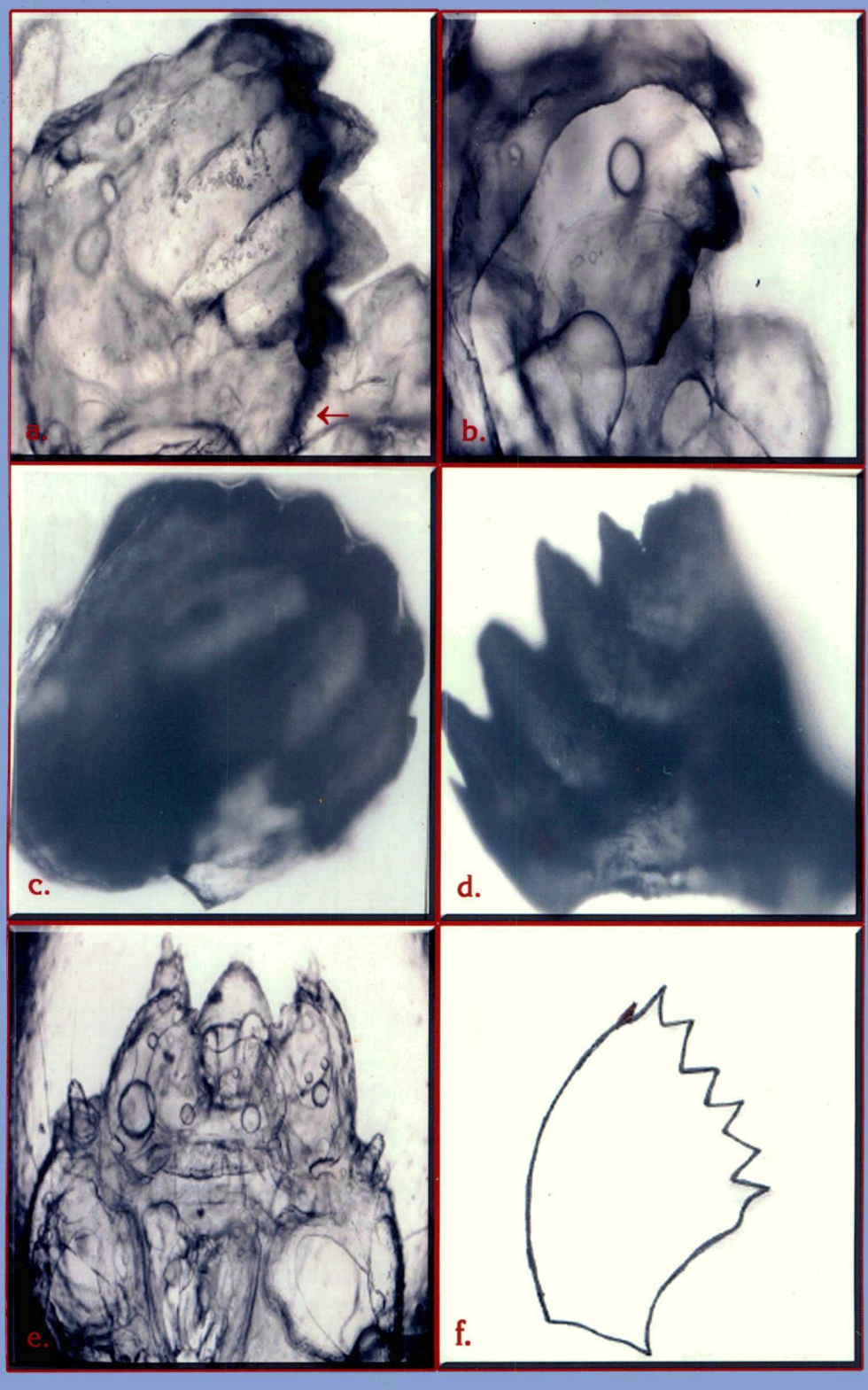


Plate. 7

of *S. litura* by various plant products (Martinez and Emden, 1999; Sahayaraj and Paulraj, 1998a, b, c and Sahayaraj *et al.*, 2001).

In general, the mortality is a concentration dependent factor (Bai and Kandasamy, 1985; Sahayaraj and Sekar, 1996; Paulraj, 2001 and Sahayaraj *et al.*, 2001) and was revealed in the present study also. The substance that is responsible for the death of the pest is available in diverse group of plants (Bernbaum, 1986) and during extraction with solvents their active compounds dissolve in the solvent. When they were provided to the insect through food, it enters into the pest larvae and then interferes with the physiological process of the pest and eventually leads to mortality of the pest in the larval, pupal and adult forms. The active compounds present in IC are Neophyatiene, 1-Decanol, Tetradecanoic acid, Pentadecane, 1-Iodo-2-methylundecane, Trans-caryophyllene, Eicosane, 2-Butenoic acid and Cholestan-3-one. Similarly VN contain 1-H-Indene, Cyclododecanol, Patchoulane, 1,2-Dihexylcyclopropene-3-carboxylic acid, 2- Heptenoic acid, (+) - Aromadendrene, Trans-caryophyllene, 7-Oxabicyclo (4.1.0) heptane, Cyclohexane, Farnesol, Pentadecane and 1-Octanol. These compounds either alone or in combination caused mortality and other effects. Further studies on these compounds' effects on *S. litura* enlighten their role in pest control programme. In this study, CE, BE and WE of VN and IC were tested and the exact compound responsible for the mortality of *S. litura* cannot be proposed and the suggestion is to isolate individual compounds from the extract and test their biopesticidal value by this part (leaves) and also in other parts of the plant.

3. 4. 2. Larval period

The larval period increased in both the plant extracts treated categories than the control. This might be due to the poor food uptake of *S. litura* larvae. Food intake plays a major role in the growth and development of insects (Bai and Kandasamy, 1985; Ananthakrishnan, 1996; Senthilkumar *et al.*, 1997; Sahayaraj and Paulraj, 1998a, b, c; Sundararajan and Kumuthakalavalli, 2000 b and Paulraj, 2001). Furthermore, the lower food intake was mainly due to the mandibular wears observed in the larval stages.

Sahayaraj *et al.* (2001) also reported the increased larval period when treated with plant extracts. In contradiction, Selvaraj (2002) observed reduction of larval period when treated with fern extracts that may be due to the presence of huge quantity of phytoecdysones in the fern extracts that in turn trigger the juvenile hormone which is responsible for molting. Behra and Satapathy (1997) also reported the prolongation of the larval period in *S. litura* treated with Indian laural, karanj and neem extracts. Similar observations were made by Govindachari *et al.* (1996) when treated with azadirachtin. Among the two plants tested, the impact was high on IC CE and minimum effect was observed in VN WE. IC CE also contain cholestan-3-one an ecdysone but its amount was not quantified.

3. 4. 3. Pupation

As the concentration of the extracts increased, the pupation rate decreased and at higher concentrations, the pupation rate was minimum. Shin-Cheng-Ien *et al.* (1995) recorded 10 - 30% pupation in *S. litura* when

treated with neem based biopesticides. Paulraj (2001) and Selvaraj (2002) also reported reduction in the pupation rate of *S. litura* when treated with different plant products. Similarly Rao *et al.* (1995) observed reduction in pupation of *H. armigera* treated with azadirachtin. The reduction in pupation rate was mainly due to the lower food intake and interruption of bioactive principles of IC and VN in digestion process.

3. 4. 4. Pupal weight

The pupal weight of *S. litura* also decreased when treated with CE, BE and WE of IC and VN. The plant extracts disturb the hormonal activity and thereby cause a reduction in pupal weight (Muraleedharan and Sheela Devi, 1992). The present study also revealed that during the larval stage, the larvae feed poorly and it was also a reason for the reduction in pupal weight. Further studies on energy budget are essential to know the feeding behavior and physiology in relation to plant extract. Rao *et al.* (1995); Govindachari *et al.* (1996); Sahayaraj *et al.* (2001) and Selvaraj (2002) also reported decrease in pupal weight of *S. litura* treated with plant extracts. Not only the plant extracts reduce the pupal weight but also the plant derived biopesticides like prococenes and neem azal reduce the pupal weight of *Galleria mellonella* (L.) (Alrubeai, 1986) and *H. armigera* (Rao *et al.*, 1995), respectively.

3. 4. 5. Adult emergence

In water control, 100% of pupae emerged into adults. Martinez and Emden (2001) observed high mortality and deformities in pupae of *S. litura.*

Sahayaraj *et al.* (2003) also reported pupal deformities in *A. jannata.* The results of the present study are in agreement with the earlier findings of Senthilkumar *et al.* (1997); Murugan *et al.* (1998); Sahayaraj and Paulraj (1998a, b, c); Paulraj (2001) and Selvaraj (2002). Pupal mortality and pupal deformities are the main reason for the poor adult emergence in the plant extract treated larvae.

Tannins play a major role in affecting the physiology of the insects and the mode of action of tannins in insects involves a reduced availability of dietary protein or a decreased activity of digestive enzymes (Goldstein and Swain, 1965; Klocke and Chan, 1982). *Heliothis zea* larvae fed on a tannin treated diet exhibited a decreased activity of the digestive protease and amylase enzymes (Klocke and Chan, 1982), reduced feeding and digestion because of the toxic compounds of the botanicals lead to mortality in the larval and pupal stages. In the present study also, tannin is present in both the botanicals that ultimately lead to the reduction in adult emergence.

3. 4. 6. Adult longevity

Both the IC and VN extracts had a great impact on the longevity of adult *S. litura.* All the extracts shortened the adult longevity that may be due to the action of the toxic compounds such as tannins, flavonoids and phenols that are present in high amount in both the plants. These compounds reduce the enzyme activity and there by reduce the growth of the insects (Goldstein and Swain, 1965; Klocke and Chan, 1982; Sahayaraj, 1998b;

Paulraj, 2001 and Selvaraj, 2002). Similar results were also reported by Govindachari *et al.* (1996).

3. 4. 7. Haemolymph protein profile of *S. litura*

An increase in number of polypeptides were seen in the haemolymph of *S. litura* when treated with benzene and chloroform extracts of IC and VN that may be the newly synthesized enzymes to detoxify the toxic substances of the plant products or the storage proteins. Sohal and Rup (1998) reported the increased band in body protein of *Lipaphis erysimi* (Kalt.) treated with methoprene a plant-based biopesticide. Sundaramurthy and Ahmed (1978) observed increased protein in the hemolymph of *S. litura* larvae treated with a biopesticide Altosid. Similarly increased band in the haemolymph of *Bombyx mori* L. was also observed, when treated with juvenile hormone analog (Kajiura and Yamashita, 1989).

3. 4. 8. *S. litura* mouthparts

One of the most intensively examined and abundantly documented structures in the animal world is the insect moth parts. The mouthparts of herbivorous insects are well adapted to feed the plant parts and the mandible play a major role (Chapman, 2000). Usually the mandibles are hard and they may not be affected by the plant allelochemicals (Berdegue and Trumble, 1996). But certain elements such as Calcium, Silica, Zinc and Manganese present in the plants may cause changes in the insect mouth parts and finally lead to the tear and wear of the mandibles and mandibular tooth (Berdegue and Trumble, 1996; Dewhurst, 1999; Keeping and Meyer, 2002 and Schofield

and Nesson, 2002). There are a number of traits that differ between diets which have the potential to influence the herbivorous feeding on them such as, differences in nutritional value (Denno and Douglass, 1985; Loader and Damman, 1991 and Haggstrom and Larsson, 1995), secondary chemicals like resin acids (Bjorkman, 1977), tannin and polyphenols (Greene, 1989) and in leaf toughness (Hoffman and McEvoy, 1986). The present study also revealed that VN and IC contain 17.5 and 28.5% of phenolic compounds. This might also interfere with the feeding and mandibular modifications.

In the leaves of IC and VN, the reports regarding the presence of certain elements (Calcium, Silica, Zinc and Manganese) are not available and they were not quantified. So further studies are essential to find out the exact reason for the changes in the mandible of *S. litura* larvae due to the treatment of IC and VN solvent extracts and also it is necessary to quantify the amount of Silica, Calcium, Zinc and Manganese in the leaves of those two plants.

3. 5. Conclusion

Both IC and VN have insecticidal property against *S. litura*. The chloroform, benzene and water extracts of these plants prolonged the larval and pupal period, decreased the pupation rate, pupal weight and adult longevity. All the extracts played a major role in the wearing and tearing of the mandibles of *S. litura* larvae. They also altered the protein content of the hemolymph. Even though all the three extracts of both the plants had an impact on *S. litura*, IC CE (0.2%) had an incredible effect. It is also

necessary to explore the bioactive compound from IC that certainly will play a massive role in the pest management and add flair to the IPM. From the present study, it is concluded that both the botanicals can be used as biopesticides and further studies are essential to identify the active compounds present in them for a promising plant based biopesticide which is safer to human beings and beneficial organisms and minimum threat to the ecosystem.

CHAPTER - 4

IMPACT OF BIOPESTICIDES ON GROUDNUT PESTS AND CROP PRODUCTION

4. 1. Introduction

Field application of the botanicals to minimize the pests and their infestation was carried out by many workers (Mohamad, 1981; Dhir *et al.*, 1992; Kalyanasundaram *et al.*, 1994; Ramamurthy and Venugopal, 1997; Ramaraju *et al.*, 1998; Senguttwan, 1999; Paulraj, 2001; Selvaraj, 2002; Sahayaraj, 2002c and Kumar and Prasad, 2002). Various plant products were tested in the groundnut field to minimize the pests such as *S. litura* and *H. armigera* (Doureesamy *et al.*, 1990; Venula *et al.*, 1999; Obulapathi *et al.*, 2000; Martinez and Emden, 2000; Paulraj, 2001; Sahayaraj, 2002c and Selvaraj, 2002) and thereby increased the groundnut production and cost benefit ratio (Kalyanasundaram *et al.*, 1994; Paulraj, 2001 and Selvaraj, 2002). *V. negundo* has pesticidal property (Abraham *et al.*, 1972; Jacobson, 1975; Chandha, 1976; Bai and Kandasamy, 1985; Campos and Quilantang, 1985; Grainge *et al.*, 1985; Tripathi and Rizvi, 1985; Kandasamy *et al.*, 1987; Manalo, 1992; Rajesus *et al.*, 1993; Sahayaraj and Sekar, 1996; Sahayaraj and Paulraj, 1998c; Paulraj, 2001 and Singh *et al.*, 2002) in the laboratory and the field application of the plant extracts greatly reduced the pest population (Ramamurthy and Venugopal, 1997; Paulraj, 2001 and Sahayaraj, 2002c). *I. carnea* is a weed plant with pesticidal property (Sahayaraj *et al.*,

2003; Pandey *et al.*, 1986; Ramamurthy and Venugopal, 1997; Kulat *et al.*, 1997; Senguttuvan, 1999 and Ramamurthy and Rajaram, 2001) and field efficacy was also tested (Ramamurthy and Venugopal, 1997).

Augmentative release of the predators is a main component in the IPM and especially the reduviids play a major role in the suppression of various pests of economic importance (Schaeffer, 1988; Ambrose, 1995; 1996; 1999; 2000; Sahayaraj, 1999b; 2002c and Sahayaraj and Martin, 2003). The reduviid predator, *P. laevicollis* was released in the coconut field to reduce the grubs and adults of *O. rhinoceros* (Antony *et al.*, 1979). Sahayaraj (1999b) released *R. marginatus* in the groundnut field and observed the suppression of lepidopteran pests and reported high groundnut yield. Grundy and Maelzer (2000) released *P. plagipennis* in the pigeon pea field and reported the control of various pests in Australia. Sahayaraj (2002c) integrated certain botanicals along with *R. marginatus* in the groundnut field and obtained a good groundnut yield. Recently, Sahayaraj and Martin (2003) reported high yield of groundnut in *R. marginatus* released field. *R. kumarii* has been reported to feed on the groundnut pests in the laboratory (Sahayaraj, 1994 and Sahayaraj and Sivakumar, 1995). Ambrose and Claver (1999b) and Claver and Ambrose (2001b, c) released *R. kumarii* in cotton and pigeon pea field cages and reported the pest suppression by the predator. However, no reports are available on the release of this predator in the groundnut field and the subsequent impact on groundnut production. The present study was undertaken to find out the impact of the crude water extracts of IC and VN

and *R. kumarii* separately on groundnut pest incidence, infestation, yield and cost benefit ratio in groundnut field.

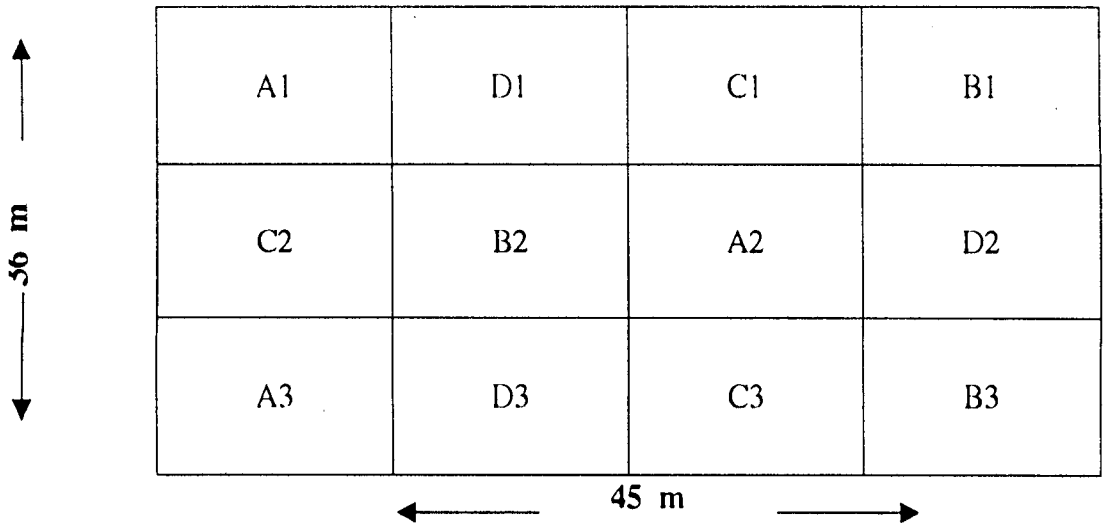
4. 2. Materials and Methods

4. 2. 1. Plot description

4. 2. 1. 1. Plant biopesticide treatment

The experiment was conducted at a farmer's field in Vellalankulam, Tirunelveli District, Tamil Nadu, India from mid January to May 2001. Groundnut (variety - TMV 7) was cultivated under well irrigation. The farmer was advised not to use pesticides or any other pest control practices. The recommended dose (gypsum - 50 - 100 kg/acre, DAP - 50 kg/acre and farm yard manure - 1 ton/acre) of fertilizers was used. From the total area under cultivation, 1620 m^2 (36 × 45m) was chosen for the present study. The chosen area was divided into 4 plots (405 m^2) (A - IC alone, B - VN alone, C - equal amount of IC + VN and D - water alone). Each plot was again divided into three subplots (130.3 m^2). Altogether there were twelve sub-plots and were arranged in a randomized block design as shown in fig. 17. Each sub-plot was separated from the adjutant sub-plot by providing one m soil bar (plate 8).

Fig. 17. Plot description for the biopesticide treatment



4. 2. 1. 2. Predator release experiment

Field experiments were conducted at Chakkamalpuram, Tuticorin District, Tamil Nadu, India from June to November 2002, in a groundnut field (variety - TMV 7) under well irrigation. The owner of the land was advised not to use pesticides or any other pest control practices. A total area of 1620 m^2 (36 x 45 m) was chosen for the present study. The area was divided into two plots (810 m^2 each) (Ia and Ib) and each plot was again divided into three sub-plots (249.26 m^2). Altogether there were six subplots (fig. 18, plate 8). Each subplot was separated from the adjacent plot by providing 1 m soil bar.

Fig. 18. Plot description for the predator released experiment

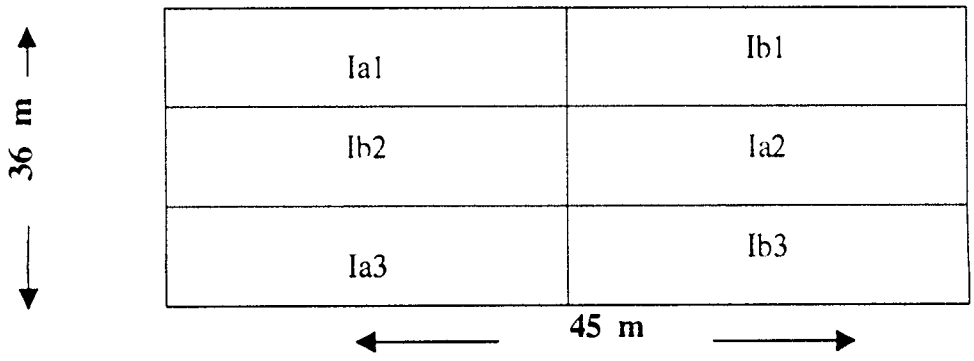


Plate. 8. Groundnut fields treated with botanicals (above) and released with *R. kumarii* (below) for experimentation

4. 2. 2. Preparation and application of plant biopesticides

The plant extract was prepared according to Nandagopal (1982) and Sahayaraj (1998a). Leaf powder (30 gm) of IC (already stored in the laboratory) was taken and mixed with 1000 ml of hot water taken in a plastic container and kept undisturbed for 24 hrs. Then it was filtered through a muslin cloth and the aqueous part was used for field application (3%). Similar procedure was followed for VN also. The plant biopesticides were sprayed on the 40^{th} and 60^{th} days after the seedling emergence (DASE) (IC, VN and IC + VN water extracts were sprayed in the sub-plots A, B and C, respectively) in the early morning hours (6:30 A.M. to 7:30 A.M.) using a hand sprayer (Amway, India) with the flow rate of 0.1 liter/min. Treatment D served as control where water alone was sprayed.

4. 2. 3. Predator release

In subplot Ia1, Ia2 and Ia3, *R. kumarii* I, II, III, IV, and IV instar nymphs and adults (360 predator in each sub-plot) were released (60 each) on the 40^{th}, 55^{th} and 65^{th} day after seedling emergence. Totally 1080 *R. kumarii* were released during the study period. The release was done during the early morning hours (6:30 A.M. to 8:30 A.M.). Sub-plot Ib1, Ib2 and Ib3 served as control where no predator was released. On the release day, the laboratory reared 24 hrs starved *R. kumarii* were taken in plastic containers (2 L capacity), covered well with lids perforated with small holes for aeration. They were transported to the release spot by bus and no food was provided and duel care was taken during the transportation. The predators were

released from the containers using a camel brush (20 cm) beneath the topmost five compound leaves.

4. 2. 4. Sampling of pests and their infestations

The sampling of the pest was done by visual observation and expressed in number of pests/plant. For the infestation by the pests, the uppermost 10 leaves were considered (Amin, 1983) and 30 plants were counted randomly in each subplot. The sampling was done four days before and after the plant biopesticide spraying and release of the predators.

4. 2. 5. Groundnut production and Cost Benefit Ratio (CBR)

On the harvest day, 30 plants were selected randomly from each subplot and one pod, two pod and three pods in each plant was recorded. After the harvest, the production of groundnut from each sub plot was estimated and expressed in $Kgha^{-1}$. The CBR was also worked according to Kalyanasundaram *et al.* (1994).

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

4. 2. 6. Percent avoidable loss

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

$$\text{Percent avoidable loss} = \frac{\text{Mean yield from protected plots} - \text{Mean yield from unprotected plots}}{\text{Mean yield from protected plots}} \times 100$$

4. 2. 7. Statistics

ANOVA was carried out using SYSTAT and STATISTICA computer package and were interpreted.

4. 3 Results

4. 3. 1. Pest incidence

In the botanicals treated field, the predominant pest observed was *Aphis craccivora* Koch (24.66, 25.12, 24.33 and 24.66 per plant for control, VN, IC and VN + IC plots for the 36th DASE, respectively) followed by *S. litura* (0.65/plant in all the treatments in the first count), *H. armigera* (0.25/plant in all the treatments in the first count) and grass hoppers like *Atractomorpha crenulata* Fab. and *Chrotogonus trachypterus* Blanch (0.12/plant for all the treatments in the first count). The incidence of all the pests gradually decreased after the application of the botanicals. Reduction in *A. craccivora* population (24.58, 16.36, 12.50/plant for VN, IC and VN + IC plots, respectively) was observed and were significant ($P < 0.05$; $P = 0.0006$) than the control. *S. litura* (0.48, 0.36 and 0.24/plant for VN, IC and VN+IC plots respectively) population was also statistically significantly ($P < 0.05$; $P = 0.0006$) reduced than that of the control (0.87/plant). The incidence of pests coincided with the application of botanicals (table 23).

The occurrence of the pest in the experimental field where predators were released is shown in table 24. Initially, the occurrence of *A. craccivora* (48.66/plant), *S. litura* (0.40/plant), *C. trachyterus* (0.06/plant), *A. crenulata* (0.04/plant), *M. pustulata* (0.70/plant) and *H. armigera* (0.15/plant) was maximum and after the release of the predators, their population was

Table 23. Impact of *I. carnea* (IC) and *V. negundo* (VN) water extracts on the incidence of groundnut pests (No. /plant)

Pests	Treatment	DASE				Mean	Significance
		36	44	56	64		
S. litura	Control	O.65	0.70	0.80	0.87	0.76	
	VN	0.65	0.59	0.64	0.48	0.59	S
	IC	0.65	0.49	0.58	0.36	0.52	S
	VN+IC	0.65	0.37	0.45	0.24	0.42	S
Grasshoppers	Control	0.12	0.12	0.13	0.13	0.12	
	VN	0.12	0.10	0.12	0.11	0.11	IS
	IC	0.12	0.11	0.12	0.12	0.11	IS
	VN+IC	0.12	0.10	0.11	0.09	0.10	IS
H. armigera	Control	0.25	0.28	0.32	0.36	0.30	
	VN	0.25	0.20	0.25	0.22	0.23	S
	IC	0.25	0.20	0.25	0.20	0.22	S
	VN+IC	0.25	0.20	0.24	0.18	0.20	S
A. craccivora	Control	24.66	34.58	49.42	63.16	42.96	
	VN	25.12	20.66	36.41	24.58	26.70	S
	IC	24.33	18.52	28.64	16.36	21.97	S
	VN+IC	24.66	16.36	24.83	12.50	19.59	S

S - Significant and IS - Insignificant (0.05 level)

Table 24. Impact of *R. kumarii* on the incidence of groundnut pests (No./plant)

Pests	Treatment	DASE						Mean	Significance
		26	34	41	49	56	64		
S. litura	Control	0.40	0.50	0.60	0.60	0.60	0.50	0.52	
	Experimental	0.40	0.20	0.40	0.30	0.30	0.10	0.28	S
C. trachypterus	Control	0.06	0.06	0.07	0.07	0.07	0.07	0.06	
	Experimental	0.06	0.04	0.05	0.04	0.05	0.03	0.04	IS
A. crenulata	Control	0.04	0.04	0.05	0.06	0.06	0.06	0.05	
	Experimental	0.04	0.03	0.05	0.03	0.04	0.03	0.04	IS
M. pustulata	Control	0.70	0.70	0.80	0.80	0.80	0.80	0.77	
	Experimental	0.70	0.70	0.80	0.80	0.80	0.80	0.77	IS
H. armigera	Control	0.15	0.18	0.20	0.21	0.23	0.25	0.20	
	Experimental	0.15	0.09	0.14	0.08	0.16	0.07	0.12	S
A. craccivora	Control	48.66	51.43	58.76	62.84	71.66	59.43	58.80	
	Experimental	48.66	25.33	38.83	19.33	50.16	24.28	34.31	S

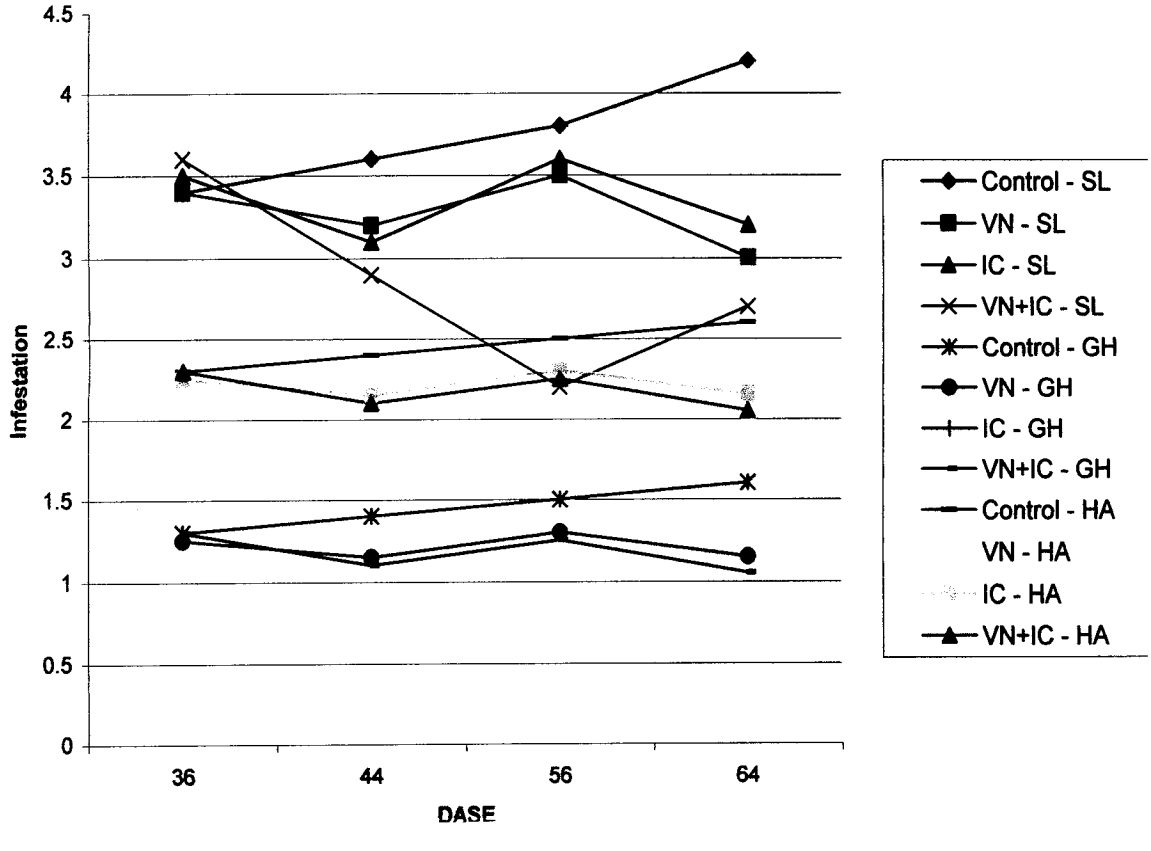
S - Significant and IS - Insignificant (0.05 level)

drastically reduced, especially that of *A. craccivora* (24.28) at 64 DASE and was highly significant ($P < 0.05$; $P = 0.0083$) than that of the control (59.43/plant). Significant reduction in the *S. litura* ($P < 0.05$; $P = 0.0086$) and *H. armigera* ($P < 0.05$; $P = 0.0077$) population were also observed. But the release of the predator did not alter the population of *M. pustulata* (0.77 for both control and reduviid released field, respectively) and eventhough it reduced the population of *C. trachyterus* and *A. crenulata* they were not statistically significant at 5% level. *R. kumarii* reduced 66.66, 40, 25, 0, 56.25, and 51.59% of *S. litura*, *C. trachyterus*, *A. crenulata*, *M. pustulata*, *H. armigera* and *A. craccivora* population, respectively.

4. 3. 2. Pest infestation

The infestation of three groundnut pests viz., *S. litura*, *H. armigera* and grasshoppers in the botanicals treated field and control were observed and are shown in fig. 19. In general, the infestation due to *S. litura* was higher (3.4, 3.5, 3.5 and 3.6 in the control, VN, IC and IC + VN plots respectively for the 36th DASE) than other two pests. The infestations due to the pests were high before the application of the botanicals and after the application of both IC and VN water extracts, it was greatly reduced and are statistically significant than control ($P < 0.05$). For instance, the infestation due to *H. armigera* before the application was 1.25/plant and after the application of VN, it was reduced to 1.15/plant. Both the botanicals greatly minimized the mean infestation of the pests solely and in combination, a great reduction in the infestation of the leaves was observed (2.85, 1.17 and 1.17 for *S. litura*,

Fig.19. Infestation in groundnut crop (No. of leaves/plant) before and after botanical application



GH – Grass Hoppers
IC – *I. carnea*
VN – *V. negundo*
HA - *H. armigera*
SL – *S. litura*

grass hoppers and *H. armigera*, respectively) and they are statistically significant to their respective control ($P < 0.05$).

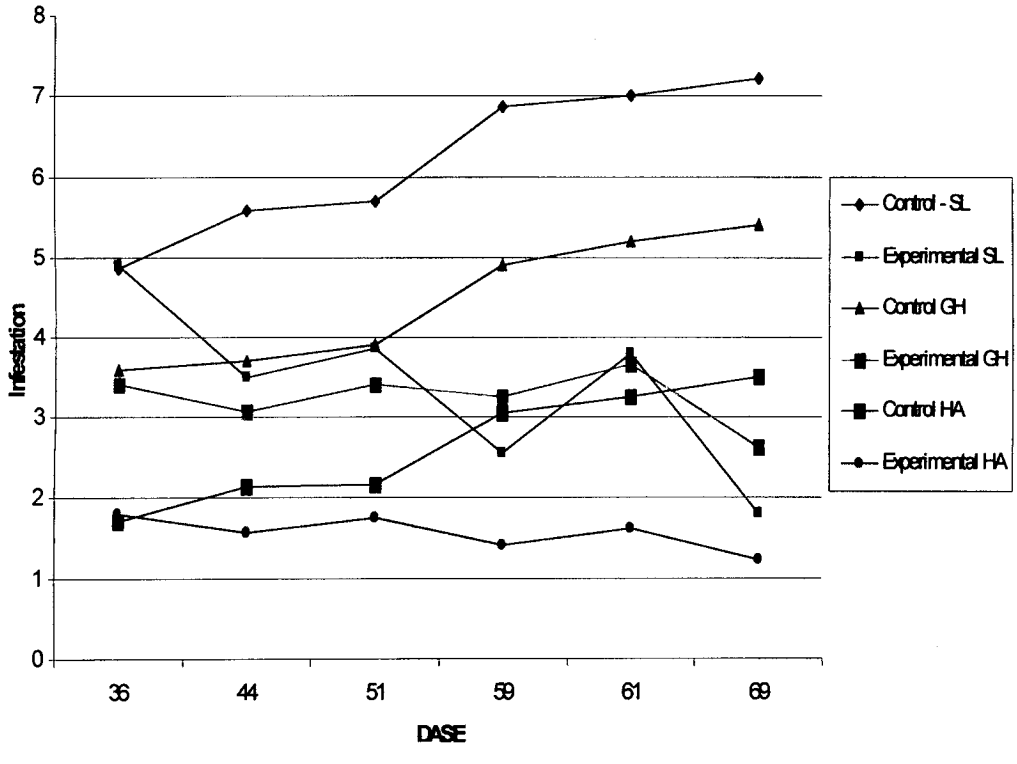
In the predator released field, the *S. litura* infestation was higher (mean 6.20 and 3.4 leaves/plant for control and experimental, respectively) followed by grasshoppers (4.45 and 3.23 leaves/plant for control and experimental respectively) and *H. armigera* (2.63 and 1.56 leaves/plant for control and experimental respectively). After the release of *R. kumarii* a significant ($P < 0.05$; $P = 0.007$, 0.0048 for *S. litura* and *H. armigera* respectively) reduction in the infestation of the *S. litura and H. armigera* were observed (fig. 20). A significant reduction in the grasshopper infestation was also observed ($P < 0.05$). The predator greatly reduced the infestation of lepidopteran pests than the orthopteran pests.

4. 3. 3. Yield and cost benefit ratio

In all the treatments, two pods was more than one pod and three pods. In the VN (0.13 ± 0.18 pods/plant) and IC + VN (0.46 ± 0.35 pods/plant) plots, three pods were also observed. Number of two pods/plant was maximum in the botanicals applied plots (14.9 ± 0.41, 16.16 ± 0.24 and 17.60 ± 0.32 pods/plant in the VN, IC and IC + VN plots, respectively) and are highly significant ($P < 0.05$) than that of control (11.53 ± 0.24 pods/plant) (table 25).

The production of groundnut was also higher in the botanicals treated plot, especially in the IC+VN plot (1386.80 $kgha^{-1}$) and is statistically significant ($P < 0.05$, $P = 0.04$) and lower in the control plot (1060.50

Fig.20. Infestation in groundnut crop (No. of leaves/plant) before and after predator release



GH – Grasshoppers HA – *H. armigera*
SL – *S. litura*

Table 25. Pod yield of groundnut in *V. negundo* (VN), *I. carnea* (IC) application and *R. kumarii* (RK) released field

Treatment	Plant/ Predator	1 Pod	2 Pod	3 Pod	Total no. of pod
Plant biopesticide	Control	2.13 ± 0.30	11.53 ± 0.24	-	13.66
	VN	1.60 ± 0.16	14.90 ± 0.41	0.13 ± 0.18	16.63
	IC	1.60 ± 0.16	16.16 ± 0.24	-	17.76
	VN + IC	0.8 ± 0.16	17.60 ± 0.32	0.46 ± 0.35	18.86
Predator	Control	3.0 ± 0.33	10.32 ± 0.90	-	13.32
	RK	1.94 ± 0.60	18.83 ± 0.33	-	20.77

Table 26. Production of groundnut and cost benefit ratio with different treatments

Treatments	Plants/Predator	Production (Kg/ha)	Cost Benefit Ratio
Plant biopesticide	Control	1060.50	1:1.43
	VN	1270.50	1:1.71
	IC	1312.50	1:1.76
	VN + IC	1386.50	1:1.87
Predator	Control	1041.0	1:1.31
	RK	1655.50	1:2.09

VN - *Vitex negundo*

IC - *Ipomea carnea*

RK - *Rhynocoris kumarii*

kgha^{-1}) (table 26). Likewise, the cost benefit ratio was also high in the IC + VN (1:1.87) followed by IC (1:1.76), VN (1:1.71) and control (1:1.43), respectively (table. 26). The botanicals greatly increased the pod yield and production than the control. As observed in the plant biopesticide treatment, the pod yield was higher in the predator released plot and two pods were higher in both the plots (10.32 in control and 18.83 in experimental) than one pod (3.0 in control and 1.94 in the experimental, respectively) (table 25). The production of groundnut was also higher in the experimental plot (1655.50 kgha^{-1}) than the control (1041.0 kgha^{-1}) and is statistically insignificant ($P > 0.05$; $P = 3.32$) (Table 26). The cost benefit ratio was also high in the experimental plot (1:2.09) (table 26).

4. 3. 4. Percent avoidable loss

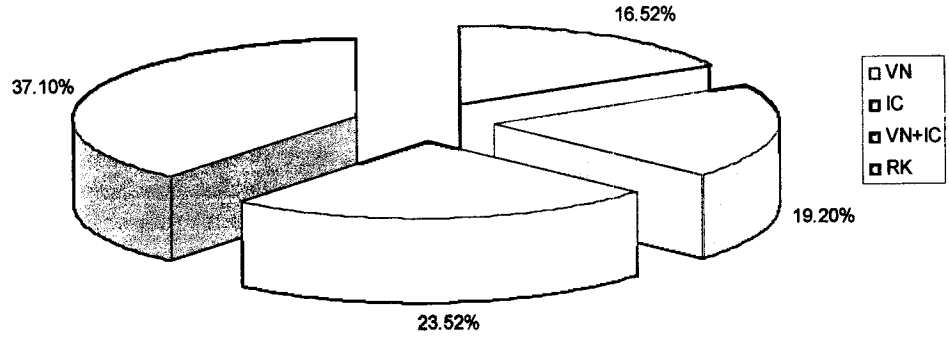
The percent avoidable loss was higher (37.10%) in predator released field, followed by VN + IC (23.52%), IC (19.20%) and VN (16.52%) respectively (fig. 21).

4. 4. Discussion

4. 4. 1. Plant biopesticide

The efficacy of various plant products was tested against different groundnut pests and the field application revealed that the botanicals greatly reduced the pest incidence and their infestation (Kalyanasundaram *et al.*, 1994; Senguttuvan, 1999; Obulapathi *et al.*, 2000; Paulraj, 2001; Sahayaraj, 2002c and Selvaraj, 2002). Plant products such as neem, vitex, pongamia and calotropis were applied in the field and they greatly reduced the infestation of *S. litura*, *H. armigera* and *A. modicella* and their population, there by greatly

Fig.21. Percentage avoidable loss in *I. carnea* (IC) and *V. negundo* (VN) treated and *R. kumarii* (RK) released fields



increased the groundnut production and cost benefit ratio (Paulraj, 2001 and Sahayaraj, 2002c). The production of groundnut and cost benefit ratio was high in the botanicals treated groundnut field (Selvaraj, 2002). The present study also revealed the same concept (i.e.) reduction in pest occurrence and increase in the groundnut production. The results were in concordance with the study of Kalyanasundaram *et al.* (1994); Paulraj (2001); Sahayaraj (2002 c) and Selvaraj (2002).

4. 4. 2. Predator release

Augmentative release of the predators, especially the reduviids is a major concept in IPM (Ambrose, 1999; Sahayaraj, 1999b, 2002c and Sahayaraj and Martin, 2003). *R. marginatus* was released in the groundnut field and it greatly suppressed the pests such as *S. litura* and *H. armigera* and there by increased the groundnut production (Sahayaraj, 1999b, 2002c and Sahayaraj and Martin, 2003). Claver and Ambrose (2001a) released *R. kumarii* in cotton field and it significantly reduced the lepidopteran pests. The field cage release of *R. kumarii* also suppressed the pest population (Ambrose and Claver, 1999b).

The field release of *P. plagipennis* (Grundy and Maelzer, 2000) and *P. laevicollis* (Antony *et al.*, 1979) were successful in reducing various pests in their released fields. Sahayaraj (2002c) integrated *R. marginatus* along with some botanicals in groundnut field and he observed the drastic reduction of pests such as *S. litura* and *H. armigera* and achieved a high yield of groundnut. In the present study, *R. kumarii* suppressed various pests in the

groundnut field as it was reported in the laboratory studies (Ambrose, 1996 and Claver, 1998). But it did not reduce the population of *M. pustulata.* But Ambrose (1999) reported that it feeds on *M. pustulata* in the laboratory. Since *R. kumarii* is a sluggish flighter than *M. pustulata,* it may be difficult to attack that in the field. In the present study, *R. kumarii* greatly suppressed the population of *S. litura, A. craccivora* and *H. armigera* (66.66, 51.59 and 56.25% respectively) and the infestation due to *S. litura, H. armigera* and grasshoppers in the groundnut crop. Furthermore, it enhanced the production of groundnut and cost benefit ratio and it is in agreement with the findings of Sahayaraj (1999b and 2002c) and Sahayaraj and Martin (2003) on *R. marginatus* released groundnut field. So *R. kumarii* could be used as a biological control agent against groundnut pests.

4. 5. Conclusion

The water extract of both the plants (IC and VN) and the life stages of the reduviid predator *R. kumarii* greatly reduced the pest population and their infestation and in turn increased the number of pods, production and cost benefit ratio. The farmers cultivating groundnut can use both the plants, and the reduviid to check the pest population and their infestation rather than using synthetic pesticides.

SUMMARY

- Laboratory mass rearing of two harpactorine reduviids namely, *Rhynocoris longifrons* Stal and *Rhynocoris kumarii* Ambrose and Livingstone was performed in the laboratory and different life stages of *R. kumarii* was released in the groundnut field. *Vitex negundo* Linn. and *Ipomea carnea* Jacq. leaf extracts were tested against *Spodoptera litura* Fabricius larvae in the laboratory and field application was made in the groundnut crop.

- Ten and twenty predators/1 L container with two alive *Corcyra cephalonica* Stainton for *R. longifrons* and *R. kumarii* respectively is the suitable predator-prey ratio for the laboratory mass multiplication.

- Heat killed *C. cephalonica* larvae enhanced the growth of both the reduviids, thereby reduced the developmental period and increased the fecundity and longevity. Frozen *C. cephalonica* larvae increased the developmental period and decreased the fecundity and longevity.

- Alive *S. litura* larvae further reduced the developmental period and increased the fecundity and longevity.

- *R. longifrons* and *R. kumarii* preferred *S. litura* and consumed in more numbers than *Helicoverpa armigera* Hubner.

- Secondary metabolites such as tannins, flavonoids, triterpenoids, phenols, lignins etc. were present in both *I. carnea* and *V. negundo*. Their distribution was different in different solvents.

- Among the three secondary metabolites quantified in both *I. carnea* and *V. negundo*, phenolic compounds constitute the higher content followed by flavonoids and tannins.

- GC-MS study of the extracts showed the presence of compounds such as 1H-Indene, Cyclododecanol, Patchoulane, 1,2-Dihexylcyclopropene-3-carboxylic acid, 2-Heptenoic acid, (+) - Aromadendrene, Trans-caryophyllene, 7-Oxabicyclo (4.1.0) heptane, Cyclohexane, Farnesol, Pentadecane and 1-Octanol in the extacts of *V. negundo* and Neophyadiene, 1-Decanol, Tetradecanoic acid, Pentadecane, 1-Iodo-2-methylundecane, Trans-caryophyllene, Eicosane, 2-Butenoic acid and Cholestan-3-one in the extracts of *I. Carnea.*

- The dose required to cause 50% mortality (LD_{50}) of *S.litura* third instar larvae was low in *I. carnea* chloroform extract and high in *V. negundo* water extract and in general, the chloroform extract of both the botanicals had more impact than the other two solvent extracts.

- All the solvent extracts increased the larval and pupal period and decreased the pupal weight and adult longevity and caused a lot of deformities in the larval and pupal forms of *S. litura.* They also altered the polypeptides of the haemolymph protein and teared and weared the mandibles of the larvae.

- Field release of *R. kumarii* and application of crude water extracts of *I. carnea* and *V. negundo* reduced the incidence and infestations of the groundnut pests such as S. litura, *H. armigera*, grasshoppers and *Aphis craccivora* Koch and significantly increased the yield of groundnut and cost benefit ratio.

Recommendations

RECOMMENDATIONS

- *R. kumarii* is found to be a potential predator on groundnut pest management and its laboratory multiplication is simple and economical, it could be used in the groundnut pest management and the farmers can make use of the technology.

- The benzene, chloroform and water extracts of both *I. carnea* and *V. negundo* have pesticidal property and they can be explored and biopesticides may be manufactured using the two botanicals.

- The farmers can use the crude water extract of both *I. carnea* and *V. negundo* to manage the groundnut pests.

Future areas of Research

FUTURE AREAS OF RESEARCH

- Mass multiplication of *R. kumarii* and *R. longifrons* using still low cost techniques either by using factitious host or oligidic diets.

- Conservation of both the reduviids through augmentation programme.

- Isolation and identification of the exact bioactive compound present in both *I. carnea* and *V. negundo* and its pesticidal property.

- Biological control potential of these reduviids and botanicals at farmers level.

- Impact of both the botanicals on the predators, beneficial arthropods in general and reduviids in particular are essential.

- Combined effect of IPM components and the role of reduviids and botanicals in the IPM of groundnut could be explored.

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