

# ABSTRACTS OF COMPLETED RESEARCH PROJECTS

*A compilation*



**ICAR - NATIONAL BUREAU OF AGRICULTURAL INSECT RESOURCES  
BENGALURU - 560 024, KARNATAKA, INDIA**



**ABSTRACTS OF COMPLETED RESEARCH PROJECTS –**

*A Compilation*

(Indian Council of Agricultural Research)

**Abstracts  
of  
Completed Research Projects**



**ICAR-National Bureau of Agricultural Insect Resources**

**P. B. No. 2491, H. A. Farm Post, Hebbal,**

**Bengaluru – 560 024**

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## **FOREWORD**

The growing concern for sustainable food production to abridge the yawning gap between production and demand, coupled with environmental safety has warranted a paradigm shift from conventional farming practices to integrated intensive farming systems with reliance on conservation of biodiversity, use of biocontrol agents, biopesticides, botanicals and useful microbial organisms for effective crop protection to sustain productivity. The ICAR-NBAIR (formerly NBAII and erstwhile PDBC) is a nodal institute for research and development to harness the rich biodiversity of useful insects, nematodes and other associated microorganisms for enhancing agricultural productivity. With passing of time, the Bureau has emerged as a front runner in insect biosystematics and barcoding and is therefore the designated insect repository of the country.

Through the years 1987-2015, the institute has carried out several research projects to address the subjects stated above encompassing the newer technologies like molecular biology, nanotechnology and information technology. The abstracts of the projects completed are testimonies to the accomplishments made by all the scientists. I wholeheartedly congratulate every one of them (both superannuated and currently working) and the officer-in-charge PME cell, for bringing out this compilation. The abridgement would enable researchers to ponder over the challenges ahead and formulate befitting programmes.

**(Abraham Verghese)**

**Dated the 6<sup>th</sup> February, 2016  
Bengaluru**

## PREFACE

The climate change, depleting resource base and onslaught of pests and diseases have become a major impediment for sustainable crop production. Consequently, the integrated intensive farming systems with reliance on ecologically sound management practices *viz.*, conservation of biodiversity with habitat management, biological control, use of semiochemicals, biopesticides and botanicals have gained significance. The impetus for all these endeavours, began with the setting up of All India Co-ordinated Research Project on Biological Control of Crop Pests and Weeds during 1977 and establishment of Biological Control Centre in 1988 and the Project Directorate of Biological Control in 1993. The Project Directorate of Biological Control was upgraded as National Bureau of Agricultural Important Insects (NBAII) during 2009, to act as a nodal agency for collection, characterization, documentation, conservation, exchange and utilization of agriculturally important insect resources (including mites and spiders) for sustainable agriculture. In the twelfth five year plan, the Bureau is re-christened as ICAR-National Bureau of Agricultural Insect Resources (ICAR-NBAIR). The ICAR-NBAIR acts as a hub of network of institutions spread across the country to harness diversity of beneficial insects and associated microorganisms for enhancing agricultural productivity.

Keeping in view, the challenges ahead in sustaining agricultural productivity without degrading the resource base, the institute (as Project Directorate and as National Bureau) had envisaged a blend of basic and applied research projects to meet the requirement of the country and the farming community in particular. During the period 1987 through 2015, a total of 90 research projects cutting across the science of insect taxonomy, biodiversity, biological control, mass production techniques of biocontrol agents, biopesticides, bio-informatics, molecular biology, nanotechnology and quarantine, were ably handled by 31 scientists at the institute and successfully concluded.

The anthology of abstracts of the completed research projects provides an insight in to the research arena of the institute mandate, the accomplishments made and the technologies generated over the years. The compilation would add to our existing knowledge and throw light on the impending future researchable issues to embark upon.

The Priority setting, Monitoring and Evaluation cell gratefully acknowledges all the scientists for the information on the projects and thank the Director, ICAR-NBAIR for the invaluable suggestions in bringing out this omnibus.

**Dated the 6<sup>th</sup> February, 2016  
Bengaluru**

**PME cell  
ICAR-National Bureau of Agricultural Insect Resources  
Bengaluru**

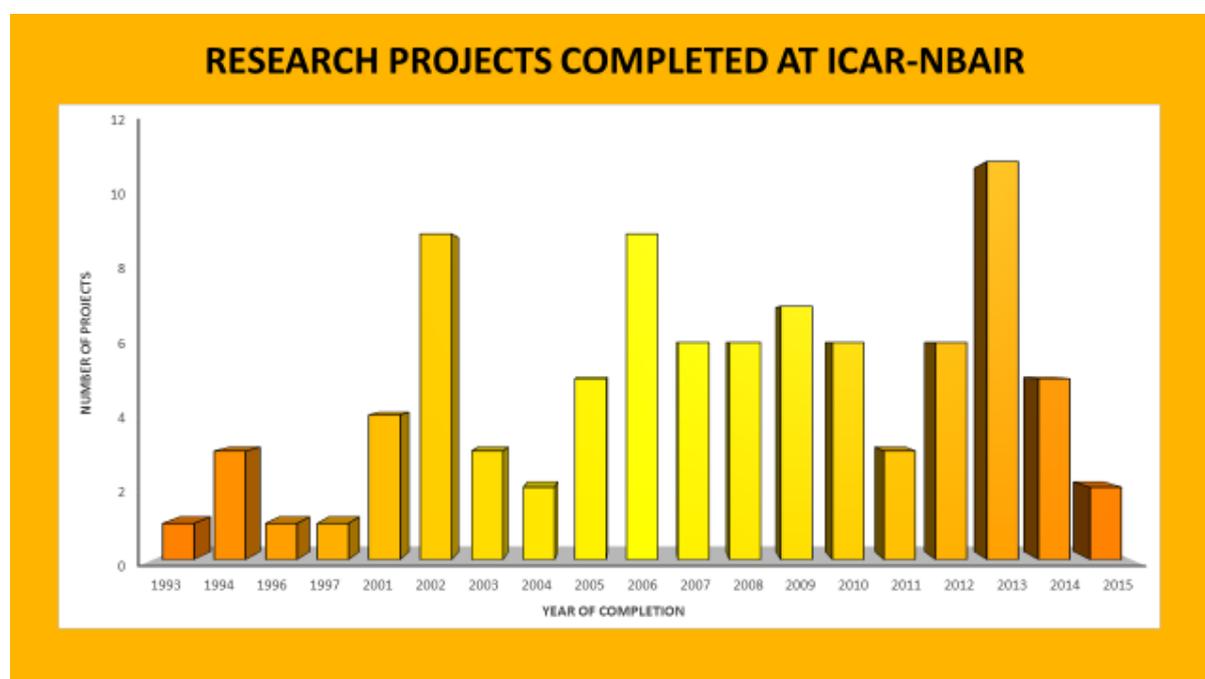
## Abstracts of Research Projects Completed at ICAR-NBAIR

### *A Compilation*

The ICAR- National Bureau of Agricultural Insect Resources is a nodal institute for the collection, characterisation, documentation, conservation, exchange and utilization of agriculturally important insect resources (including mites, spiders and related arthropods) for sustainable agriculture.

The research Projects were initiated in the year 1987 under the All India Co-ordinated Research Project on Biological Control of Crop Pests and Weeds. Subsequent to the establishment of Biological control centre in 1988, Project Directorate of Biological Control in 1993 and upgradation as National Bureau of Agriculturally Important Insects in 2009, the institute now rechristened as NBAIR has successfully completed 90 research projects which is the fruition of dedicated work of 31 scientists.

The Projects have dealt with basic as well as applied aspects of entomological research including molecular biology, nanotechnology and information technology for minimising the crop losses and enhancing the agricultural productivity.



The research carried out has covered various aspects of agricultural pests, parasitoids, predators, entomopathogens, EPNs, weed insects, invasives and utilizing these for effective management of crop pests, diseases and weeds. Technologies have been developed for the mass multiplication several parasitoids and predators, their storage and packaging. Protocols were standardized to rear parasitoids and predators on artificial diet.

Natural enemies have been imported from different countries for the control of several crop pests and weeds and have been released against the target pest/weed after ensuring proper quarantine measures.

Development and use of insect viruses for the management of major pest complex of cruciferous crops, development of improved formulations of NPV for management of *H. armigera* and *S. litura* in tomato and long term management of red hairy caterpillar (*Amsacta albistriga*) by creating epizootics of nuclear polyhedrosis virus has been a landmark in the effective pest management.

Biological control of soil borne plant pathogens by antagonistic bacteria, development of bacterial biocontrol agents, management of bacterial wilts of tomato and brinjal caused by *Ralstonia solanacearum* through *Bacillus* spp., biological control of Alternaria leaf blight of tomato, efficient formulations of *Trichoderma* sp. and entomofungal pathogens with prolonged shelf-life, identification of *Trichoderma* isolates with enhanced biocontrol potential, biological control of plant parasitic nematodes with fungi and bacteria with special reference to *Paecilomyces lilacinus* and *Pasteuria penetrans*, nematode-derived fungi and bacteria for exploitation in agriculture, mass production and exploitation of entomopathogenic nematodes against white grubs from diverse habitats, mass production, formulation and field-testing of entomopathogenic nematodes against important lepidopterous pests, biocontrol of insect pests using entomopathogenic fungi, development of mycoinsecticides and biological suppression of plant parasitic nematodes exploiting antagonistic fungi and bacteria in specific cropping systems are some of the salient achievements through the projects.

Studies on bee pollinators in crop-ecosystems with special reference to pulses and oilseed crops, in situ conservation of natural enemies and pollinators in pigeonpea and sunflower ecosystem, host derived kairomones to enhance the efficiency of natural enemies and formulations of pheromones of important borer and other crop pests and kairomones for natural enemies using nanotechnology have helped in better usage of natural enemies. Software has also been developed for identifying and suggesting biocontrol measures of different crop-pests.

Cataloguing of insect fauna of India is the main mandate of the institute. Traditional taxonomy of different groups of insects as well as molecular characterization have been done extensively. Biosystematic studies on predatory coccinellids, development of interactive identification key for important families of insect parasitoids and predators, taxonomic studies on lesser known Coccinellidae of the Indian Subcontinent, cataloguing of insect fauna of India, with emphasis on minor orders, and biosystematics of *Trichogramma* and *Trichogrammatoidea* are great milestones in the field of traditional taxonomy of insects. Molecular characterization of trichogrammatids, *Chrysoperla carnea* and *Cryptolaemus montrouzieri*, isolation, identification and characterization of endosymbionts of trichogrammatids and their role on the fitness attributes, molecular characterization of Indian Coccinellids, development and evaluation of improved strains of trichogrammatids, *Cheilomenes sexmaculata* and *C. carnea* tolerant to insecticides, temperature and high host searching ability and molecular characterization and identification of endosymbionts of chrysopid predators and their functional role on the biological attributes are turning points in molecular characterization of insects.

Development of high temperature and insecticide tolerant strain of *Trichogramma* has been a breakthrough in the field of biological control as it enhanced their ability to fit into integrated pest management programmes across different crop ecosystems. This has enabled the farming community immensely in mitigating the use of pesticides, in addition to the conservation of biodiversity.

The abstracts of several research projects completed at the institute up to 2015 showcase the salient achievements made by the scientists during the past 26 years for the effective management of pests across various agro-ecosystems. This also paves a path for formulating future research projects on the areas less explored.

## List of Projects

Sl. No.	Project title	Name of the PI	Date of start	Date of completion	Page no.
1.	Introduction and evaluation of natural enemies of important homopterous pests	Dr. Sushil Kumar Jalali	01/04/1987	31/03/1994	9
2.	Introduction and evaluation of natural enemies of tissue borers	Dr. Chandish R Ballal	01/04/1987	31/03/1997	10
3.	Introduction and studies on natural enemies of polyphagous lepidopterous pests	Dr. Chandish R Ballal	01/04/1987	31/03/1994	11
4.	Studies on trichogrammatids	Dr. Surinder Pal Singh	01/04/1987	31/03/1994	11
5.	Studies on insect viruses/pathogens	Dr. Kandaswamy Narayanan	01/04/1989	31/03/1996	13
6.	Biosystematic studies on Chrysopidae	Dr. Uma Narasimham	01/04/1990	31/03/1993	14
7.	Development of mass production techniques for parasitoids	Dr. Thiruvengadam Venkatesan (1994 to 1996) Dr. Chandish R Ballal (1996 to 2002)	01/04/1994	01/04/2002	15
8.	Evaluation of improved and selected species/ strains of egg parasitoids	Dr. Sushil Kumar Jalali	01/04/1994	31/03/2002	16
9.	Evaluation and development of artificial diet for important lepidopterous pests	Dr. Kotilingam Srinivasa Murthy	01/04/1994	31/03/2002	17
10.	Evaluation of artificial diet, release rates and genetic improvement of important predators	Dr. Thiruvengadam Venkatesan	01/04/1996	31/03/2002	17
11.	Software development for identifying and suggesting biological control measures for vegetable crop-pests using a PC	Mr. Santi Ranjan Biswas	01/04/1994	31/03/2008	18
12.	Development of mass production techniques for predators	Dr. Sunil Joshi	04/11/1994	01/03/2001	18
13.	Survey, identification and utilization of entomopathogenic nematodes against some important lepidopterous and coleopterous pests	Dr. Syed Shahabuddin Hussaini	01/04/1996	31/03/2001	19
14.	Use of semiochemicals to improve the efficiency of important predators	Dr. Nandagopal Bakthavatsalam	01/04/1996	31/03/2002	21
15.	Biological control of plant parasitic nematodes with fungi and bacteria with special reference to <i>Paecilomyces lilacinus</i> and <i>Pasteuria penetrans</i>	Dr. Chellappa Sankarnarayanan	01/04/1996	31/03/2001	21
16.	Survey, identification and utilization of plant pathogens for the biological control of weeds with particular reference to parthenium and water hyacinth	Dr. Sreerama Kumar Prakya	01/09/1996	31/03/2003	21
17.	Biological control of soil borne and other plant pathogenic fungi by antagonistic fungi and development of biofungicides	Dr. Ravulapalli Durga Prasad	08/09/1996	08/09/2001	23

18.	Behaviour ecology of potential parasitoids to enhance their efficacy in biological suppression of key crop pests	Dr. Purshotam Lal Tandon	01/10/1996	31/03/2002	23
19.	Biological control of soil borne plant pathogens by antagonistic bacteria and development of bacterial biocontrol agents	Dr. Rajagopal Rangeshwaran	01/04/1997	31/03/2002	24
20.	Biosystematic studies on predatory coccinellids	Dr. Janakiraman Poorani	01/04/1997	31/03/2006	25
21.	Development of national information system on biological suppression of crop pests	Mr. Santi Ranjan Biswas	01/04/1997	31/03/2005	25
22.	Introduction and studies on the exotic natural enemies of some dipterous and homopterous insect pests	Dr. Basavaraj Shidlingappa Bhumannavar	01/04/1997	31/03/2002	25
23.	Introduction and studies on the exotic natural enemies of some lepidopterous insect pests	Dr. Srinivasan Ramani	01/04/1997	31/03/2002	27
24.	Software development for identifying and suggesting biocontrol measures of different crop-pests using PC	Mr. Santi Ranjan Biswas	01/04/1997	31/03/2005	28
25.	Knowledge-base system of <i>Helicoverpa armigera</i> and its natural enemies	Dr. Maria Pratheepa	01/10/1999	31/03/2003	29
26.	Decision support system of safer pesticides for natural enemies	Dr. Maria Pratheepa	01/06/2000	31/05/2003	29
27.	Biocontrol of insect pests using entomopathogenic fungi and development of mycoinsecticides	Dr. Bonam Ramanujam	01/04/2001	31/03/2004	29
28.	Biological suppression of plant parasitic nematodes exploiting antagonistic fungi and bacteria in specific cropping systems	Dr. Mandadi Nagesh	01/04/2001	31/03/2006	30
29.	Development of mass production techniques for dipteran (Diptera: Cecidomyiidae) and acarine (Arachnida: Acarina) predators for use in biological control programmes	Dr. Prashanth Mohanraj	01/04/2001	30/04/2006	31
30.	Biosystematic studies on Indian Tachinidae	Dr. Srinivasan Ramani	01/08/2001	31/08/2006	32
31.	Development and evaluation of artificial diets for <i>Opisina arenosella</i> and <i>Plutella xylostella</i> and studies on host-parasitoid interrelations	Dr. Kotilingam Srinivasa Murthy	01/04/2002	31/03/2006	33
32.	Development and formulation of artificial diets for the rearing of coccinellids and anthocorids	Dr. Thiruvengadam Venkatesan	01/04/2002	31/03/2006	33
33.	Host derived kairomones to enhance the efficiency of natural enemies	Dr. Nandagopal Bakthavatsalam	01/04/2002	31/03/2007	34
34.	Introduction and studies on the exotic natural enemies of some important crop pests and weeds	Dr. Basavaraj Shidlingappa Bhumannavar	01/04/2002	31/03/2007	34

35.	Mass production, formulation and field-testing of entomopathogenic nematodes against important lepidopterous pests	Dr. Syed Shahabuddin Hussaini	01/04/2002	31/03/2006	36
36.	Rearing and evaluation of natural enemies with special reference to scelionid, braconid, ichneumonid and anthocorid groups	Dr. Chandish R Ballal	01/04/2002	31/03/2007	37
37.	Development and use of insect viruses for the management of major pest complex of cruciferous crops	Dr. Kandaswamy Narayanan	01/04/2002	31/10/2005	38
38.	Development and evaluation of improved strains of trichogrammatids, <i>Cheilomenes sexmaculata</i> and <i>Chrysoperla carnea</i> tolerant to insecticides, temperature and high host searching ability	Dr. Sushil Kumar Jalali	01/04/2002	31/03/2007	41
39.	Herbivore induced plant synomones and their utilization in enhancement of the efficiency of natural enemies	Dr. Purshotam Lal Tandon	31/05/2002	30/04/2007	41
40.	Development of interactive identification key for important families of insect parasitoids and predators	Dr. Janakiraman Poorani	01/08/2002	31/07/2004	42
41.	Evolving and testing superior strains of <i>Steinernema</i> sp. and <i>Heterorhabditis</i> sp. against <i>Spodoptera litura</i> in field	Dr. Syed Shahabuddin Hussaini	01/09/2002	31/08/2005	43
42.	Development of improved formulations of NPV for management of <i>Helicoverpa armigera</i> and <i>Spodoptera litura</i> in tomato	Dr. Veenakumari Kamalanathan	01/12/2002	31/03/2007	44
43.	Identification of pathogens of phytophagous mites and assessment of their potential in microbial control	Dr. Sreerama Kumar Prakya	01/04/2003	30/06/2008	45
44.	Development of a data base on microbial biopesticides	Dr. Maria Pratheepa	01/07/2003	30/06/2006	47
45.	Efficient formulations of <i>Trichoderma</i> sp. and entomofungal pathogens with prolonged shelf-life	Dr. Subbaraman Sriram	01/07/2004	31/03/2009	47
46.	Evaluation of fungal pathogens against onion thrips	Dr. Bonam Ramanujam	01/07/2004	30/06/2006	48
47.	Identification of effective entomofungal pathogens for the management of sugarcane woolly aphid	Dr. Bonam Ramanujam	01/07/2004	31/03/2005	48
48.	Identification of <i>Trichoderma</i> isolates with enhanced biocontrol potential	Dr. Subbaraman Sriram	01/10/2004	31/03/2008	48
49.	Isolation, characterization and toxicity of indigenous <i>Bacillus thuringiensis</i> strains against lepidopterous pests	Dr. Rajagopal Rangeshwaran	01/11/2004	30/11/2010	48
50.	Development of novel mass production, storage, and packaging techniques for <i>Cryptolaemus montrouzieri</i>	Dr. Sunil Joshi	01/03/2005	01/03/2009	49

51.	Isolation and characterization of plant growth promoting endophytic bacteria and development of improved formulations	Dr. Rajagopal Rangeswaran	01/03/2005	31/03/2010	50
52.	Mass production and field evaluation of <i>Micromus</i> sp.	Dr. Sunil Joshi	01/03/2005	01/03/2009	50
53.	Selection of superior strain of <i>Chrysoperla carnea</i> and <i>Cryptolaemus montrouzieri</i> from different agroecosystems and their molecular characterization	Dr. Thiruvengadam Venkatesan	10/04/2006	31/03/2010	52
54.	Selection of superior strains of certain parasitoids and their characterization	Dr. Kotilingam Srinivasa Murthy	01/04/2006	31/03/2010	52
55.	Taxonomic studies on lesser known Coccinellidae of the Indian Subcontinent	Dr. Janakiraman Poorani	01/04/2006	31/03/2009	53
56.	In vitro cloning of NPV for genetic improvement	Dr. Kotilingam Srinivasa Murthy	01/04/2006	31/03/2009	53
57.	Biological control of Alternaria leaf blight of tomato	Dr. Bonam Ramanujam	01/07/2006	30/06/2010	53
58.	Database on Entomopathogenic Nematodes	Dr. Maria Pratheepa	01/07/2006	31/03/2013	54
59.	Biosystematics of <i>Trichogramma</i> and <i>Trichogrammatoidea</i>	Dr. Prashanth Mohanraj	01/07/2006	31/03/2013	55
60.	Interaction within the natural enemy guilds of <i>Ceratovacuna langera</i> and <i>Maconellicoccus hirsutus</i>	Dr. Srinivasan Ramani	01/09/2006	31/03/2008	55
61.	Biological and molecular characterization of inter and intra specific variation in trichogrammatids	Dr. Sushil Kumar Jalali	01/04/2007	31/03/2010	55
62.	Attractants for natural enemies of rice pests for use in conservation of natural enemies	Dr. Deepa Bhagat	01/04/2007	30/06/2011	57
63.	Conservation of natural enemies of Rice pests through habitat manipulation techniques	Dr. Nandagopal Bakthavatsalam	01/04/2007	30/06/2009	57
64.	Development of production protocols and evaluation of anthocorid and mite predators	Dr. Chandish R Ballal	01/04/2007	31/03/2012	58
65.	Effect of different edaphic factors on EPN activity and refinement of packaging for EPN formulations	Dr. Syed Shahabuddin Hussaini	01/04/2007	31/10/2008	59
66.	Long term Management of red hairy caterpillar ( <i>Amsacta albistriga</i> ) by creating Epizootics of Nuclear polyhedrosis virus	Dr Veenakumari Kamalanathan	01/05/2007	30/08/2008	60
67.	Formulations of pheromones of important borer and other crop pests and kairomones for natural enemies using nanotechnology	Dr. Deepa Bhagat	01/04/2008	31/03/2013	62
68.	Nematode-derived fungi and bacteria for exploitation in agriculture	Dr. Mandadi Nagesh	16/04/2008	31/03/2012	63

69.	Mass production and exploitation of entomopathogenic nematodes against white grubs from diverse habitats	Dr. Mandadi Nagesh	16/04/2008	31/03/2012	65
70.	Establishment of <i>Puccinia spegazzinii</i> on <i>Mikania micrantha</i>	Dr. Sreerama Kumar Prakya	01/07/2008	30/06/2009	66
71.	Phytophagous mites as a source of microbes for harnessing in pest management	Dr. Sreerama Kumar Prakya	01/07/2008	30/06/2011	66
72.	Management of bacterial wilts of Tomato and Brinjal caused by <i>Ralstonia solanacearum</i> through Bacillus spp.	Dr. Gopalsamy Sivakumar	01/01/2009	31/03/2013	68
73.	Molecular characterization of Indian Coccinellids	Dr. Ramasamy Gandhi Gracy	01/01/2009	31/12/2011	69
74.	Cataloguing of insect fauna of India, with emphasis on minor orders	Dr. Janakiraman Poorani	01/04/2009	31/03/2013	69
75.	Studies on bee pollinators in crop-ecosystems with special reference to pulses and oilseed crops	Dr. Sundararaju Dheravaraju	01/04/2009	31/03/2012	69
76.	Standardization of solid state fermentation conditions and development of prototypes with semi-automation for the mass production of <i>Trichoderma</i> spp.	Dr. Subbaraman Sriram	01/04/2009	31/03/2012	70
77.	Influence of elevated levels of carbon dioxide on the tritrophic interactions in some crops	Dr. Nandagopal Bakthavatsalam	01/07/2009	31/03/2014	70
78.	Polymorphism in pheromone reception in <i>Helicoverpa armigera</i>	Dr. Nandagopal Bakthavatsalam	01/07/2009	31/03/2013	70
79.	Isolation, identification and characterization of endosymbionts of trichogrammatids and their role on the fitness attributes	Dr. Sushil Kumar Jalali	01/04/2010	31/03/2013	70
80.	Molecular characterization and identification of endosymbionts of chrysopid predators and their functional role on the biological attributes	Dr. Thiruvengadam Venkatesan	01/04/2010	31/03/2013	73
81.	Studies on <i>Trichogramma brassicae</i> and <i>Cotesia plutellae</i> interaction with their host in cabbage ecosystem	Dr. Kotilingam Srinivasa Murthy	01/04/2010	31/03/2014	73
82.	In situ conservation of natural enemies and pollinators in pigeonpea and sunflower ecosystem	Dr. Timalapur Maharudrappa Shivalingaswamy	01/06/2010	31/05/2013	73
83.	Semiochemicals for the management of coleopteran pests	Dr. Nandagopal Bakthavatsalam	01/11/2010	31/03/2015	74
84.	Evaluation of fungal pathogens on <i>Aphis craccivora</i> in cowpea and <i>Bemisia tabaci</i> in Tomato and Capsicum	Dr. Bonam Ramanujam	01/10/2010	31/03/2014	74
85.	Interactions of microbial control agents in diverse soil types	Dr. Sreerama Kumar Prakya	01/10/2010	30/09/2013	75
86.	Bio-intensive management of root-knot nematode and /Fusarium disease complex in tomato and okra using PGPR	Dr. Rajkumar Manikappa Gond	22/11/2010	22/11/2013	76

87.	Studies on Thrips Components Influencing The Epidemiology Of Tospoviruses	Dr. Subbaraman Sriram	01/04/2012	11/12/2012	77
88.	Genetic diversity, biology and utilization of entomopathogenic nematodes (EPN) against cryptic pests	Dr. Mandadi Nagesh	01/04/2012	10/11/2014	77
89.	Insect vector components influencing phytoplasma diseases	Dr. Sreerama Kumar Prakya	01/04/2012	31/03/2015	78
90.	Mechanism of insecticide resistance in certain mealybugs	Dr. Thiruvengadam Venkatesan	01/04/2013	31/05/2013	80

## Abstracts of Institute Research Projects Completed at NBAIR

### A Compilation

#### 1. Introduction and evaluation of natural enemies of important homopterous pests (01/04/1987 to 31/03/1994) PI: Dr. Sushil Kumar Jalali

The parasitoid *Adelencyrtus mayurai* (Subba Rao) alone was very effective in bringing down pest population of sugarcane scale insect compared to the predator, *Sticholotis madagassa* Weise, controlling 78.8 and 53.4% scales, respectively. In all multiple combinations higher parasitism was obtained but predator *S. madagassa* population was reduced drastically.

It was evident that 5°C and 10°C were not suitable for storing adults of *S. madagassa*. However, 15°C was found to be ideal temperature. Adults could be stored up to 60 days without significant mortality. Out of seven hosts tested, *S. madagassa* was able to develop on 4 hosts. It showed marked preference for *Melanaspis glomerata* (Green) egg, larval and pupal period were shorter on it. Similar daily consumption of scale was @ 30/adult, which was much higher than other accepted hosts. Predator readily fed and accepted *Qudraspidiotus perniciosus* (Comstock) completing egg, larval and pupal period in 6.5, 22.3 and 7.9 days respectively. On other acceptable hosts, viz., *Hemiberlesia lataniae* (Signoret) and *Aonidiella aurantii* Maskell no egg laying was observed for 45 days.

It was observed that *A. aurantii* did not lay eggs in absence or in low density host population, though it continued to feed on host or adult food available. Per cent mortality in absence of the host was 4.0, 15.0, 54.0, 85.0, 93.1 and 100 in 7, 15, 21, 30, 45 and 60 days whereas corresponding mortality of low density and high density host was 0.0, 4.4, 12.0, 21.2, 39.3, 59.9 per cent and 0.0, 0.0, 0.0, 10.0, 20.4 and 53.0 per cent, respectively.

Screening of pesticides against *Aphytis proclia* Walker dimethoate, phosphamidon and monocrotophos caused 86.6, 92.0 and 96.6 per cent mortality within one hour of exposure and subsequently 100% after 2 hours. Fenvalerate inflicted 38.0, 62.0, 97.6 and 100 per cent in 1, 2, 6 and 24 hours of exposure, respectively. All fungicides tested were found to be safe. A low mortality was obtained on Bavistin (26.0%), Dithane M 45 (28.0%), Foltof (27.0%), Baycor (14.0%), Fytolan (16.0%) and Captan (30.0%) after 24 hours of exposure compared to control with no mortality. All fungicides were found to cause less mortality, but parasitism obtained was significantly lower than control batch. It was concluded that fungicides have some repellent action which inhibits parasitoid to parasitize host scale insect.

*A. proclia* accepted two out of six hosts tested, the per cent parasitism on *Q. perniciosus* was 86.6% and on *H. lataniae* 32.3%. The developmental period varied significantly with the host *Chilocorus bijugus* Mulsant. It developed faster (33.5 days) on *Q. perniciosus* than on *M. glomerata* (41.4 days); *Chilocorus nigrita* (Fabricius) (32.3 days) on *Q. perniciosus* to *H. lataniae* (35.1 days).

*Heteropsylla cubana* Crawford was recorded in severe form on almost all varieties of subabul and in all locations where observations were recorded. Per cent infestation varied from 80.0-100.0 per cent. The extensive sucking by adults and nymphs resulted in defoliation and in severe attack plants die. From the infested field a number of predators like mantids, spiders, *Cheilomenes sexmaculata* (Fabricius) and a species of *Chrysoperla* were recorded. *Curinus coeruleus* (Mulsant) was released in Hebbal plantation at five spots. During November to December 1988, 2018 grubs were released in 4 spots in an area of 100 m<sup>2</sup>. Recovery studies were carried out after 4 months after making releases. At Hebbal, during March - April 1989 observation revealed presence of 20-30 adults per tree at random sampling of 10 trees at each spot. Observation was also recorded after 6 months on its dispersal. It was observed that adults have moved in 2½ sq. km area in about 6-8 months.

During the year 1989, *H. cubana* population was very high during the months August, September and October. Pest population started declining after increase in population of introduced coccinellid predator *C. coeruleus*. Pest population remained low from Nov., 1989 to August-1990. It started building up again after Sep. 1990 and remained high till Nov. 1990. However, pest population was significantly low during 1990 in comparison to 1989. Population of *C. coeruleus* also fluctuated in different months. High population was recorded from September 1989 to March 1990 and

population declined sharply with low pest population. Again during August 1990 population build up was noticed and it remained high till March, 1991. Thus, *C. coeruleus* was able to suppress high *H. cubana* population. In life-table studies, egg laying started after 19 days of pre oviposition period and occurred up to 67 days after emergence. Peak egg laying period was between 23<sup>rd</sup> to 41<sup>st</sup> day. Net reproductive rate ( $R_o$ ) was 155.36,  $T_c$  37.123,  $r_c$  0.1359,  $r_m$  0.1261,  $T$  40.00 and  $\lambda$  1.134. Average longevity of female adult was 45.67 day with minimum 34.0 and maximum 73.0 days and sex ratio (male: female) was 1: 1.02.

## **2. Introduction and evaluation of natural enemies of tissue borers (01/04/1987 to 31/03/1997) PI: Dr. Chandish R Ballal**

The project covered studies on natural enemies of tissue borers viz., *Chilo partellus* (Swinhoe), *Chilo infuscatellus* Snellen, *Chilo auricilius* Dudgeon and potato tuber moth (PTM) *Phthorimaea operculella* (Zeller). Adult females of the exotic parasitoid *Allorhogas pyralophagus* Marsh could be stored for 20 days at 5°C and at 10°C for up to 50 days and maximum progeny production was by the females emerging from cocoons stored for 7 days at 5°C. Fecundity of adults from cocoons stored at 10°C reduced when stored beyond 28 days. There were no females among the progeny in the case of storage at 5°C and 10°C for 35 days. Host preference studies were conducted on *A. pyralophagus*. Percent parasitism on different hosts was recorded as 37.41 on *C. partellus*, 39.35 on *C. infuscatellus*, 38.48 on *C. auricilius* and 17.38 on *Corcyra cephalonica* (Stainton). This experiment proves the efficiency of the host insects mentioned above, except *C. cephalonica* as laboratory hosts for rearing *A. pyralophagus*. Insecticides, dimethoate, phosphamidon, endosulfan and fenitrothion were highly toxic to *A. pyralophagus* adults, followed by decamethrin and cypermethrin. Fenvalerate and monocrotophos were moderately toxic, both caused 51.5% mortality followed by oxydemeton methyl (23.3%). Phosalone and the fungicides Mancozeb and Zineb were safe to *A. pyralophagus*, while dicofol was the safest. Interaction studies between *A. pyralophagus* and *Cotesia flavipes* (Cam.) showed that *C. partellus* larvae parasitised by *A. pyralophagus* were not at all preferred for parasitisation by *C. flavipes*. However, larvae parasitised by *C. flavipes* were pricked by *A. pyralophagus*. Cocoons of only one parasitoid could be obtained till the time interval between the exposures to the two parasitoids was 11 days. In one instance, when the larva parasitised by *C. flavipes* was exposed to *A. pyralophagus* after 13 days, cocoons of both parasitoids could be obtained on a single larva.

Among six laboratory hosts tested, the egg-larval parasitoid *Chelonus blackburni* Cameron completed its development in only three hosts, viz., *C. cephalonica*, *P. operculella* and *Achroia grisella* (Fabricius). Adults of *C. blackburni* could be stored for 10 days at 5°C and 30 days at 10°C. Age specific fecundity showed that maximum fecundity of *C. blackburni* was recorded on the first day of exposure (38.7) after which there was a steep fall (2.7) on the second day. The parasitoid was capable of laying eggs throughout its life time and the mean fecundity was observed to be 206.5.

An artificial diet for *C. partellus* was developed and standardised. The mean percent survival in the artificial diet was 70% in comparison to about 50% when fed on natural diet. The performance of the field collected larvae on natural diet and on artificial diet was compared. Though there was no reduction in pupae weight from the first generation on artificial diet to the fifth generation, a gradual reduction in longevity and fecundity was observed due to continuous rearing on artificial diet. The effect of storage of artificial diet on percent survival was studied. When compared to fresh diet, storage for even up to 10 days did not cause any significant reduction in the survival of *C. partellus* larvae transferred into the vials containing stored artificial diet. The cost of producing the semi-synthetic diet which can be used to rear about 350-400 larvae was found to be Re. 45.27. Considering the average per cent survival upto pupation from hatching to be about 70, the cost of producing one *C. partellus* pupa was calculated as Rs.0.16 - 0.18.

Studeis were conducted to determine the optimum host parasitoid ratio for the laboratory multiplication of *Copidosoma desantisi* Annecke & Mynhardt, exotic parasitoid of PTM. At 50 eggs per parasitoid, maximum number of mummies was obtained, which ranged from 23 to 29 per parasitoid. Mummy formation was reasonably good (range 9 to 17.5) in the first four exposures. The 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> exposures yielded lesser number of mummies ranging from 4.5 to 7.0 mummies. From the eighth exposure there was a drastic reduction in the number of mummies formed.

The Indonesian strain of *C. flavipes* obtained under import permit from Texas, USA, during July 91 was maintained successfully on *C. partellus* under quarantine conditions for more than 12 generations. The number of adults produced per bunch of cocoons varied from 60-80. In the case of Indian strain, continuous rearing was possible for only two generations. The number of adults per bunch was found to vary from 24-56 and the males were generally found to be more than the females.

Studies were conducted on the parasitizing ability (measured in terms of percent larvae producing parasitoid adults) of *C. blackburni* (reared on *P. operculella* and *C. cephalonica*) on *Helicoverpa armigera* (Hübner) eggs on some selected host plants. Parasitism was generally low, with the maximum being on tomato whether the adults were reared from *C. cephalonica* or *P. operculella*. The results indicated that generally per cent parasitism on *H. armigera* was very low whether the adults of *C. blackburni* reared from *P. operculella* or *C. cephalonica* were used.

### **3. Introduction and studies on natural enemies of polyphagous lepidopterous pests (01/04/1987 to 31/03/1994) PI: Dr. Chandish R Ballal**

Studies were focused on the natural enemies of some important lepidopteran pests. A time-temperature schedule was formulated to break diapause in prepupae of the exotic braconid *Cotesia kazak* (Telenga). The preferred host plants of the exotic parasitoids *Telenomus remus* Nixon, *Cotesia marginiventris* (Cresson) and *Hyposoter didymator* (Thunberg) were identified. Cold tolerance of the exotic parasitoids *H. didymator* and *C. marginiventris* was studied. Life table and fertility table statistics of the exotic scelionid egg parasitoid *T. remus* was studied in both individual and group rearing systems. Effect of release of different species of *Trichogramma* and *Campoletis chloridae* (Uchida) against *H. armigera* on chickpea was studied. Biological parameters of the local strain of *Diadegma semiclausum* (Hellén), larval parasitoid of diamond back moth *Plutella xylostella* (L.) were found to be on par with the Indonesian strain. Host preference and host plant preference of *C. marginiventris*, *C. kazak*, *C. blackburni*, *T. remus* etc., were worked out. Improved mass rearing techniques for *Spodoptera litura* (Fabricius) and *H. armigera* were standardised and the cost of production on semi-synthetic diet was worked out. Different geographical strains of *C. chloridae* were compared and the superior strain was identified.

### **4. Studies on trichogrammatids (01/04/1987 to 31/03/1994) PI: Dr. Surinder Pal Singh**

During the project period (1987-1994) 164 shipments of nine different Trichogrammatids were sent to 38 centres all over country covering 14 states and a total of 3,76,85,700 parasitized eggs were sent. During the project period 1,63,22,270 moths were collected, 9,450 cc eggs were obtained.

The mechanical collection method (MCM) proved its superiority over hand collection method (HCM) by increasing the moth collection efficiency by 32.3%. The MCM also reduced moth escape by 6.17 times. It was observed that in HCM not more than 10 moths could be collected at one time and to prevent escape from collection tube repeated tapping was required this in turn gave jerk to boxes resulting in more escape. In comparison in MCM even 200 to 300 moths could be collected in collection tube (20 cm x 3 cm) without giving jerk. Similar in HCM number of moths got damaged because of pushing with finger tips while in MCM such damage was not noticed. There was no difference in egg laying efficiency in both the treatments.

Result of adult mortality test revealed that insecticides, dicofol, phosalone and fenvalerate caused less initial mortality of adults of *Trichogrammatoidea armigera* Nagaraja in comparison to other insecticides tested. After six hours of constant exposure, dimethoate, fenitrothion, monocrotophos, phosphamidon, endosulfan, and decamethrin caused 100% mortality. Dicofol caused 11.2, phosalone 13.7, fenvalerate 71.2 and cypermethrin 75.0% mortality. After 24 hours of constant exposure dicofol and phosalone were less toxic, all other insecticides caused 100% mortality. In dimethoate adult *Trichogramma chilonis* Ishii could parasitise to the tune of 10.0% eggs, in monocrotophos 5.0% phosphamidon 2.0%, dicofol 70.0%, endosulfan 3.0%, phosalone 60.0%, whereas in control parasitism was 98.0%, thus insecticides significantly reduced the parasitising efficiency. Synthetic pyrethroids were less toxic initially but parasitoids failed to parasitize the egg in this treatment. Adult emergence recorded from dimethoate (54.0%), dicofol (86.0%), edosulfan (33.0%) and phosalone (80.0%), Fecundity of adults obtained from treatments showed that adults

from dicofol, phosalone and endosulfan produced a progeny of 45.0, 44.0 and 28.0 and lived for 6.0, 6.0 and 4.0 days, respectively. When the effect of the pesticides on the immature stage of the parasitoid inside *H. armigera* eggs was examined, the greatest effect was on the pupal stage and the lower mortality in the larval stage. Fenitrothion and endosulfan caused 100% mortality of all three stages, whereas dicofol and phosalone caused low mortality in all the stages compared to other insecticides. Fungicides were found to be safe to all the stages. The fecundity of emerging adults was drastically affected by dimethoate, fenitrothion, monocrotophos and endosulfan in comparison to the other pesticides tested. Parasitoids emerging from individuals treated at the larval stage could parasitise significantly more than those treated in the egg or pupal stages. In trials of residual toxicity to adult *Tr. armigera* mortality pattern differed significantly for different pesticides. Carbendazim, methyl thiophenate, and carboxingave mortality after 7 days and dicofol was found to be non-persistent. Twenty-one days after treatment, dimethoate, phosalone, fenvalerate, and fluvalinate were no longer causing any mortality and the percentage mortality was down to 13.8% for decamethrin and 22.2% for cypermethrin. These compounds were slightly persistent. However, the toxicity of endosulfan, monocrotophos, phosphamidon and fenitrothin persisted for more than one month. Very low parasitism was recorded for all other pesticides. Based on the parasitism pattern obtained at 7 days after treatment, residues of dicofol, carbendazim, methyl thiophenate and carboxin were classified as short-lived. Low parasitism was obtained up to 15 days after treatment for all insecticides except phosalone and fluvalinate where parasitism was 44.7% and 49.3% respectively, so these two insecticides were classified as slightly persistent. Thirtyone days after treatment, parasitism in dimethoate was 73.0%, decamethrin 73.9%, cypermethrin 71.0%, fenvalerate 70.1%, monocrotophos 56.2% and phosphamidon 60.0%, these compounds were classified as moderately persistent. Fenitrothion and endosulfan were however classified as persistent, with only 25-39 per cent parasitism 31 days after treatment.

Results revealed that replin caused 72.4% adult mortality of *T. chilonis* followed by neem cake 63.3% neem kernel 53.1%, neem rich I 45.9%, ahook 38.5%, nimbecide 37.4%, neem gold 32.9%, neem mark 32.8% , and neem rich II 30.9% comparing control where mortality was 22.5% after 24 hours of exposure. In general immature stages were safe to all products as mortality in egg stage ranged from 6.3-11.7 per cent, in larval stage 4.2-8.9 per cent and in pupal stage 4.2 – 7.7 per cent. Larval and pupal stages were comparatively less affected than egg stage. Adult parasitoids could parasitise to the tune of 98.4-99.9 per cent eggs treated by various neem products. Thus high parasitism occurred in all neem products. Results of the study when both testing unit and egg cards were sprayed before exposure of *T. chilonis* reveal high parasitism in neem rich II – 92.2%, ahook – 89.4%, neem on 85.5%, replin 86.1%, neem guard 85.2%, neem kernel suspension 77.6% and neem goa 73.14%, in other products it was less than 60%. Neem products to some extent inhibit *T. chilonis* to parasitise eggs. Thus the neem products can be safely used along with release of *Trichogramma* in management of *H. armigera*.

The effect of various Bt products on *T. chilonis* revealed that bitoxibacillin, lipidocide and dipel caused 60.7, 63.1 and 36.4 per cent mortality of *T. chilonis* adult after 24 hours of constant exposure. None of the compounds caused any hindrance in parasitism, which ranged from 93.4 – 98.2 per cent Emergence of parasitoids from all treatments was normal and at par. Therefore, both *B.t* products and *T. chilonis* were compatible and can be released / sprayed together for effective biosuppression. Screening of neonate larvae against B.t products revealed different response of products. Lepidocide, thuricide and delfin caused 93.6, 83.8 and 78.04 per cent mortality of larvae within 3 days on hatching. BTK –I and II and dipel caused 59.5, 50.7 and 50.2 per cent mortality, respectively. In Bitoxibacillin 18.4% larvae pupated which was significantly less than control where 38.0% larvae pupated.

In temperature tolerance studies, it was observed that *Tr. armigera* and *Trichogrammatoidea bactrae* Nagaraja were not amenable to low temperature storage, *Trichogramma achaeae* Nagaraja & Nagarkatti and *Trichogrammatoidea eldanae* Viggiani can be stored up to 35 days, while *Trichogramma japonicum* Ashmead for 21 and 28 days, respectively at 10°C. Fecundity of emerging adult parasitoids was drastically reduced after low temperature store at 10°C, in *Tr. armigera* it was after 7 days and in *Tr. bactrae* Nagaraja within 7 days. Hence *Tr. armigera* and *Tr. bactrae* can not be stored at low temperature and other species can be stored for maximum of 21 to 30 days only if both emergence and fecundity was taken into account.

Superior strain selection by collecting *T. chilonis* from cotton ecosystem from Anand, Ludhiana, Coimbatore, Bangalore, Rajahmundry and Nagpur and was reared separately on the eggs of *C. cephalonica* and life table studies were carried out. Simultaneously, observations on longevity and sex-ratio were also recorded. Irrespective of the collection area, per cent emergence in the laboratory was at par in all the ecotypes. However, there were variations in fecundity and longevity among the ecotypes.

Results of interaction studies between *T. chilonis* and *T. remus* on *S. litura* eggs indicated that when eggs were parasitised by parasitoids alone or in egg mass no desiccation of eggs was observed. However, in all multiparasitised sequences, 30.0-90.3 per cent eggs were desiccated in various treatments. It was also noted that more than 2 adults also emerged from single egg. In all single layer host egg parasitised sequences *T. chilonis* was found to be superior over *T. remus*.

#### **5. Studies on insect viruses/pathogens (01/04/1989 to 31/03/1996) PI: Dr. Kandaswamy Narayanan**

A polyhedrosis virus from greater wax moth, *Galleria mellonella* (Linnaeus) was isolated for the first time in India. The infection was found to be both in blood cells and in bodies. The larvae took nearly 5-7 days to succumb to the disease.

Isolation and purification of granulosis virus from tobacco caterpillar *S. litura* was standardized by repeated differential centrifugation with low and high speed, sedimented the capsules. Using 1% glycerol by radiant centrifugation technique, the capsules were purified. For extracting the virions, the granules were dissolved in 0.1 M Na<sub>2</sub> CO<sub>2</sub> for 30 minutes at room temperature and the virions and capsules proteins were separated by sucrose gradient centrifugation. For extraction of DNA and capsid protein, the methods were standardized so as to characterize the genome of baculoviruses using various restriction enzymes.

The effect of NPV of *H. armigera*, on the total haemocyte counts and differential haemocyte counts was studied. Standardization was perfected on the dose and age of caterpillar. Ingestion of 4x10<sup>6</sup> PIB/ml, to late fourth instar larvae was found to be ideal. Various blood cells were identified, viz., prohaemocytes, plasmatocytes granulocytes and vermiform cells in healthy *H. armigera*. On ingestion of NPV of *H. armigera*, the infection of NPV of *H. armigera* was located both in plasmatocytes and granulocytes 96 hr after post infection.

The minimum acquisition feeding period needed for maximum kill of *S. litura* revealed that 12 to 16 hr. feeding resulted in maximum kill of *S. litura* by the granulosis virus (GV). A preliminary study conducted on the interaction of NPV and GV of *S. litura* revealed that the dominant feature of NPV over the GV both in dose and time. It was found that on ingestion of 4 x 10<sup>6</sup> pib/larva, the incubation period ranged from 7 to 9 days in the case of late fourth instar larvae, when compared to more than 10 days incubation period found in the case of GV infected *S. litura*.

Field trials conducted with the help of CTRI, Rajamundry for the control of *S. litura* both in nursery and in main field revealed that three rounds of NPV spray @ 100 LE/acre at an interval of ten days were found to be effective for the control of *S. litura* in tobacco nursery. The percentage seedlings damaged was almost 0 and ranged from 0 to 0.1%. In contrast, the distant control plot registered 5 to 30% seedlings damaged with insect infestation of more than 200 larvae. The trial conducted at main field revealed that the percentage of seedling damage in NPV treated plot varied from 0-10 per cent in contrast to 40 to 45 per cent seedling damage in distant control plots. Similarly demonstration trials conducted on various cultivators field on crops like gram, tomato, knol-khol etc. with the use of NPVs of both *S. litura* and *H. armigera* have revealed the effective check of the above pests wherever sprayed.

The field trials conducted with the help of Agricultural Research Station, Durgapur, Jaipur, for the control of *H. armigera* in chickpea revealed the significant differences in NPV treated and control plots with regard to percent pod damage as well as in grain yield.

Ascovirus was for the first time isolated both from *Heliothis armigera* Hübner (*H. armigera*) and *S. litura*. For the first time in India we have isolated, the asco virus from the field collected gram pod borer *H. armigera*. The diseased larvae also showed the characteristic symptoms of retardation in the development when compared to control and difficulty in sluffing off the skin especially in third and fourth instar.

Studies on the effect of NPV on the blood were being continued and there was a reduction in the total plasmacytes count due to the infection of NPV. On clinical haematological observation it was found that most of the plasmacytes and granulocytes were infected with NPV. To find out the site of multiplication of granulosis virus of *S. litura*, several sections were cut to 4 to 5 microns in size and stained following method of Hamm (1966) technique and it was found that the virus multiplication was found to be heavily concentrated in the fat bodies.

For the first time in India, we have isolated a protozoa parasite, viz., *Mattesia* sp. from tomato pod borer *H. armigera*. On inoculation by oral method it was shown that it was highly infective and cause cent percent mortality on second and third instar larvae by diet surface contamination technique.

Large scale production of nuclear polyhedrosis viruses of both *H. armigera* and *S. litura* was done. Nearly 50,000 larvae of both *S. litura* and *H. armigera* were inoculated with their respective baculoviruses. And nearly 30,000 larval equivalent of NPV s of both *S. litura* and *H. armigera* were harvested for conducting field demonstration trials and supply against both the pests.

For the first time in India, attempts were made to establish insect cell line of *S. litura*. Primary culture was initiated with embryos of *S. litura*. Insect embryos were macerated and trypsinated under condition and seeded to the insect medium containing antibiotics of 100-200 units of penicillin and streptomycin and 40 gm/ml kanomy, which was later partially overcome with addition of some of the reducing agents. Contamination of fungal and probably yeast was later eliminated with addition of nystation.

Attempts were made to bring the cell line of *Spodoptera* sp. to serum free medium since serum alone takes nearly 80% of the cost. Our attempts to bring insect cells from 10% faetal bovine serum to serum free medium by weaning methods shows promise.

For characterization of viral DNS from *Heliothis* nuclear polyhedrosis virus, isolation and purification of viral DNA following phenol-chloroform extraction procedure was standardized. DNA from nuclear polyhedrosis virus of *H. armigera* was isolated and purified.

Conserved sequence of polyhedron as well as 20-40 primer needed for DNA probing was identified. DNA was isolated from the NPV of *H. armigera* for further study.

## **6. Biosystematic studies on Chrysopidae (01/04/1990 to 31/03/1993) PI: Dr. Uma Narasimham**

Historically the classification of Chrysopidae has relied mainly on colour patterns on the adult head and thorax, at the species level and on wing venation at higher taxonomic levels. Recent generic revision of Chrysopidae has revealed male genitalia as the most useful character, which has also proved to be automorphic. However, only recently investigations were made to include broader range of characters in systematic analysis of this group.

Traps baited with honey and protinex, L-tryptophane and methyl eugenol were observed at BCC, during May and June 1992 and Dec. 1992 and Jan. 1993. Although chrysopid eggs were collected on foliage in the vicinity of the traps, no adults were collected from the traps. Traps (4 to 5 at a time) with Nuvon, in addition to the different baits were observed in a mango grove during January to March 1993. A single *Apertochrysa* female was collected in the orchard during the period of the study. Use of these food lures proved to be poor means of collecting Chrysopids. A light trap was operated at the institute campus during May to Sept., using a 100 w bulb and during the period a single male of *Chrysoperla carnea* (Stephens) was collected. During the period eggs of Chrysopids were collected from foliage in the campus. It appears that this might be useful only when the population was particularly high in the field. During the period under report 22 adults of Chrysopids were attracted to light in a farm house at Faridkot, Punjab, amidst fields of sunflower, lucerne and vegetables.

Studies on *C. carnea* from 4 widely separated geographical areas in India, to gain an understanding of intra-specific variations were carried out. Morphology of larvae of *Mallada boninensis* (Okamoto) was studied. Detailed taxonomic studies on *Mallada astur* (Banks) and an *Apertochrysa* sp. were carried out. Biological traits such as development period, debris-carrying behaviour, wing stridulation, egg deposition pattern etc. that could serve as a supplementary taxonomic character were also explored. Collections were made in following agrosystems: cotton, mango, citrus, guava, coffee, castor, sunflower, coconut, okra, casuarina, ragi, jackfruit, butter fruit,

canna, *Drasenea*, field beans, sapota, grapes, spider lily, mulberry, *Syzigium cumini* (L.) croton, *Atriplex hortenses* L., mahogany and polyalthia. Special efforts were made to collect chrysopids in sunflower, cotton and tomato fields in Bangalore area. In sunflower fields, *C. carnea*, *M. boninensis* and *Apertochrysa* sp. were found while in cotton in addition to these species, *M. astur* was also collected. No chrysopids were collected on tomato. 32 collections of colonies of host material were made, mainly aphids, mealybugs and scale insects. However no chrysopid was reared from these colonies.

Egg burster occurs in embryos of insects belonging to several orders. But this structure has not been adequately studied for its taxonomic value. Hence egg bursters of four species of Chrysopids, viz., *C. carnea*, *M. boninensis*, *M. astur* and *Apertochrysa* sp. were studied. The parameters studied were found to be homogenous in the egg bursters obtained from different females of same species in all four species studied and interspecific difference indicated this structure to be of value as supplementary character.

Morphology of larvae, including chaetotaxy of three spp. of field collected chrysopids, colonised in the laboratory was studied and descriptions of I and III instar larvae of *Apertochrysa* sp., *M. astur* and *M. boninensis* were prepared along with pencil sketches.

Material belonging to the following taxa were sorted- Retipenna, *Cuntophrysa* and *Tumepochrysa indica* Needham and *Chrysopa pallens* (Rambur), based on nongenitalic as well as genitalic characters, using literature or authentically identified material in BCC, collection.

Pupal mandibles and labrum were studied in five species of Chrysopids, viz., *C. carnea*, *M. boninensis*, *M. astur*, *Apertochrysa* sp. and *Ankylopteryx* sp. 1. Seven parameters were identified for morphometric studies, of which three parameters were significantly or highly significantly different between any two species that were considered, showing its potential as a taxonomic tool. However labrum in the five species studied was more consistent in its chaetotaxy and proved to be of little value.

## **7. Development of mass production techniques for parasitoids (01/04/1994 to 01/04/2002) PIs: Dr. Thiruvengadam Venkatesan (1994 to 1996) Dr. Chandish R Ballal (1996 to 2002)**

This project concentrated on the standardisation of mass rearing techniques for some potential parasitoids and also the relevant host insects. An acrylic multicellular rearing unit was devised as a prototype for mass rearing *S. litura* and *H. armigera*. The unit provided 80 to 90 per cent survival of the transferred larvae and the trays were supplied to different research organisations. A method was standardised for rearing *S. litura*, wherein the larvae could be reared completely on semi-synthetic diet.

Evaluation of *T. remus* and *T. chilonis* - Release of *T. remus* on *S. litura* eggs on sunflower resulted in around 25% parasitism; *T. remus* can perform well on different varieties of soybean providing upto 70% parasitism of *S. litura* eggs. *T. chilonis* could parasitise 70% *H. armigera* eggs on soybean.

Evaluation of *Eriborus argenteopilosus* (Cameron) and *C. chloridae* indicated that one female parasitoid: five host larvae was the optimum ratio for obtaining higher per cent parasitism in *E. argenteopilosus* and in the case of *C. chloridae*, 1: 5 and 1: 10 were the ideal ratios.

Method developed to identify male and female of *C. chloridae* and *E. argenteopilosus* in the cocoon stage, based on which it can be ascertained that enough female cocoons could be included while sending consignments.

Fertility tables were constructed for field collected and lab reared *C. chloridae*. *C. chloridae* parasitism was more on host larvae when they were feeding on host plants in comparison to freshly released larvae, indicating the role of chemical cues in promoting parasitism.

Optimum storage temperature was identified for cocoons of *E. argenteopilosus* they could be stored for up to 10 days at 11°C and up to 15 days at 15°C, without any adverse effect on the biological parameters of the parasitoid.

Effect of continuous rearing of parasitoids was studied; continuous rearing of *C. chloridae* led to deterioration in characters like progeny production and sex ratio after 10 generations. In the case of *E. argenteopilosus*, this deterioration occurred within four generations.

Variations were observed in the performance of *C. chlorideae* on populations of *H. armigera* obtained from different cotton growing areas. Interaction studies between *C. chlorideae* and *E. argenteopilosus* indicated that *C. chlorideae* was the dominant parasitoid. On chickpea, at a parasitoid (*C. chlorideae*): host ratio of 1: 5, approximately 33% parasitism was obtained. *E. argenteopilosus* had a higher preference for pulse crops.

#### **8. Evaluation of improved and selected species/ strains of egg parasitoids (01/04/1994 to 31/03/2002) PI: Dr. Sushil Kumar Jalali**

The cultures of egg parasitoids, i.e., 11 species of trichogrammatids and five strains were maintained on laboratory host *C. cephalonica* and on *H. armigera* eggs.

The experiment on *in-vitro* rearing of trichogrammatids was carried out by preparing two sets of artificial diet. Diet I contained *H. armigera* larval haemolymph (50%), egg yolk (25%) and milk suspension (25%) and streptomycin sulphate (0.15%) was added to the diet. Diet II contained *H. armigera* larval haemolymph (50%), egg yolk (20%) and new born calf serum (10%) and streptomycin sulphate (0.12%) was added to the diet. Egg laying was obtained on ovipositional stimulant egg capsules. Partial development up to pupal stage was observed on diet no.1. However, further development was not observed due to drying up of the diet.

Selection of egg parasitoids for tolerance to insecticides by adult exposure method: The results suggested that after 34 generation of exposure parasitoids have become tolerant to 1.5 ml/lit of monocrotophos also besides endosulfan (2.5 ml/lit). The parasitism obtained was more than 90% and per cent mortality less than 20 after six hours of constant exposure to both insecticides. Similarly, exposure to fenvalerate for 26 generations resulted in parasitism and mortality of more than 90 and less than 20 per cent, respectively, after six hours of constant exposure at 0.05 ml/lit solution. Experiment was started with 'Endogram' strain to develop multiple insecticide tolerant strain of *T. chilonis*. The results suggested that this strain has now become tolerant to 2.75 ml / lit of endosulfan, 1.5 ml / lit of monocrotophos and parasitoids were now shifted to 0.10 ml / lit of fenvalerate.

Development of high temperature tolerant strain of *T. chilonis*: The experiment was conducted in BOD incubator with constant temperature and varying humidity. Studies were continued at 33°C and 70-80 per cent humidity. Results showed that after 38 generations, parasitism increased from 10 to 98 per cent and longevity from 1 to 7 days. Parasitoids were shifted from 33 to 36°C and results at 36°C showed that after 48 generations of constant rearing parasitoids was now showing adaptability to this temperature with constant parasitism and increased longevity. The parasitism obtained was >80% and longevity was increased from 2 days to 6 days. Results showed that after 55 generations of constant rearing parasitoids showed adaptability to 36±1.5°C. The parasitism obtained at first generation at this temperature was 41% and survival of <1 day in comparison to less than 5.0% parasitism by other susceptible strains.

*S. litura* eggs were exposed to *T. remus* at 27°-36°C in BOD chambers. Results showed that after 30 generations of constant rearing parasitoids gave 72.2% parasitisation and longevity was 7.8 days. However, sex-ratio, which was female biased up to 32°C, became male biased when parasitoids were shifted to high temperatures.

Life-table and parasitizing efficiency of *Tr. bactrae* at various temperatures: The net reproductive rate ( $R_0$ ) of 23.2 was highest at 25°C and lowest (3.59) at 35°C. The finite rate of increase varied from 1.17 to 1.36females/day at various temperatures. At 15°C, though low parasitism was observed but parasitoids failed to emerge from the eggs.

Five different species, viz., *Tr. armigera*, *Trichogramma pretiosum* Riley (Ar), *T. chilonis*, *Trichogramma evanescens* Westwood and *T. pretiosum* were reared on eggs of *H. armigera* in the glasshouse and laboratory conditions. The developmental period was 7 - 9 days from February to May, 11 - 12 days from June to October and 13 - 15 days from November to January. *Tr.armigera* always took 2-4 days to complete its development in corresponding periods in glasshouse. In the laboratory, however, all *Trichogramma* species completed its development in 8 - 10 days in different months. In the net house per cent parasitism was significantly lower from February to May (summer season) in all the species in comparison to June to January. *Tr. armigera* was most affected by change in temperature comparing *Trichogramma* species.

Strain identification of insecticide tolerant strains of *T. chilonis* through electrophoresis techniques was carried out. Studies conducted on the characterization of endosulfan, fenvalerate, monocrotophos tolerant strains of *T. chilonis* and susceptible trichogrammatid indicated that there were clear protein banding patterns in the pesticide tolerant strains and susceptible *T. chilonis* revealing that there were changes in proteins of pesticide tolerant strains and susceptible *T. chilonis*. Three new protein bands were appearing in pesticide tolerant *T. chilonis* compared to susceptible strain. The molecular weights of the proteins present in pesticide tolerant strains of *T. chilonis* and susceptible species ranged between 3000 to 43,000 KDa.

Selection for high host searching ability strain of *T. chilonis* for use against *H. armigera* on tomato suggested that after 30 generations of rearing in insect rearing cage (30 cm<sup>3</sup>), parasitoids were able to parasitize > 95% eggs when 30 adults were released from initial level of 40% when about 500 adults were released. SAS parasitoids have significantly higher host searching ability and they could parasitize 50.6 to 77.0 per cent eggs compared to 4.0 to 34.0 per cent by check species, i.e., *T. pretiosum*.

Evaluation of multiple insecticide tolerant strain against cotton bollworms in Gujarat, Karnataka and Tamil Nadu: The field trial at Coimbatore, where parasitoids were released three times at 15 days interval revealed mean per cent parasitism of 9.4 (range 4.0-12.0 per cent) and it varied significantly with untreated control where no egg parasitism was recorded. Per cent fruiting bodies damage was also significantly low, 4.0% compared to 10.5% in untreated control. Results indicated that multiple insecticide tolerant strain was highly effective against bollworms.

#### **9. Evaluation and development of artificial diets for important lepidopterous pests (01/04/1994 to 31/03/2002) PI: Dr. Kotilingam Srinivasa Murthy**

Different artificial diets were evaluated for cost effective rearing of important lepidopterous insect pests, viz., *S. litura*, *Spodoptera exigua* (Hübner), *Opisina arenosella* Walker and *P. xylostella*. *S. litura* was reared on three experimental diets based on the leaf powders of castor, cauliflower, and cabbage and compared with that of control diet (without leaf powder).

Cabbage leaf powder based diet resulted in 96% pupation, 52% female emergence and 70% adult emergence. Castor leaf powder based diet was also effective. Combination of diets indicated that kabuligram + groundnut cake based diet was found to be the best in terms of increased pupation (95%), adult emergence (92.5%) and fertile eggs/female (390 eggs).

*O. arenosella* was reared on three experimental diets based on leaf powders of host plants namely, coconut, wild date palm and toddy palm in combination with defatted soya and compared with the prescribed diet of Jayanth and Sudha nagarkatti (1974). Toddy palm based diet was found effective for rearing *O. arenosella*. The shelf life of the diet maintained at 26 ± 1°C at 65% RH was 9-11 days.

Castor leaf powder based diet and other leaf powders viz., cauliflower, knolkhol, radish and kidney beans in combination with defatted soya were evaluated for rearing *S. litura*. Cabbage leaf powder with soya based diet was effective for rearing *P. xylostella*. The adaptation of the insect to the diet over a period of time would enhance the biological attributes comparable to those reared on natural host leaves.

#### **10. Evaluation of artificial diet, release rates and genetic improvement of important predators (01/04/1996 to 31/03/2002) PI: Dr. Thiruvengadam Venkatesan**

*C. carnea* larva (one day old) was successfully reared on beef liver-based lyophilised artificial diet for 15 generations. The mean per cent survival and adult emergence of the *C. carnea* reared on artificial diet were 87.9 and 85.5 per cent, respectively. The same on the *Corcyra* reared predator was 88.8 and 85.5 per cent, respectively. The artificial diet could be stored up to 150-160 days at 5°C without any deleterious effects on the biological attributes of *C. carnea*. The results of the toxicity tests with the adults of *C. carnea* reared on artificial diet indicated that spinosad 48SC was highly toxic to adults. At 24 hrs, spinosad caused 100% adult mortality. Field studies revealed that predatory efficiency of artificial diet reared *C. carnea* was comparable with the *Corcyra* reared *C. carnea* in suppressing cotton pests. *Cryptolaemus montrouzieri* Mulsant was reared on a freeze-dried artificial diet based on

beef liver and egg yolk. Mean adult emergence of the predators reared on artificial diet was 58.0%. Aphid predator, *C. sexmaculata* were reared on a pig liver based artificial diet. Mean per cent adult emergence, longevity and fecundity on the above diet were 47.0%, 42 days and 85 eggs/female respectively, as compared to natural diet (aphids) 81%, 76 days and 403 eggs/female, respectively. *Cardiastethus exiguus* Poppius was reared on an artificial diet based on beef liver, defatted soybean and milk.

**11. Software development for identifying and suggesting biological control measures for vegetable crop-pests using a PC (01/04/1994 to 31/03/2008) PI: Mr. Santi Ranjan Biswas**

A software was developed for identifying and suggesting biological control measures for vegetable crop-pest using a PC, information on 16 pests of brinjal, 12 pests of bean, nine pests of cabbage, six pests of tomato, 10 pests of cowpea, two pests of potato were catalogued. The information on the pests, their natural enemies, distribution maps and different IPM measures adopted for the control of these pests were put in the form of expert system. The software was developed in MS-Access and the first screen display the crops list. The list of pests would be displayed while selecting the crop. It was a very easy menu driven system and could be used by any body using a PC. The information is available in the form of CD.

**12. Development of mass production techniques for predators (04/11/1994 to 01/03/2001) PI: Dr. Sunil Joshi**

Biotic-potential of three coccinellid predators, viz., *Coccinella septempunctata* Linnaeus, *Coccinella transversalis* Fabricius and *C. sexmaculata* was studied on six species of aphids, viz., *Aphis craccivora* Koch, *Aphis gossypii* Glover, *Aphis nerii* Boyer de Fonscoimbe, *Lipaphis erysimi* (Kaltenbach), *Rhopalosiphum maidis* (Fitch) and *Uroleucon compositae* (Theobald). These three coccinellid predators accepted all the hosts, but *U. compositae* was the least preferred resulting in no egg production in *C. septempunctata* and *C. transversalis*. Developmental period on different aphid hosts, viz., *C. septempunctata* and *C. transversalis* varied from 16.5 to 21.4 and 15.2 to 18.0 days, respectively. *C. sexmaculata* voraciously predated and bred well on all these hosts including *U. compositae*, indicating its wide host range. But, it developed faster on *A. nerii* (12.2 days) than other hosts and was more fecund on *A. craccivora* and *A. craccivora* was the most preferred host for all the coccinellids with higher rate of consumption and higher fecundity.

Studies were carried out on the biology and feeding potential of six species of syrphid predators, viz., *Betasyrphus fletcheri* Ghorpade, *Betasyrphus linga* Ghorpade, *Dideopsis aegrota* (Fabricius), *Ischiodon scutellaris* (Fabricius), *Paragus serratus* (Fabricius) and *Paragus yerburiensis* Stuckenberg feeding on cowpea aphid, *A. craccivora*. Adult mating and larval feeding behavior was described. At 26±2° C and 75±2% RH, developmental period of these predators from egg to adult emergence ranged from 20.10 to 25.80 days. There were three larval instars and the last instar was the most voracious. *I. scutellaris* was found most suitable for laboratory multiplication.

Populations of *Paragus serratus* and *P. yerburiensis* were studied to assess their growth potential. The female *P. serratus* had an average oviposition period of 15.3 days and a generation time of 34.55 days. In *P. yerburiensis*, the corresponding values were 8.6 and 30.07 days. The net reproductive rate was also higher in *P. serratus* (12.33) than in *P. yerburiensis* (4.85). The intrinsic rate of natural increase was 0.0737 and 0.0509 in *P. serratus* and *P. yerburiensis*, respectively. The population of *P. serratus* and *P. yerburiensis* multiplied 1.18 and 1.13 times per day and doubled in 4.14 days, and 5.74 days, respectively. The maximum contribution towards stable age distribution of both the predators was made by larval stages.

Effect of different storage temperatures and duration on longevity and fecundity of *Brumoides suturalis* (Fabricius) was studied. Fecundity declined with increase in storage period. Mortality was high at 5, 10, and 15°C. However, 20°C was ideal temperature for storage for up to 30 days with affordable reduction in fecundity and survival. Fecundity of adults stored at 20°C for 30 days was reduced by 13% over those adults which were not subjected to cold storage and provided with *Ferrisia virgata* (Cockerell) throughout their life. Fecundity of adults stored for 45 days at the same

temperature was reduced by 19.51% indicating an additional 33.36% reduction when stored for a further period of 15 days.

*Sticholotis cribellata* Sicard was recorded for the first time as a predator of *M. glomerata*. Its host range and seasonal incidence were detailed and the immature stages described and illustrated. The egg, larval and pupal stages lasted for 6.9, 19.5, and 8.6 days, respectively, and adult longevity was 40.41 days. The average fecundity was 48.9 eggs/female. The larvae and adults consumed on an average 653.95 and 1822.49 *M. glomerata* crawlers, respectively, throughout their life span. *S. cribellata* appears to be a promising bioagent of *M. glomerata* in view of its high feeding potential and amenability to large scale mass multiplication.

The exotic ladybird beetle, *C. coeruleus* was reared in the laboratory for seven successive generations using two different hosts, viz., natural psyllid host, *H. cubana* Crawford and on alternative host, *F. virgata*. *F. virgata* was found to be suitable for *C. coeruleus* in supporting larval growth and development. Fecundity and adult longevity increased with advancing generations on *F. virgata*. Pupae reared on *H. cubana* were heavier than *F. virgate* reared pupae. However, fecundity, adult longevity and sex ratio did not differ in advancing generations in the two rearing regimes. High larval survivorship could be obtained on either host and it ranged from 82 to 96% on *H. cubana* and 86 to 96% on *F. virgata*. The *F. virgate* reared predators were of acceptable quality and exhibited high feeding potential, larval survivorship and fecundity after transferring back to natural host even after seven successive generations of rearing on alternative host. One cage containing one fully infested pumpkin could yield 50 to 60 beetles. It was possible to rear about 1000 to 1500 adults per month with half an hour of labour per day. The method developed for rearing can be employed anywhere, irrespective of availability of subabul fields. Natural host based procedure fails during summer, as there was a decline in psyllid population, whereas alternative host based procedure was feasible even during summer. It was economical and minimised handling of insect stages, thus saving time and labour.

### **13. Survey, identification and utilization of entomopathogenic nematodes against some important lepidopterous and coleopterous pests (01/04/1996 to 31/03/2001) PI: Dr. Syed Shahabuddin Hussaini**

One hundred and six soil samples were collected from different crop habitats and agro climatic zones covering 10 states

Information on the type of soil, elevation and annual median rainfall of the locations from the soil samples were collected were also reduced. *Steinernema spp.* was isolated from the localities with an elevation range of 107 m to a maximum of 2200 m. the annual median rainfall in these localities ranged between 714 mm to 923 mm. *Steinernema spp.* was found to occur in sandy loam and clayey loam soils.

*Heterorhabditis spp.* was isolated from soils collected from Delhi and Hyderabad, where the elevation was found to be 107m to 545 m, annual rainfall 714.2mm and 764.4mm and the soil type clayey loam and gravelly loam respectively. There was no correlation between occurrence of either of the species of entomopathogenic nematodes and elevation, annual rainfall and type of soil.

Identification: The collected entomopathogenic nematodes were fixed in TAF and processed by slow glycerin method. Permanent slides made were analyzed for morphometrics of infective, and males.

Infective juveniles: morphometric analysis of Ijs of isolates KND1 and KNC h 25 was done. The average length of Ijs was found to be 446.43  $\mu\text{m}$  with an average width of 22.11  $\mu\text{m}$ . the excretory pore was located at distance of 40.89 from tip of head and the nerve ring was at 99.19. The ratios of A, B, C, D and E was found to be 20.27, 3.28, 10.13, 0.301 and 0.93 respectively.

Males: The morphological characters like the rounded tail tip and presence of mucron confirmed that isolates, viz., *Steinernema carpocapsae* (Weiser) 6.11, *Steinernema sp.* Adigenalli 6.2 were *Steinernema sp.* The spicule colour was found to be yellowish brown in all isolates with blunt tip., mucron length ranged from 1.67 $\mu\text{m}$  (KNK1) to 4.14 $\mu\text{m}$  (*S. carpocapsae* 6.11) and for *Steinernema sp.* Adigenalli 6.2 isolates it was 2.88, the D%, EN, SW, GS for isolates *Steinernema sp.* KNK1 was nearly the same.

Indigenous isolates of ten *Steinernema* and six *Heterorhabditis* were tested in the lab. Per cent mortality, 72h after exposure was recorded and was presented. Per cent mortality of *S. litura* was maximum with *Steinernema bicornutum* Tallosi, Peters & Ehlers PDBC EN3.2 and *S. carpocapsae* PDBC EN 6.61 (88.89%) followed by the other *Steinernema* spp. ranging from 44.44 to 77.78 per cent. Among *Heterorhabditis indica* Poinar, Karunakar & David, PDBC EN13.3 and 6.71 caused 100% mortality followed by other isolates (0.00-77.78 per cent). In *H. armigera* maximum mortality was caused by *Steinernematami* Luc, Nguyen, Reid & Spiridonov PDBC EN2.1 and *H. indica* PDBC EN6.71 and 13.3 (100%). Ranges of mortality caused by *Steinernema* and *Heterorhabditis* spp. were 0.00-88.89 per cent and 33.33-88.89 per cent, respectively. *S. tami* PDBC EN2.1 and *Steinernema abbasi* Elawad, Ahmad & Reid PDBC EN 3.1 were the best for potato tuber moth, *P. operculella* with 100% mortality followed by other *Steinernema* sp. (0.00-66.67 per cent). In diamond back moth *P. xylostella* maximum mortality (100%) was caused by *S. carpocapsae* PDBC EN1.3. *S. bicornutum* PDBC EN3.2, *S. carpocapsae* PDBC EN1.3, *S. carpocapsae* PDBC EN7.2 were effective against *O. Arenosella* (66.67%) followed by *S. tami* PDBC EN2.1 (33.33%).

Bioefficacy of *Steinernema glaseri* (Steiner), *S. carpocapsae*, *S. bicornutum* and *H. indica* against different insect pests: *S. glaseri*, *S. carpocapsae* PDBC EN 6.11, PDBC EN 1.3, *S. abbasi* PDBC EN 3.1 and *H. indica* PDBC EN 13.3 were tested against *S. litura*, *H. armigera*, *O. arenosella*, *P. xylostella* and *P. operculella* by soil column assay. Among the isolates tested *S. abbasi* PDBC EN 3.1 and *H. indica* PDBC EN 13.3 showed consistent result by recording highest mortality (80-100 per cent) against all insects tested. Bioefficacy of all the isolate tested against *P. operculella* and *P. xylostella* was found to be on par with 80-100 per cent mortality.

The mass production of *Steinernema* spp. and *Heterorhabditis* sp. was attempted with different media. The suitability of the different media was evaluated by the total yield of IJs/250 ml flask. The mass production of *Heterorhabditis* sp. was done with *H. indica* PDBC EN 6.71 in Wout's medium and a yield of 35 lakhs IJs/ flask was obtained.

Calcium alginate formulation with EPN embedded were prepared and tested in laboratory for feeding by larvae of *S. litura* and *H. armigera*. The efficiency of the formulation of different *Steinernema* spp and *Heterorhabditis* spp. was evaluated by the per cent mortality of final instar larvae of *S. litura* and *H. armigera* exposed to formulation for 72h. Among the formulation talc + china clay and alginate was better in retaining the viability of IJs, for a longer period than talc formulation. Per cent survival of IJs was maximum in talc china clay followed by Alginate encapsulation.

In a collaborative experiment with CTRI, Rajahmundry, alginate formulation of *S. carpocapsae* and *H. indica* were tested against *S. litura* in FCV tobacco nurseries. Application at the rate of 1000 capsules per sq. m significantly reduced the population of late third instar of *S. litura* and numbers of seedlings damaged were reduced.

Bioefficiency against *S. litura* in tobacco nurseries: Talc based formulation of *S. carpocapsae* PDBC EN 6.11 and *H. indica* 13.3 was tested, @ 0.5, 1.0 and 2.0 billion/ac against *S. litura* in FCV tobacco nurseries at Rajahmundry and were found very effective. *S. carpocapsae* PDBC EN 6.11 and *H. indica* 13.3 was sprayed against *H. armigera* on pigeon pea at Tandur, Medak Dt. under Acharya N. G. Ranga Agricultural University, Hyderabad, and encouraging results were obtained.

Field evaluation of indigenous isolates of EPN against brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenée: Preliminary field trial with the isolates *S. carpocapsae* PDBC EN 6.11 and *H. indica* PDBC EN 6.71 @ 0.5, 1.0, 2.0 billion/ac on brinjal indicated that higher the concentration of infective juveniles per dose, higher the reduction in borer holes on brinjal fruits and the results were comparable with sprays of neem seed kernel extract. Between the two species evaluated, isolate *S. carpocapsae* PDBC EN 6.11 was found to be more effective in reducing the fruit damage in terms of number of fruits with borer holes and increase in yields.

Efficacy of *Steinernema* spp. against maize tissue borer, *C. partellus* on maize in pot culture: Pot culture experiment was conducted to find out the efficacy of *Steinernema* spp. against *C. partellus* on maize. The occurrence of *C. partellus* was less in pots sprayed with 1000IJs/pot. Pupation of larvae was reduced to 2 in 1000 IJs/pot whereas it was 7 in check. The tunnel length was also minimized in the treated plants. Plants sprayed with EPN recorded maximum height compared to untreated check.

**14. Use of semiochemicals to improve the efficiency of important predators (01/04/1996 to 31/03/2002) PI: Dr. Nandagopal Bakthavatsalam**

Under the project kairomones for the chrysopids were developed. Chrysopids were attracted to the honey dew secretions of the aphids. On chemical analysis it was observed that compounds like L-tryptophan were important attractants for chrysopids. A method was developed to hydrolyse tryptophan using hydrochloric acid and the acid hydrolysed L-tryptophan was found to act as an attractant to chrysopids under laboratory and net house conditions. Later the same compounds were tested on cotton at field conditions and the acid hydrolysed L-tryptophan attracted the chrysopids. However the results were not encouraging on the experiments conducted with the substitution of hydrogen peroxide instead of acids and amino acid oxidisers. The waiting period was not reduced through the use of amino acid oxidisers.

The larvae of chrysopids use the scales and egg deposits of the lepidopteran insects for locating their hosts. On chemical analysis it was observed that compounds like n-tricosane, pentacosane, hexacosane were important compounds which attract chrysopid larva. Laboratory studies were conducted to find out the best solvent with least phytotoxicity. Hexane was found to be better solvent. The concentration of 0.1% Tricosane was found to be better in evoking good behavioural response to chrysopid larva. Field trials with this compound proved higher predation by chrysopid larvae at field conditions on cotton.

**15. Biological control of plant parasitic nematodes with fungi and bacteria with special reference to *Paecilomyces lilacinus* and *Pasteuria penetrans* (01/04/1996 to 31/03/2001) PI: Dr. Chellappa Sankarnarayanan**

The culture filtrate of *Pseudomonas fluorescens* (Flügge) was found toxic to plant parasitic nematodes. Mortality of the juveniles was observed from 12 h. Mortality increased with exposure time. At 48 h, mortality rates for *Meloidogyne incognita* (Kofoid & White), *Heterodera cajani* Koshi and *Rotylenchulus reniformis* Linford and Oliveira were 94, 85 and 95 per cent, respectively. *Verticillium chlamydosporium* Goddard cultured on sorghum grain and applied @10g/plant obtained least galls of *M. incognita* and the reduction was 73% over nematode inoculated control tomato plants. Also 41 to 70 per cent reduction in egg masses of *M. incognita* were observed by application of *V. Chlamydosporium* @ 10g/ plant. In sunflower, *V. Chlamydosporium* cultured on sorghum grain and applied @ 10g/plant as well as fungus cultured on wheat grain applied @ 10 g/plant was found to be superior to other substrates in terms of parasitisation of eggs of *R. reniformis*. Increase in growth of tomato plants recorded either with individual inoculations of *Pasteuria penetrans* (Thorne) or *P. fluorescens* or with combined inoculation of both bacterial antagonists. Least number of galls, egg masses/g root and nematode population of *M. incognita* were recorded in combined inoculation of both the bacterial agents than individual inoculations. Considerable reduction of galls and egg masses of *M. incognita* was recorded with inoculation of *Fusarium oxysporum* Schlecht. Fungal isolate I was effective in suppressing galls, egg masses and nematode population than isolate 2. About 65.7% parasitisation of egg masses and 68.5% parasitisation of eggs were recorded with *F. Oxysporum* isolate 1.

**16. Survey, identification and utilization of plant pathogens for the biological control of weeds with particular reference to parthenium and water hyacinth (01/09/1996 to 31/03/2003) PI: Dr. Sreerama Kumar Prakya**

Extensive and intensive pathogen surveys were undertaken for diseases of parthenium (*Parthenium hysterophorus* L.) in Bangalore Rural, Bangalore Urban, Bellary, Bidar, Chitradurga, Dharwad, Gulbarga, Mandya, Mysore and Raichur districts of Karnataka. Targeted disease symptoms were various stem and leaf spots, blights, lesions, mildews and other major categories of pathogenic damage. The most common disease afflicting parthenium was powdery mildew (*Oidium parthenii* Satyapr. & Ushar.), which was widespread during August-April. *Alternaria* spp. were equally dominant during the monsoons. A blight disease, incited by *Rhizoctonia solani* J.G. Kühn, was also consistently isolated. For the first time, association of *Nigrospora spherical* (Sacc.) with a leaf spot of

parthenium was unravelled. *Pestalotia* sp. originated from leaf spots, *Sclerotium rolfsii* (Curzi), isolated from wilted parthenium plants, was highly pathogenic to the weed. Plants applied with at least four sclerotia drooped from the fourth day and collapsed on the sixth day. *Sclerotinia sclerotiorum* (Lib.) was also found to be infecting the weed.

*Lasiodiplodia theobromae* (Pat.) (= *Botryodiplodia theobromae* Pat.) and *Nigrospora oryzae* (Berk. & Broome) (teleomorph: *Khuskia oryzae* H.J. Huds.) were studied further. Lesion-inducing *Phoma chrysanthemicola* (Hollós) and *Phoma eupyrena* Sacc. were investigated for their potential as mycoherbicides. Inoculation assays on detached leaves and on 45-day-old pot-grown parthenium plants with a conidial suspension of the pathogens produced symptoms typical of those found in field plants. A pathogenic bacterium was isolated and purified from leaf spots and leaf blights. The other widespread disease was phyllody, incited by a mycoplasma-like organism, and leaf curl, a viral disease.

In the course of trials with many pathogens, *Fusarium pallidoroseum* (Cooke) [*Fusarium semitectum* (Berk. & Ravenel)], a leaf-spotting pathogen, showed the most desirable characteristics for development as a mycoherbicide for parthenium. The most pathogenic isolate [WF (Ph) 30] was taken up for further investigations. All the growth stages of parthenium were susceptible to *F. pallidoroseum*, younger plants being more susceptible than older ones. The preliminary host-range testing determined that all the test plant species, including those of the Compositae, were not susceptible to *F. pallidoroseum*. A total of 21 cultivars/ accessions of sunflower were also found safe.

Parthenium populations from all over Karnataka were found susceptible to the isolate WF (Ph) 30 with the overall mean susceptibility of 91.1%. All the parthenium biotypes from six different states were also susceptible to *F. pallidoroseum*. Absolute susceptibility was recorded in the case of parthenium biotypes from Rajahmundry, New Delhi and Pune.

The effect of some common surfactants on the pathogenicity of *F. pallidoroseum* was assessed. The best was Tween 80, with a necrotic leaf area of 90.6%. The utility of some hydrophilic substances in promoting leaf wetness and thereby increasing the pathogenicity of *F. pallidoroseum* to parthenium was examined. Gum arabic enhanced the pathogenicity to obtain the maximum necrotic leaf area of 94.6%.

For the first time, mass production of *F. pallidoroseum* was undertaken. Robust macroconidia were formed in shake culture with a final conidial yield of  $2.2 \times 10^7$ /ml. The mean colony-forming units (CFU) recorded was  $1 \times 10^7$ /ml. The mean wet and dry weights of the biomass were 7.35 g and 0.34 g/100ml, respectively. A bench-top fermentor of 3-litre capacity was used for fermentation studies. The wet and dry weights recovered were 8.88 g and 0.48 g/100 ml, respectively. A count of  $6 \times 10^7$  CFU/ml was obtained at the end of fermentation. The biomass obtained through fermentation was formulated into four different products (bioherbicides), viz., powder, oil emulsion, alginate pellets and pesta granules. In the greenhouse, two sprays were better than a single spray with regard to all the formulations tested. Among the one-spray treatments, alginate granule formulation produced the highest (34.7%) necrotic leaf area, followed by powder formulation (26.5%). In the field experiment at Singapura near Jalahalli in Bangalore Urban district, the talc-based powder formulation caused the maximum damage (14.7%).

Pre- and post-emergence mycoherbicidal activity of *Gliocladium virens* J.H. Millculture filtrate towards parthenium was investigated. On blotters, filtrate-treated seeds were unable to produce radicles suggesting the deleterious effect of fungal metabolites on root development. In soil, the pronounced effect on emerging radicles indicated the rhizogenic toxicity of the culture filtrate. Soil-incorporated *G. virens* drastically reduced (62.4%) parthenium seed germination.

Lakes in and around Bangalore were surveyed for water hyacinth, *Eichhornia crassipes* (Mart.) pathogens. *Alternaria eicchorniae* and *Alternaria alternata* (Fr. Keissler) were dominant on the weed. In Hebbal Lake, the incidence of the species was up to 35%. *Cercospora* spp. were the other dominant fungi causing extensive damage to the weed in waterbodies in and around Bangalore. *L. theobromae* and several species of *Drechslera*, *Fusarium*, *Phoma* and *Nigrospora* were also isolated frequently.

*A. eicchorniae*, *A. alternata* and *Cercospora* sp. were investigated for their effect on water hyacinth damaged by weevils (*Neochetina* spp.) and mite (*Orthogalumna terebrantis* Wallwork). The highest disease severity (6.67) was achieved through inoculation of mite-damaged water hyacinth leaves with *A. eicchorniae*. In experiments on the interactive effect, the most potent (disease severity

8.0) treatment was the combination of all the three pathogens. In host-specificity studies, species belonging to 10 plant families (Malvaceae, Papilionaceae, Poaceae, Euphorbiaceae, Rosaceae, Palmae, Rutaceae, Rubiaceae, Anacardiaceae and Myrtaceae) were not susceptible to the three pathogens.

Mass production studies were carried out for *A. alternata*. In shake culture, mean wet and dry weights obtained were 20.95 g and 0.91 g/100 ml, respectively, with a mean CFU of  $1.3 \times 10^4$ /ml. In fermentation studies, mean wet and dry weights were 4.97 g and 0.16 g/100 ml, respectively, with mean CFU of  $7.8 \times 10^4$ /ml. The biomass (only mycelia) obtained through fermentation was formulated into four different bioherbicides, viz., powder, oil emulsion, alginate pellets and pesta granules. Open-air trials were conducted with all the four formulations in buckets. The formulations fared better when sprayed two times than when used once. Among the single-spray treatments, maximum disease severity (7.8) was recorded with the powder formulation and the minimum (4.2) was obtained with pesta granule formulation. Among the two-spray treatments, maximum (8.6) disease severity was achieved with the powder formulation.

Water fern (*Salvinia molesta* D.Mitch.), water lettuce (*Pistia stratiotes* L.) and alligator weed (*Alternanthera philoxeroides* Griseb.) were not susceptible to *Alternaria* and *Cercospora* spp.

#### **17. Biological control of soil borne and other plant pathogenic fungi by antagonistic fungi and development of biofungicides (08/09/1996 to 08/09/2001) PI: Dr. Ravulapalli Durga Prasad**

*Trichoderma* and *Gliocladium* spp. were isolated from rhizosphere samples of various crops. The identified antagonistic fungi included *Trichoderma* (10 species), *Gliocladium* (3 species), *Chaetomium globosum* Kunze (2 species) and *Verticillium lecanii* (Zimmerman) (1). Maximum biomass (1224 mg) was obtained in potato dextrose broth after 96 h of fermentation. Molasses-soy medium (MSM) also gave maximum yield of biomass, viable propagules. Among three carrier materials tested on shelf life of *Trichoderma harzianum* Rifai, talc and kaolin retained more than  $10^6$  viable propagules up to 90 days. Seed treatment with kaolin and talc formulation recorded >50% plant stand. Conidial formulation retained optimum amount of viable propagules ( $>10^6$  cfu/g) after 180 days of storage at room temperature. Against *S. rolfisii* of sunflower *T. harzianum* isolate PDBCTH 2 gave 61.4% inhibition of mycelial growth. The three *T. harzianum* isolates and the *T. viride* isolate (PDBCTV 4) were superior under greenhouse conditions with PDBCTH 8 showing maximum disease control (66.8%) followed by PDBCTH 7 (66.0%), PDBCTV 4 (65.4%), PDBCTH 2 (61.6%) and were even superior to the fungicide, Captan. Studies were also conducted during this period to know the efficacy of various doses of *T. harzianum* at various *Fusarium udum* Butler levels in field. At higher pathogen levels disease control achieved ranged between 22 to 35.3% with soil treatment. Soil application of the bioagent was found to be more effective than seed treatment. The biocontrol efficacy of two isolates of *T. harzianum* was tested against wilt of pigeonpea in a wilt sick plot. Soil amendment with two bioagents (PDBCTH 10 and 15) resulted in a disease incidence of 22.5 and 28.8%, respectively, whereas in seed treatments 35.3 and 40.3% disease incidence was recorded. A rapid *in vivo* bioassay method for screening and selection was developed using *Rhizoctonia solani* J.G. Kühn-chickpea system and *P. capsici* -bell pepper system. *T. viride* (PDBCTV 32) and *T. harzianum* (PDBCTH 10) were rated as efficient bioagents against *R. solani* and three *T. viride* isolates (PBCTV 6, 31, 33, 34 and) were found efficient against *P. capsici*. The results recorded through faster *in vivo* bioassay methods were validated under greenhouse against *R. solani* of chickpea and *P. capsici* of bell pepper. *T. viride* (PDBCTV 32) showed minimum (13.3%) pre-emergence mortality and no post emergence mortality. Bell pepper crown/root rot and fruit rot was significantly reduced by *T. viride* (PDBCTV 31) under greenhouse. Studies on management of black spot of rose caused by *Diplocarpon rosae* F.A.Wolf showed that highest vigour index was recorded in *T. harzianum* treatment. Highest flower production was recorded in *C. globosum*-chlorothalonil treatment (4.33) followed by *T. harzianum* alone and *T. harzianum*-chlorothalonil treatments (4.00).

#### **18. Behaviour ecology of potential parasitoids to enhance their efficacy in biological suppression of key crop pests (01/10/1996 to 31/03/2002) PI: Dr. Purshotam Lal Tandon**

Mixture of four plant volatiles, *i.e.*, pentacosane + Tricosane + heptacosne + octocosane in the ratio of 1:3:1:1 at 50% concentration was quite attractive to four day old *C. carnea* adults as indicated by mean excess proportion index. Eleven plant volatile compounds bioassayed for their activity at six different concentrations revealed that ten of them were effective at 0.05 and 0.1% concentrations. Above these, they were having negative effect. Linalool was effective only at 0.05%. At higher concentrations *i.e.* 0.2, 0.3, 0.4 and 0.5% the insects were turning away from cue and cleaning antennae very fast and frequently. Response of six flowers, *i.e.*, rose, tuberose, goldenrod, *Allium* sp. (Ladies lace) and yellow flower volatiles to *C. carnea* indicated its preference to golden rod and Ladies lace over all other flowers.

Volatiles trapped from bolls of Bt, Non-Bt, Bt with Gauche and non-Bt Gauche were identified using GCMS system. Maximum compounds (20) were identified from Non-Bt cotton, followed by Bt with Gauche and Non-Bt with Gauche (14 each). Minimum compounds (11) were identified from Bt cotton. Non-Bt cotton contained alpha-pinene, undecane, decane and eicosane, which were absent in Bt and other combinations. Octadecane was found alone in Bt cotton in this variety. However, quantitative changes were observed in some compounds like dodecane, limonene and linalool.

Volatile profile of male flowers of different varieties/hybrids of maize: volatiles from fifteen varieties/hybrids of maize. Linalool-L was the most common volatile present in all the 15 varieties/hybrids of maize and varies from 4.23 to 78.44%. Highest quantity was trapped from Godavari Shubham. Limonene was another most prevalent compound identified from 14 varieties and quantity varied from 0.05 to 87.61 per cent. Highest quantity as recorded from American seed Popcorn. Pentanol was another most common compound found in male flower of 12 varieties and the proportion varied from 0.01 to 64.24 per cent. Highest quantity was found in variety Kesari King. Highest quantity of 1-Hexanol was found in variety Seedtek-740 (86.81%). Maximum volatile diversity was found in variety American Popcorn (19), followed by Seedtek Suraj (18).

Volatiles trapped from buds of *Tagetes erecta* L.: The most common compounds were Cis-ocimene, Beta- ocimene, Cis-bisabolene, Beta- bisabolene, Sabinene, Myrcene, Methyl chavicol, Alpha-terpiniole and Phenol 2, 4-bis.

Olfactometric response of *T. chilonis* to five volatile compounds namely, linalool, limonene, alpha- pinene, myrcene, methyle salicylate and combination of linalool+ limonene + M. salicylate was evaluated. Maximum response was recorded towards myrcene (58.66%), which was on par with methyl salicylate (48.66%), alpha-pinene (47.99%), and Limonene (43.33%).

Volatile organic compounds (VOCs) were trapped, isolated and identified from Fern, *Chenopodium* sp. and tulsi leaves (*Ocimum sanctum* L.). Twenty-eight compounds were identified from fern leaves and the major fractions were: trans, trans-2, 4-decadienal, tetradecenal, tetradecanoic acid, hexadecanal, 2-methyl, heneicosane, octadecanoic acid, 9, 17-octadecadienal and muskolectone. In *Chenopodium* sp. most common compounds were plant hydrocarbons. However, main VOCs of tulsi leaves were: citral, methyl eugenol, methyl chavicol,  $\beta$ -caryophyllene and  $\alpha$  and  $\beta$ -bisabolene. These compounds were known for their attractions.

## **19. Biological control of soilborne and other plant pathogens by antagonistic bacteria and development of bacterial biocontrol agents (01/04/1997 to 31/03/2002)** **PI: Dr. Rajagopal Rangeswaran**

A total of 109 rhizospheric isolates were selected as plant growth promoting rhizobacteria (PGPR) from 300 isolates. The isolates were identified as *P. fluorescens* (22), *Bacillus subtilis* Cohn (1), *Burkholderia cepacia* (Palleroni & Holmes) (2), *Pseudomonas putida* Trevisan (1), *Pseudomonas* spp. (28), *Bacillus* sp. (12), *Acaligenes* sp. (1), *Alcaligenes odorans* (Mitchell and Clarke) (1), *Streptomyces* sp. (1) and Fluorescent pseudomonads (30). In greenhouse studies when tested against *S. rolfisii* of sunflower the number of disease free plants was highest (63%) in *P. fluorescens* (PDBCAB 2) and *P. putida* (PDBCAB 19) seed treatments. Against chickpea root pathogens *P. putida* (PDBCAB 19) and *P. fluorescens* (PDBCAB 2) were able to inhibit *F. oxysporum* f. sp. *ciceris* and *R. solani* both under *in vitro* and in greenhouse conditions. Under potted conditions more than 40% inhibition of the pathogens was observed. In pigeonpea, against *F. udum*, *P. putida* (PDBCAB 19) showed 90% inhibition under dual culture and under potted 64% disease control was noticed but *P. fluorescens* (PDBCAB 2) could exhibit only 38% inhibition under potted conditions. Mass

production methodology was standardized for multiplication and formulation of bacterial antagonists. Tryptic Soya Broth (TSB) was found to be suited for all the antagonists tested. Talc with pH amended to 7.0 was a suitable carrier with moisture content of 12 to 14%. Calcium carbonate and carboxymethyl cellulose was used for pH and as binding agent respectively. Field efficacy studies showed that *P. fluorescens* (PDBCAB1 and PDBCAB 2) were effective against both *S. rolfii* of sunflower as well as against *F. oxysporum* f. sp. *ciceri* and *R. solani* of chickpea and suppression was up to 30%. *P. putida* (PDBCAB19) suppressed *F. udum* by 28% and increased yield by 10%.

**20. Biosystematic studies on predatory coccinellids (01/04/1997 to 31/03/2006)  
PI: Dr. Janakiraman Poorani**

An identification guide for about 150 species of coccinellids commonly found in the agroecosystems of the Indian subcontinent was prepared. The guide provides information on the current nomenclature, synonyms, a brief diagnostic description, geographic distribution, prey / associated habitat, seasonal activity and important references pertaining to taxonomy and biology / economic importance, for all the species were included. Colour photographs or illustrations of the habitus were provided for all the species, along with illustrations of other diagnostic characters and genitalia, wherever possible.

A website “Coccinellidae of the Indian Subcontinent” was constructed and hosted on the internet to serve as an identification aid for lady beetles of India and neighbouring countries. The site has image galleries of over 100 species of common coccinellids of the Indian region and their natural enemies. It also provides a checklist of the coccinellids of the Indian region and an account of their morphology to help beginners in coccinellid systematics. URL: [www.angelfire.com/bug2/j\\_poorani/index.html](http://www.angelfire.com/bug2/j_poorani/index.html)

An interactive key to 10 common species of *Chilocorus* of the Indian region was constructed on Lucid Phoenix software and was hosted at [www.lucidcentral.org](http://www.lucidcentral.org)

A compendium of fact sheets on ~140 common species of coccinellids found in the Indian region prepared with details on diagnosis, distribution, hosts, and other relevant information along with colour illustrations A CD entitled “A Pictorial Guide to Insect Natural Enemies in Biological Control” (including common Coccinellidae) was brought out as a priced publication of PDBC for the benefit of economic entomologists, students and biocontrol workers.

**21. Development of national information system on biological suppression of crop pests (01/04/1997 to 31/03/2005) PI: Mr. Santi Ranjan Biswas**

The software INFOBASE, a user friendly-menu driven, self explanatory software containing information on biocontrol resources in the country was developed. This software contains details about different industries manufacturing different biological control agents in the country and is useful for farmers, extension workers, industry, entomologists, biocontrol practitioners, students, teachers and research managers to enhance their knowledge base.

**22. Introduction and studies on the exotic natural enemies of some dipterous and homopterous insect pests (01/04/1997 to 31/03/2002) PI: Dr. Basavaraj Shidlingappa Bhumannavar**

*Liriomyza trifolii* (Burgess): Culture of *L. trifolii* was maintained on the seedlings of french bean and cotton. The seedlings of these two host plants were raised in pots and were enclosed in acrylic sheet cage. Ten days old seedlings were infested with freshly emerged adults from the field infested leaves. New seedlings were raised once in 15 days to maintain the culture of *L. trifolii*.

*H. cubana*: Culture of *H. cubana* was maintained on small bushes of subabul. The branches were pruned to regulate the availability of new and tender shoots/ leaves, which were required for the breeding of the psylla.

*Aleurodicus disperses* Russell: The culture of *A. dispersus* was raised on seedlings of cotton and seedlings of *Bauhinia* after it failed to develop on canna plants. It was not possible to mass rear *A.*

*dispersus* in captivity on any of the hosts tried though a small population was always maintained on cotton seedlings.

Identification of suitable host plant for the mass rearing of *L. trifolii*: The seedlings of french bean, cowpea, bottle gourd, pumpkin, gherkin, snake gourd and cucumber were used for testing. It was found that only French bean seedlings were more suitable than cucurbits. The main problem in cucurbits was unhealthy seedlings, improper leaf structure and insufficient leaf area. French bean seedlings were found more suitable than cowpea and 22 generations of *L. trifolii* were reared.

Adult survivability studies revealed that when food was provided, females had longevity up to 19 days with an average of 10.1 days whereas male longevity was up to 17 days with an average of 6.3 days. When food was not provided females and males lived only for 2 days. Male and female with food survived for 11 days (average 9.7 days).

Oviposition studies indicated that pre-oviposition period varied from 4 to 11 days (mean 7.2 days) and oviposition period from 4 to 14 days (mean 9.4 days). A female laid as low as 31 and as high as 227 eggs (mean 122.2 eggs) during her life span. Adult longevity varied from 12 to 19 days (mean 16.6 days).

Mass rearing the American serpentine leaf-miner, *L. trifolii* in the laboratory unit consisted of the larval/ pupal recovery units with a funnel supported with a stand, enclosure with a lid. The narrow end of the funnel is placed in a glass/ plastic vial. The seedlings were kept in top enclosure and lid was placed. It was advisable to keep a maximum of 6 polythene bags in slanting position in each enclosure. The larva wriggles out of the mine and passes through the funnel and finally gets collected directly into the vial. Some of the larvae pupate over the leaf surfaces and were collected manually by a wet brush. The vial containing larvae/ pupae were replaced with empty vial every day. The pupae collected each day were kept for adult emergence. Pupae can be stored in lower compartments of refrigerator for the augmentation of continuous adult supply for oviposition.

Culturing the promising indigenous natural enemies of *L. Trifolii* indicated two of the parasitoids could be multiplied for two generations and thereafter, only one species of the parasitoids was multiplied successfully. The available keys suggest that the parasitoids might be *Chrysonotomyia ?appanni*. This parasitoid appears to be poor performer as the per cent parasitisation in the test samples was less than 5%. Efforts were made to improve the per cent parasitisation by altering the host population and as well temperature and humidity. More than two generations were reared till March 1996. The available keys for the identification of parasitoids of *L. trifolii* did not include Asian species. Due to this the indigenous species could not be identified. However Dr. Farooqui, IARI, New Delhi had developed a key for Asian species and also identified few parasitoids of *L. trifolii*. The comparison of the identified species revealed that the species which we were multiplying could be *Chrysonotomyia? appanni*. Exotic natural enemy *Diglyphus begini* (Ashmead) was received during March, 1997 from California. It was successfully quarantined and pure culture was developed in quarantine laboratory. Import permit for the importation of *Encarsia? haitiensis* Dozier was obtained in 1997, no funds were available for importing it from Guam as it was costing \$ 2000 (Rs. 10 lakhs approximately). Lakshadweep islands were surveyed for the natural enemies of spiralling whitefly, *A. dispersus*. Five islands, namely, Kalpeni, Kavaratti, Minicoy, Viringili and Andrott, were visited. The farthest island in the south was Minicoy, which was closest to Maldives where an effort to establish *Encarsia* spp. was made a few years ago. Spiralling whitefly was recorded on 11 host plants (banana, papaya, crotons, *Thevetia neriifolia* Juss., chillies, capsicum, *Erythrina indica* Lam., coconut, bread fruit, *Gliricidia* and *Hibiscus tiliaceus* L.) from Kavaratti and on 32 hosts (guava, *Antigonon*, *Ficus benghalensis* L., *Ficus* sp., *Plumeria alba* L., hibiscus, *Polyalthia longifolia* Sonn., brinjal, *Terminalia catappa* L., *Calotropis gigantea* (L.), castor, *Abutilon indicum* L., *Acalypha indica* L., mulberry, *Bidens pilosa* L., *Cleome pentandra* L., *Syzygium* sp., *Ochrosia* sp., neem and two undetermined hosts, besides those recorded in Kavaratti) from Minicoy. Whitefly incidence was not recorded in Andrott and Kalpeni. Parasitised whitefly samples and the parasitoid, *Encarsia* spp. were collected. At least two species were present and at least one of them seems different from the species found in the mainland in Kerala and in Bangalore. Attempts to identify them were made. Only two predators, namely, *Axinoscymnus puttarudriahi* Kapur & Munshi and *Cybocephalus* sp. were recorded on *A. dispersus*.

Two aphelinid parasitoids, *Encarsia guadeloupeae* Viggiani (reported for the first time) and *Encarsia* sp. nr. *haitiensis* Dozier were found in the Minicoy Island in addition to some predators.

They were introduced serendipitously into our country. They were also successfully introduced into the mainland and established very well in areas in and around Bangalore. *Encarsia* sp. nr. *haitiensis* has already been reported from Bangalore and Kerala.

The islands of Minicoy, Amini, Kadmath, Agatti and Kavaratti were surveyed during March 2000. The whitefly was found only on 11 host plants in Minicoy, 34 host plants in Amini and 54 host plants in Kavaratti. The pest was found to be severe on banana, papaya, tapioca, guava, etc. A host plant list for the islands with the whitefly was prepared. The presence of the coccinellid predator, *A. puttarudriahi* was found in one plot of banana in Kavaratti where the infestation was also found to be less. Amongst the five islands in the Lakshadweep the infestation of the whitefly was only seen in Minicoy, Kavaratti and Agatti and the parasitoids were not seen in the last two islands and the infestation very severe in them. The activity of the parasitoids, *Encarsia* (?) *haitiensis* and *E. guadeloupae* was seen in Minicoy and the infestation of whitefly was also found to be less severe as compared to last year. *A. puttarudriahi* and *Cybocephalus* sp. was also seen active there. The Agric. Dept. officials were fully briefed about the method of parasitisation, identification of the adult parasitoids and parasitised whitefly nymph, differentiation of the emergence holes of the parasitoid and the pest, etc. so that they can collect the same and redistribute the same to other islands like Kavaratti and Agatti, where the infestation was severe and the parasitoids were not present. Introduction of these parasitoids were initiated in the islands of Kavaratti and Agatti. Survey in and around Bangalore for natural enemies revealed in addition to the above two aphelinids, the presence of *A. puttarudriahi*, *Cybocephalus* sp., *C. montrouzieri*, *Acletoxenus* sp., *Notobiella* sp., *Leucopis* sp. and an unidentified coneopterygid. The aphelinids had spread to many areas and were observed up to Tumkur and also recorded in Amruthahalli and near by areas. The presence of the whitefly was seen in many areas and the pest was recorded on tapioca (61.4 nymphs/leaf), guava (14.7 nymphs/leaf and 22.5 nymphs/leaf), *Cassia* sp. (10.1/leaf).

The biology of *A. puttarudriahi* and *Cybocephalus* sp. when fed on the whitefly revealed that the egg period was 4 days (both), larval period- 7-8 (both) and pupal period 5-6 days and 16-17 days, respectively. The total period for completing the cycle from egg to adult was 16-18 days for the coccinellid and 27-29 days for the nitidulid. The females of *A. puttarudriahi* lived for 31-47 days and laid 51-134 eggs while those of *Cybocephalus* sp. lived for 90 days and laid 112 eggs. The feeding potential of *A. puttarudriahi* was worked out by providing 8 whitefly nymphs per day and they fed on 124-133 nymphs during their adult life of 48-64 days. A larval parasitoid *Zeteticontus* sp. of *Cybocephalus* sp. was recorded from the field.

### **23. Introduction and studies on the exotic natural enemies of some lepidopterous insect pests (01/04/1997 to 31/03/2002) PI: Dr. Srinivasan Ramani**

*A. pyralophagus*, a Mexican larval braconid parasitoid was imported in to our country earlier for the control of graminaceous stem borers was taken up for certain investigations. The longevity of 15 females as also the per cent larvae of *C. partellus* parasitized and adult parasitoids emerged was presented. The results showed that *A. pyralophagus* adult females lived on an average for 40.87 days (Range 18-47 days) and each female parasitized 48.49% of the larvae presented to them during their lifetime. There was a high proportion of females in the adults emerged from these parasitized larvae with an average of 8.19 proportion of females and an average production capacity of 52.3 adults per female parasitoid was recorded. The maximum larvae were parasitized (100, and 93.3 per cent) by 7-9 days old females and this slowly reduced as the age advanced to 10% by females (36 days old). The mean larvae parasitized were 56.49%. Similarly the number of adults emerging per larva parasitized was also high for 7-9 days females, being 12.40 and 6.87 adults /larva with an average of 4.41 adults / larva for the entire life of the females. The trend for proportion of females was also similar with 7-9 day old females showing maximum proportion of females (22.25 and 13.71).

Influence of *C. partellus* larval weight on parasitization by *A. pyralophagus* showed that an average of 7.94 adults (Range 1 to 24) emerged from each larva irrespective of the weight. There was a greater proportion of females amongst the emerged adults (7.56). A correlation was worked out to know the influence of larval weight on adults emerged and proportion of females in them. There was a strong positive relationship between the weight of larvae and emergence of parasitoid adults per

larva ( $r=0.296^{**}$ ). The relationship was also positive between the larval weight and proportion of females emerged from each larva ( $r=0.285^{**}$ ) when the regression equations were plotted.

The host suitability of the laboratory host *C. cephalonica* to rear the Mexican parasitoids *A. pyralophagus* indicated that *A. pyralophagus* could parasitise *C. cephalonica* also but the adult yield and per cent adult emergence were poor. Though *C. cephalonica* could be used as an alternate host the performance of the parasitoids was not as good as when *C. partellus* was used as a laboratory host.

*C. partellus* larvae of uniform size were provided to mated females of the parasitoids *C. flavipes* to study the ovipositional and other aspects of the life cycle of the parasitoids. The results revealed that the egg and larval period of the parasitoids was 14.56 days (Range 13-17 days) when they formed cocoons outside the host larva. The pupal period was 8.36 days (7-11 days). The number of cocoons from each host larva was 36.73 (5-80) with a per cent adult emergence of 76.82. The sex ratio was female biased with 3.82 females for every male parasitoid emerged. *C. partellus* larvae were individually weighed and presented to mated females of *C. flavipes* in a patch of diet. The data on the weight of host larva, the total emergence of parasitoids and the number of females emerged was subjected to correlation analysis to know the influence of larval weight on parasitoids emergence. There was no great influence of the host weight on the number of females amongst them. The 'r' values recorded were 0.1313 and 0.2183, respectively, which were not significant. No linear relationship between the weight of larva and parasitoid adult emergence was observed.

The results on performance of *T. chilonis* on average size egg clusters of *C. partellus* showed that as the number of parasitoid females increased from 1 to 5 there was a steady increase in per cent eggs parasitized with 1 female recording 37.83%, 3 females 82.53% and 5 females 82.82%. Similarly there was also an increase in the number of adults emerged with 1 female recording 4.8 adults/cluster, 3 females 6.3 adults/cluster and 5 females 11.50 adults/cluster. However, there was no great variation in the sex ratios of the emerged adult parasitoids with the treatments recording about 1: 0.5 (female: male).

The results on biological studies on *Cotesia plutellae* (Kurdjumov), a larval parasitoid of *P. xylostella* showed that an average of 21.12% of the larvae were only parasitized (Range 4.00 to 50%) while 84% of the cocoons formed emerged as adults. However, overall only 17.88% of the larvae presented were parasitized and parasitoid adult emergence was successful showing that the per cent parasitism by *C. plutellae* was low. The parasitized larvae were observed for cocoon formation individually and the emerging adults sexed to find out the larval and pupal periods of male and female parasitoids. The males had a larval period of 7.52 days and a pupal period of 3.76 days while the female parasitoids took 7.72 days to complete larval development and 4.05 days to emerge as adults after cocoon formation. The influence of parasitoid female age on per cent larvae parasitized and adult emergence from them showed that the parasitism was bimodal with 2 day old female parasitizing about 35% larvae which reduced to almost 3% in 4 day old females. This subsequently increased to nearly 50% in days old females and then gradually tapered down as the female age increased to 11 days.

Two parasitoids of the coffee berry borer were imported from Mexico for quarantine screening in the laboratory in a collaborative attempt with Coffee Board. The two parasitoids, *Cephalonomia stephanoderis* Betrem and *Prorops nasuta* Waterston were brought from Mexico during the return from there and later two more consignments were received from there. Rearing procedures were perfected using berry borer infested berries, parasitoids were released at a ratio from 1:5 to 1:10 parasitoids to infested berries and placed in ventilated bread boxes. Parasitoids emerged from 21 days after setting up the cultures and continued to emerge even up to 80 days. The emerged parasitoids were utilised for further multiplication. A higher humidity level of 70-90 per cent gave good emergence with temperature being 28-30°C. *C. stephanoderis* could be fed on honey for short periods but *P. nasuta* needs to be fed with berry borer stages alone as adults. Field releases of both the parasitoids were made in Kodagu and recoveries were made after releases. The cultures were being handed over to the Coffee Board for further multiplication and field testing.

**24. Software development for identifying and suggesting biocontrol measures of different crop-pests using PC (01/04/1997 to 31/03/2005) PI: Mr. Santi Ranjan Biswas**

Software BIOCOT was developed for accessing information on biological control of cotton pests, BIORICE was developed for accessing information on biological control of rice pests, SUGARCANE BIOCONTROL was developed for accessing information on biological control of sugarcane pests. The information is available on the pests of crops, their natural enemies, distribution maps, different IPM and biocontrol measures. The information is provided in MS-Access mode is menu driven and easily accessible using a PC.

**25. Knowledge-base system of *Helicoverpa armigera* and its natural enemies (01/10/1999 to 31/03/2003) PI: Dr. Maria Pratheepa**

‘Helico-Info’ is a windows-based unique computer database which enables researchers to have easy access to information on aspects of bionomics, distribution in India, host plants, effect of abiotic factors on the population and list of natural enemies relating to *H. armigera*. This database was developed in MS-Access97. A Biocontrol measure for the pest on different crops is also provided. The database gives a comprehensive list of other *Helicoverpa/Heliiothis* species. ‘Helico-Info’ is user-friendly computer software and persons having basic computer knowledge could utilize it effectively.

**26. Decision support system of safer pesticides for natural enemies (01/06/2000 to 31/05/2003) PI: Dr. Maria Pratheepa**

Decision Support System (DSS) for “Saferpesticides in Biocontrol” was developed to provide information on the identification of natural enemies, the safer pesticides on natural enemies and price list of natural enemies maintained at NBAIR and the dosage of safer pesticides for the control of the pest and safety to natural enemies. This DSS was developed by using Visual Basic 6.0 and the development of databasewas in MS-Access. The ODBC connector was used for connecting or accessing the MS-Access database in the Visual Basic. Forms were generated and visual controls were created in Visual Basic. Computer programs were written in Visual Basic to access the information in a user friendly manner. The user can get the list of natural enemies with photographs and safer pesticides for those natural enemies. The recommended dosage of biocontrol agents, on various pests with application methods were given in the software for the specific crop, pest and biotic agent. This software gives information about the live cultures of parasitoids, predators and pathogens maintained at NBAIR (formerly PDBC) for the supply for research and utility purposes.

This software is available in the form of CD and this CD is a handy tool for the students, researchers and farmers to know about the natural enemies and pesticides safer to them.

**27. Biocontrol of insect pests using entomopathogenic fungi and development of mycoinsecticides (01/04/2001 to 31/03/2004) PI: Dr. Bonam Ramanujam**

Sabouraud’s Maltose Agar Yeast (SMAY) medium incorporated with chloramphenical (50 ppm) or rosebengal (100 ppm) can be used for isolation of *Nomuraea rileyi* (Farl.) without contamination from the field infected larvae of *H. armigera* and *S.litura*.

Thirty six isolates of *N. rileyi*, 12 isolates of *Beauveria bassiana* (Bals.-Criv), seven isolates of *Metarhizium anisopliae* (Metchnikoff), 18 isolates of *V. lecanii* from various hosts and an isolate of *Paecilomyces fumosoroseus* (Wize), *Fusarium sambucinum* (Fr.) Sacc. and *F. pallodoroseum* were collected and added to the germplasm collection of PDBC. Among the different isolates tested for their virulence against *H. armigera*, Nr-26 and Nr-17 of *N. rileyi* and against *S. litura*, Nr-17, Nr-26, Nr-3, Nr-7 and Nr-12 showed 100% mortality of the 3<sup>rd</sup> instar larvae. Bb-6 and Bb-1 isolates of *B. bassiana*, and Ma-5 and Ma-1 of *M. anisopliae* showed maximum mortality of *P. xylostella* (88.62, 84.47, 78.44 and 76.23 per cent respectively) in laboratory bioassay studies.

*F. sambucinum* showed adult mortality of 73.6% and nymphal mortality of 63.8% of rose aphid (*Macrosiphum rosaeiformis* Theobald). B.b-5a isolate of *B. bassiana* showed maximum mortality of three aphid species, viz., *A. craccivora*, *A. gossypii* and *R. maidis* (74.00, 80.80 and 50 per cent mortality respectively).

*M. anisopliae* (Ma-4) showed maximum mortality of sugarcane wooly aphid in the field trials (31.68% mycosis). Yeast granules were found to be suitable for mass production of *N. rileyi* on rice grains which was much cheaper than the yeast extract. Addition of 25 ml of 5% yeast granule solution to the basal medium of rice gave good sporulation of *N. rileyi*.

Among the different liquid media tested for mass production of five isolates of *N. rileyi*, Rice extract (5%) + Yeast granules (5%) showed maximum sporulation of all isolates. Optimum incubation temperature for maximum conidial production of five isolates of *N. rileyi* on Rice+ 5% Yeast granules was found to be at 25°C. Optimum humidity range for maximum conidial production of five isolates of *N. rileyi* on Rice+ 5% Yeast granules was found to be at 70-90 per cent RH. Spores of all five isolates of *N. rileyi* remained viable in sufficient numbers ( $10^8$  /g) for a period of four months when stored in Rice flour at room temperature. After six months of storage in rice flour, the spore count reduced drastically for all isolates both at room temperature ( $10^5$ - $10^6$ /g) and refrigerator temperature ( $10^6$ - $10^7$ /g). No significant reduction in the infectivity of *N. rileyi* spores stored in Rice flour for a period of 120 days at room and refrigerated temperature was observed. Maximum conidial germination of *N. rileyi* was observed in sunflower oil (79.8%), Gingelly oil (75.3%), Diesel (71.5%) and Kerosene (68.3%) which can be used in preparation of oil formulation of *N. rileyi*.

B.b-5a, Ma-4 and VI-5 isolates were identified as fast growing and highly sporulating among the different isolates of *B. bassiana*, *M. anisopliae* and *V. lecanii* based on the studies in Potato Dextrose Broth (PDB) and Rice. Among the oil cakes tested, maximum sporulation of *B. bassiana*, *M. anisopliae* and *V. lecanii* were observed with pongamia ( $3 \times 10^9$ ,  $2 \times 10^9$  and  $1.1 \times 10^9$  spores/g respectively) followed by castor cakes ( $2 \times 10^9$ ,  $2 \times 10^9$  and  $1 \times 10^9$  spores/g respectively). Dried silk worm pupal powder also served as good substrate for *B. bassiana* ( $1.3 \times 10^9$  spores/g) and *M. anisopliae* ( $3.6 \times 10^9$  spores/g) apart from sorghum grain.

Spent malt + brewers yeast (10: 1) gave good sporulation of *M. anisopliae* ( $1.7 \times 10^9$  spores/g). Mass production of *V. lecanii* using soya flour molasses medium in laboratory grade fermentor was standardized. A talc based formulation of *V. lecanii* was developed using fungal biomass from fermentor. Copper oxy chloride (0.3%), Wettable sulphur, (0.3%), Bordaux mixture (1%), was found to be less toxic to *N. rileyi* isolates while Fenvelerate was compatible with *N. rileyi*.

## **28. Biological suppression of plant parasitic nematodes exploiting antagonistic fungi and bacteria in specific cropping systems (01/04/2001 to 31/03/2006) PI: Dr. Mandadi Nagesh**

Survey for indigenous isolates of antagonistic fungi and bacteria: In *toto* six isolates of *Pochonia chlamydosporia* (Goddard); four of *Paecilomyces lilacinus* (Thom); eight isolates of *Trichoderma*; one isolate of *Arthrobotrys oligospora* Fresen. and one isolate of *P. fluorescens* were isolated under this project from different crop rhizospheres and agro-climatic situations. These isolates were effective against cyst, reniform and root-knot nematodes.

A serial dilution technique with water agar/CMA on specific selective media for isolating nematode egg-mass infective fungi from soil/root homogenate and for evaluating the antagonism against the nematode eggs was evolved. A simple and rapid technique to evaluate several indigenous isolates of antagonistic fungi for consistent results on their effect on root colonization, parasitism and multiplication in rhizosphere was evolved.

Molecular identity of native isolates *P. chlamydosporia* at PDBC established through sequencing the  $\beta$ - tubulin gene (1 to 233 bases) was deposited and registered in the Genebank, NCBI, Maryland, USA. Broken barley and corn were identified as ideal solid media for mass production of *A. oligospora* (PDBC AOI) based on the production cycle, yield per cycle and caking. The pH and temperature optimum for *A. oligospora* (PDBC AOI) were found to be 5.5 to 7.5 and 25 to 35<sup>o</sup> C, respectively.

Identified native isolates of *P. lilacinus* and *Trichoderma* sp. that can tolerate common soil fungicides *viz.*, copper oxy chloride, and metalaxyl mancozeb 72 WP and potassium phosphonate at different ppm isolates. Studies using split – root technique indicated that the plant growth was promoted (in terms of plant height, fresh weight and dry weight) in treated plants than in untreated, but the resistance to nematodes infection induced by *P. fluorescens* at the dose of applied was

localized and did not record systemic resistance. Identified talc, sawdust and vermiculite as best carrier material for *P. lilacinus* for better spore viability even up to 10 minutes of storage.

Solid media, viz., malt waste, wheat bran and sorghum grain identified to yield higher number of *P. chlamydosporia* chlamydospores per g of substrate. Among liquid media corn meal extract, barely salt broth and cotton seedmeal extract yielded higher chlamydospores. Solid, liquid and di-phasic media from natural sources were identified and protocols for enhancing their production cycles and yields in a given set of conditionals was standardized.

Incorporation of *P. lilacinus* or *P. chlamydosporia* talc formulation at 20-30 Kg/ha along with 200kg / ha of vermicompost in furrows before sowing gherkin seeds recorded 54-72 per cent infection of egg masses, reduction in the nematode population by 22-38 per cent in soil and 38-66 per cent reduction in root-knot nematode infection in roots, in 75 days of crop growth.

Demonstrated effective control of root-knot nematode on gherkins in farmers' field, using a combination of talc formulation of *P. lilacinus* and neem cake, which was comparable to application of phorate/phenamiphos. Demonstrated talc formulations of *P. lilacinus* and *P. chlamydosporia* reduced potato cysts by 62-68 per cent in 2 years in potato, and by pigeon pea cyst nematodes by 59-60 per cent in pigeon pea. Phytophthora root-knot nematode wilt complex in black pepper was effectively controlled by planting pre-treated cuttings with mancozeb 72 WP and akomin in combination with native isolates of *Trichoderma* sp. and *P. lilacinus*. Incorporation of *P. lilacinus* and *T. harzianum* prior to tomato transplantation effectively controlled Fusarium wilt in tomato even in presence of root-knot nematodes.

**29. Development of mass production techniques for dipteran (Diptera: Cecidomyiidae) and acarine (Arachnida: Acarina) predators for use in biological control programmes. (This project was reoriented to focus on the sugarcane woolly aphid and its natural enemies) (01/04/2001 to 30/04/2006) PI: Dr. Prashanth Mohanraj**

*Stethorus pauperculus* Weise and *Oligota* sp., two predators of spider mites were studied. While the fecundity and longevity of the former were 162 eggs and 14 days that of the latter were 63 eggs and 12 days when reared on *Tetranychus neocaledonicus* André.

A rearing protocol was developed to produce *Amblyseius longispinosus* Evans for use in the biological control of mites. The phytophagous mites *T. neocaledonicus* André were first multiplied on cowpea seedlings. From an initial population of two spider mites per leaf a population of 50-55 mites could be built up in one week. *A. longispinosus* could then be multiplied on these mites. At a predator: prey ratio of 1:50, 400 to 500 phytoseiids could be produced from a pair in 12 days. Prey density was found to be a crucial factor in increasing egg laying by *S. pauperculus* females. Higher prey densities resulted in higher egg laying. Wet sand was found to be essential for pupation by *Oligota* sp. pupal mortality was nevertheless very high with less than one per cent of the pupae developing into adults. Cotton and vermiculite were found to be unsuitable for pupation. Regulation of moisture levels in the sand was the most important factor for pupation.

*S. pauperculus* and *Oligota* sp. were more voracious than *A. Neocaledonicus*, while *S. pauperculus* fed on 16 – 44 mites per day, *Oligota* sp. fed on 18 – 44 mites per day. *A. neocaledonicus* on the other hand fed on only on 6 – 7 mites per day. *S. pauperculus* was therefore the most promising of the three predators from the rate of multiplication point of view. But *A. neocaledonicus* was the species that could be multiplied with the greatest ease.

This project was subsequently reoriented and work on the sugarcane woolly aphid and its natural enemies was commenced as this pest assumed pest proportions in Karnataka, Maharashtra and other sugarcane growing regions of the country. Surveys were initially conducted in the northern districts of Karnataka and western Maharashtra to monitor for the intensity of infestation by the sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner as well as the occurrence of natural enemies. The natural enemies collected from the field were all predators – *Dipha aphidivora* (Meyrick), *Dideopsis aegrota* (Fabricius), *C. sexmaculata*, *Scymnus* sp. and *Anisolemnia dilatata* (Fabricius).

*Synonycha grandis* (Thunberg) were collected from colonies of the bamboo aphid *Pseudoregma bambusicola* (Takahashi) as they were reportedly successful in suppressing the

sugarcane woolly aphid in China. *Micromus igorotus* Banks was later collected from almost all localities in N. Karnataka and Pune, Maharashtra.

In the effort to develop multiplication techniques as well as to have predators for initial test of efficacy against the woolly aphid the life cycles of *A. dilatata* ( $19.3 \pm 1.2$  days), *S. grandis* ( $21.3 \pm 1.3$  days on *P. bambusicola* and  $21.3 \pm 0.5$  days on *A. craccivora*), *Scymnus* sp. ( $19.3 \pm 1.5$  days), *M. igorotus* ( $17.5 \pm 1.2$  days) and *D. aphidivora* ( $5.4 \pm 1.7$  days) were studied, while *Micromus* and *D. aphidivora* could be reared successfully on *A. craccivora* as alternative prey *S. grandis* and *A. dilatata* had to be given a diet of *P. bambusicola* to complete their life cycles. *D. aphidivora* could be reared on frozen woolly aphids but the larval period was extended by 2-3 days there was over 60% reduction in pupation. Adult emergence was also reduced by 15%. *D. aphidivora* also completed its life cycle on *F. virgata*, but it fed only on crawlers and not on mature adults of the mealybug.

District wise surveys in N. Karnataka and in Pune indicated that the predators occurred at different intensities at different places. Seasons also had an impact on the population levels of the predators with the syrphids occurring in the cooler months while *Dipha* and *Micromus* occurred throughout the year.

A comparative study to determine soldier allocation between *P. bambusicola* and *C. lanigera* indicated a larger proportion of soldiers in colonies of the bamboo aphid (22 – 24 per cent) than in those of the sugarcane woolly aphid (10 – 12 per cent). Colony size and colony density were positively correlated to number of soldiers. No relationship was found with the number of natural enemies. Within plant distribution of the sugarcane woolly aphid indicated that the greatest concentration of aphids was on the middle leaves (7<sup>th</sup> to 9<sup>th</sup>) than on others.

A freeze dried liver based semisynthetic diet was developed in collaboration with the Biotechnology laboratory, NBAIR (formerly PDBC). This diet needs further refinement for large scale production of the moth.

Both field and laboratory studies were conducted to test the efficacy of *S. grandis* as a biocontrol agent for the sugarcane woolly aphid. It was found that the lady beetles failed to lay eggs in confinement while they were not effective in suppressing populations of the Woolly aphid of sugarcane in the field even when confined in net enclosures.

### **30. Biosystematic studies on Indian Tachinidae (01/08/2001 to 31/08/2006)**

**PI: Dr. Srinivasan Ramani**

Specimens belonging to three subfamilies of Tachinidae, viz., Goniinae, Tachininae and Proseninae were studied. Specimens from lepidopterous hosts belonging to the genera *Carcelia*, *Palexorista*, *Sturmiopsis*, *Goniophthalmus* and *Winthemia* were examined for characters and placement. Tachinids reared from *H. armigera* feeding on cotton collected from Jalgaon, Barwah, Khandwa, etc. were found to belong to *Carcelia* and *Palexorista*. A species of *Winthemia* was found attacking the fruit piercing moth, *Othreis maternal* (L.). Other hosts from which tachinids were collected were *C. infuscatellus*, *Bombyx mori* (Linnaeus), *Spilosoma* spp., *Amsacta albistriga* (Walker), *Trichoplusia* spp. The higher classification of family was analysed. The family contains four subfamilies and can be recognised by the following characters. Goniinae – hairy eyes, parasitoids of Lepidoptera; Tachininae – hairy eyes, prosternum bare, parasitoids of Lepidoptera, Coleoptera, Orthoptera; Dexiinae (=Proseninae) - bare eyes, parasitoids of Coleoptera; Phasiinae – bare eyes, parasitoids of Hemiptera.

Tribes of the subfamily Goniinae include Acemyini, Blondeliini, Carceliini, Eryciini, Exoristini, Goniini, Sphonini, Sturmiini and Winthemiini and species from all these tribes were studied. Tribes of the subfamily Tachininae studied include Tachinini, Ernestini and Voriini and specimens studied from all these tribes. Tachinidae (Phasiinae) from some hemipteran hosts were recorded for the first time. *Lophosia excise* Tothill (Cylindromyiini) was recorded from *Cyclopelta siccifolia* Westwood from Shimoga and *Cylindromyia rufipes* Meigen (Cylindromyiini) from *Tolumnia* sp. (Pentatomidae) from Bangalore. There were nearly 10,000 described species of Tachinidae in the world but the actual size of the family was much larger because the Neotropical, Afrotropical, Oriental and Australasian regions contain large numbers of undescribed species.

*Sturmiopsis inferens* Townsend widespread in the Oriental Region – recorded on Pyralidae and Noctuidae. *S. inferens* introduced to Africa, Madagascar and Mauritius. *Sturmiopsis parasitica*

(Curran), *Sturmiopsis emdeni* Mesnil found in Africa and Israel. *Sturmiopsis angustifrons* Mesnil synonymised with *S. parasitica*. *S. parasitica* present in CIBC collections (introduced to India in 1974). Based on descriptions of *S. emdeni*, it seems to be a synonym of *S. inferens*.

The checklist for the Indian Tachinidae including all taxa under the family recorded from the Indian subcontinent was prepared. The family was grouped under four subfamilies. The checklist has the following numbers of tribes, genera and species under different subfamilies, 325 species under 163 genera, 33 tribes and 4 subfamilies.

*Chaetogena raoi* (Mesnil), *Drino (Palexorista)* sp. and *Exorista xanthaspis* Wiedemann were recorded on the groundnut red hairy caterpillar, *A. albistriga* from Pavagada and other areas in Tumkur. *Palexorista solennis* Walker was recorded on *Crocidolomia pavonana* (Fabricius). Parasitism by *Carcelia illota* Curran to an extent of 11% and *Goniophthalmus halli* Mesnil to an extent of 2% was recorded on *H. armigera* on red gram and lab lab. Parasitism by *S. inferens* to an extent of 5% was recorded on *C. partellus*. In the chickpea crop infested with *H. armigera*, *C. illota*, *Drino (Palexorista) laxa* (Curran) and *Exorista japonica* Townsend were recorded while from the same pest on redgram was parasitized by *C. illota*, *D. laxa*, *G. halli* and *Peribaea orbata* Wiedemann.

**31. Development and evaluation of artificial diets for *Opisina arenosella* and *Plutella xylostella* and studies on host-parasitoid interrelations (01/04/2002 to 31/03/2006)  
PI: Dr. Kotilingam Srinivasa Murthy**

Developed semi-synthetic diets for important lepidopterous insects, viz., *O. arenosella*, *P. xylostella*, *S. exigua*, *Crocidolomia binotalis* Zeller and for *Parthenium* beetle, *Zygomma bicolorata* Pallister in the laboratory. Toddy palm leaf powder with soya powder diet developed for mass production of *O. arenosella*. The shelf life of the diet at 25-27°C and 65% RH was 20-22 days. The cost of diet reared pupa was Rs. 0.78 (73.2% pupation). The late larval parasitoid *Goniozus nephantidis* (Muesebeck) and pupal parasitoids *Brachymeria nosatoi* Habu and *Brachymeria nephantidis* Gahan were multiplied on the artificial diet reared host. The percentage parasitism was on par with those when reared on natural hosts.

Cabbage leaf powder and defatted soya based diet was suitable for *P. xylostella*. The biological attributes were comparable with those reared on natural host (Cabbage). The cost of rearing a pupa worked out to Rs. 0.38, based on 62.6% pupation. The shelf life was 30 days at 5°C. The parasitoid *Cotesia vestalis* (Haliday) (= *plutellae*) was multiplied on the artificial diet reared host. All the biological attributes when reared on semi synthetic diet were comparable with natural diet.

The artificial diet for *P. xylostella* was effective for rearing *C. binotalis*. *S. exigua* was reared on Kabuli gram powder and defatted soya based diet. Leaf extract of *Parthenium* based diet was developed for *Z. bicolorata*. The grub survival and pupation on diet was 48.2 and 36.4%, respectively. Artificial diets substitute the necessity to rear the insects on natural plants and facilitate continuous availability of the host for rearing their natural enemies.

**32. Development and formulation of artificial diets for the rearing of coccinellids and anthocorids (01/04/2002 to 31/03/2006) PI: Dr. Thiruvengadam Venkatesan**

Among different semisynthetic diets tested, beef-liver based artificial diet was effective in rearing *C. sexmaculata* and the per cent survival was 63 and there was no significant difference between artificial and natural diet (68.5 %) reared predators. Aphid reared adults survived for 78.0 days, which was significantly different as compared to artificial diet reared predators. Larvae reared on artificial diet consumed 238.0 aphids during the larval period, which was at par with natural diet (245.0). Pupation and adult emergence of *C. montrouzieri* on artificial diet were 75.9 and 69.4 per cent, which was significantly less when compared to natural diet (90 and 89.02 per cent) respectively. Maximum number of *C. montrouzieri* larvae was obtained in natural diet reared *C. montrouzieri* (253.7 larvae/female), which was significantly more than the artificial diet reared (238.25). Two-three days old larvae of *C. nigrita* were reared on artificial diet and the per cent pupation and adult emergence was 62 and 57, respectively, as compared to natural diet (*H. lataniae* scales) 81.0 and 75 per cent respectively. *C. exiguus* was reared on artificial diet. Adult formation was highest in *Corcyra* eggs (91%), which was significantly more than the *O. arenosella* (87.0) and artificial diet reared

(80.4). *Orius tantillus* (Motschulsky) could be reared on a combination of artificial diet plus maize pollen and green beans. Three-four days old *D. aphidivora* larvae were reared on artificial diet and the per cent survival was 45. The adult moth lived for 7 days and laid fertile eggs.

**33. Host derived kairomones to enhance the efficiency of natural enemies (01/04/2002 to 31/03/2007) PI: Dr. Nandagopal Bakthavatsalam**

Based on the earlier studies, the kairomones based on L-tryptophan and n-tricosane for increasing the abundance of chrysopid was identified. The kairomone impregnated septa based on Tricosane were used for increasing the searching efficiency of the larva using the compounds such as n-tricosane along with the supplementary diet like *Corcyra* eggs. For inducing the oviposition by the adult chrysopids, compounds such as acid hydrolysed L-tryptophan were utilized. In the field trials conducted for subsequent 3 years with the above kairomone compounds, the abundance of *C. carnea* significantly increased in the kairomone treated plots compared to treated control (with inundative release of chrysopid larvae) and untreated control. The incidence of the pests and bollworm infestation was comparatively lower than the treated and untreated control.

Other attempts made with the substitution of acids with weak acids like citric acid and acetic acid did not register much oviposition by adult chrysopids. However, Indole acetaldehyde showed promise as an attractant for *C. carnea* at 10mM concentration.

The kairomone impregnated rubber septa with active ingredients like tricosane recorded higher parasitization by *T. chilonis* than the treated check (inundative release of *T. chilonis*) on cotton in the field trials conducted consecutively for the past 2 years. The incidence of the bollworm and damage was less in the kairomone treated plots. Same kairomone formulation containing n-tricosane along with supplementary diet increased parasitization by *T. japonicum* on rice in field trials conducted at Tamil Nadu. The incidence of stem borer was comparatively lesser than the treated check (with inundative release of *T. japonicum*) and the untreated check.

Methyl salicylate was attempted as an ovipositional attractant for predators like chrysopids, coccinellids and anthocorids. Cotton plants exposed to methyl salicylate recorded more oviposition than unexposed plants by chrysopids. However other groups of predators like anthocorids and coccinellids did not respond.

A kairomonal solution containing 70mg of larval wash of *S. litura* improved the performance of *C. chloridae* on the *S. litura* at laboratory conditions. This was a boon for the mass production of *C. chloridae*.

Compounds containing linalool and unsaturated hydrocarbons like pentacosane increased the parasitization efficiency of *T. chilonis*. Similarly compounds containing caryophyllene oxide along with the compounds like pentacosane and hexacosane recorded higher parasitization by *T. chilonis*. There was variation in the behaviour of strains of *T. chilonis* to the kairomonal compounds. Strains such as 15, 73, 106 recorded significantly higher response compared to the other strains. *T. chilonis* adults exposed to compounds such as hexacosane before release recorded higher parasitization on *H. armigera*.

**34. Introduction and studies on the exotic natural enemies of some important crop pests and weeds (01/04/2002 to 31/03/2007) PI: Dr. Basavaraj Shidlingappa Bhumannavar**

A culture of the gall fly, *Cecidochares connexa* (Macquart) was established in the quarantine at NBAIR (Project Directorate of Biological Control), Bangalore from 28 females and 30 males received from Indonesia (import permit from PPA, GOI No. IP-12/2002 PQD dated 19.9.2002). This colony was used in the experiments and the future field colonies in India shall be constructed to were derived from this colony. A pair of mated flies was enclosed along with the plant (with more than 14 shoots) in a cage. The oviposited plant was maintained in the same cage and observation on the development of galls recorded until the emergence of adult flies from the galls. From a shipment of 30 males and 28 females received from Indonesia, 54 males and 42 females were produced in the first generation and 127 males and 133 females in the second generation. A pure culture of *C. connexa* was thus established for further studies.

Plants were chosen from the earlier list of plants tested at Marihat Research Center, Sumatra, Indonesia, for host specificity test. Following the internationally accepted phylogenetic centrifugal method, suitable substitutions were made for plants not cultivated in India, and other plants were included while finalizing the list of plants for host-specificity tests. This was done in consultation with botanists at UAS, GKVK, Bangalore. Several of these test plants were raised from seeds or stem cuttings, and few of them procured from scientific nurseries. In all 76 host plants belonging to 29 families were tested for their suitability for oviposition and feeding by the stem gall fly and the observations were made on visits to the plant, oviposition attempts and gall formation by paired and under no-choice tests. Among the 76 plants belonging to 29 families, oviposition was not observed on 75 plants either in free-choice or in no-choice tests except on *Chromolaena odorata* (L.). Host-specificity tests in India revealed that *C. connexa* could lay eggs and complete its life cycle only on *C. odorata* and this proved beyond doubt its safety to other plants was proved in other parts of the world.

Naturally growing *C. odorata* of about two hectares area in the University of Agricultural Sciences, GKVK, Bangalore, was selected for the initial limited field release studies. Field release was done by individual oviposition method and by mass cage method after slashing during July-August, 2005. The gall fly was also released near village Tataguni during 2005-06. By utilizing 23 females, 371 shoots were got oviposited between 18.7.2005 and 5.8.2005. Females survived for 1-14 days (mean 7.43 days). The maximum number of galls produced by a single female was 50 with an average per female of 19.60. These 23 females produced 173 terminal and 278 axillary galls. On a single shoot maximum of six galls (one terminal and five axillary) were produced during the one hour oviposition period. There was a significant reduction in plant height 30 days after oviposition (11.61%) and 60 days after oviposition (16.72%) in galled plant as compared to control. There was significant reduction in number of branches per plant (35.62%), number of panicles per plant (45.43%), number of capitula per panicle (12.07%) and number of seeds per head (10.89%) in galled plants over control plant.

The density of gall fly in second and subsequent generation was estimated by counting the number of fresh galls in a ten minute search by an individual in the released field and in the adjoining plot. In ten minutes intensive search one could count 9.1 fresh galls on 9.12.2005 (45 days after oviposition) in the released plot by second generation. The gall density was less than one, 50 m away from the released spot in south, east and west directions. One could count 5.55 galls in ten minutes search by the fourth generation around the release spot. The gall density increased from 2.5 on 15.4.2006 to 98.3 on 6.11.2006 at GKVK, Bangalore, whereas the gall density increased from 1.6 on 29.4.2006 to 156 on 28.10.2006 at Tataguni village.

The spread of gall fly in second and subsequent generations was recorded by closely examining all the plants for the presence of galls at 25, 50, 75 and 100 metres away from the released spot in the east, west, north and south directions. Freshly emerging adults were observed on 17.10.2005 (90 days after oviposition). Close examination of shoots on 21.12.05 (60 days after adult emergence) revealed presence of second generation galls, which confirmed the field establishment of this gall fly. Fresh galls were observed at 25 m distance in north and at 50 m distance in south, east and west directions indicating the spread of this gall fly in its second generation, whereas The galls were observed at 50 m distance in the north and at 25 m in south, east and west directions by the third generation. However, in the fourth generation, the galls were observed beyond 100 m distance in the north, east, west and south directions from the release spot. The gall fly was released at the fag end of the growing season of *C. odorata*, its spread in first and second generation got affected due to low winter temperatures and dry weather condition. The gall fly could successfully overcome the dry period from January to April in larval stage and fresh galls were observed during May, 2006 indicating its establishment. The gall fly could spread to a distance of 1 km from initial release spot at GKVK, Bangalore north, whereas it could spread to a distance of 2 km at Tataguni village.

A consignment of *Trichogramma* sp. nr. *mwanzai* Schulten and Feijen was received during April, 2004. Pure culture was raised from these parasitised eggs. Till 31<sup>st</sup> March, 2005, the parasitoid had undergone more than thirty generations. Extent of parasitisation on *H. armigera* eggs glued on chickpea and pigeon pea leaves was studied and the parasitisation ranged from 3.3 to 6.7 and 5.0 to 32.5 when *H. armigera* eggs were glued on chickpea and pigeon pea respectively, with more parasitism noticed on pigeon pea. Repeated studies gave unsatisfactory (less than 5%) egg

parasitisation of *H. armigera* by *T. mwanzai* on chick pea and pigeon pea. *T. mwanzai* could be tried against other crop pests.

A shipment of *Trichogramma brassicae* Westwood was received on 29.1.2005 from Beneficial Insectary Canada, 60, Taggart Streets, Guelph, ON, Canada. Pure culture was raised on eggs of *C. cephalonica*. Part of the culture was maintained on eggs of *P. xylostella*. The percent parasitisation was 47.4, 69.5, 91.3, 93.4 and 62.0 on eggs of *P. xylostella*. Further, dose fixation and interaction with *T. brassicae* and *Tr. bactrae*, interaction between *T. brassicae* and *Tr. bactrae*, importation and studies on *Eriborus trochanteratus* (Morley), *Glabromicroplitis croceipes* (Cresson) was studied. A shipment of *Z. bicolorata* was exported to Sri Lanka for the biological suppression of parthenium. A shipment of *S. inferens* was exported to ICIPE, Nairobi, Kenya for trials on the biological suppression of *C. partellus*. Studies on *Cybocephalus indicus* Tian & Ramani, like biology, morphometrics, studies on new species of *Cybocephalus* feeding on *A. dispersus*, interaction studies between the natural enemies of *A. dispersus*, monitoring the population of *A. dispersus* and its parasitoid and establishment of quarantine facility at NBAIR (PDBC), Bangalore, were also carried out under this project.

### **35. Mass production, formulation and field-testing of entomopathogenic nematodes against important lepidopterous pests (01/04/2002 to 31/03/2006) PI: Dr. Syed Shahabuddin Hussaini**

A rapid and cheap mass production method using an environmentally compatible porous material, vermiculture was developed for EPN. The multiplication was fast with raw whole egg in vermiculture. Scale up *invitro* of native isolates on modified egg yolk medium gave positive correlation between yield and volume up to 1000 ml. Media containing beef fat, kidney and liver found suitable for *Heterorhabditis* spp. with beef kidney as most suitable for *H. indica* 6.71 and *Heterorhabditis bacteriophora* Poinar.

Bioefficacy tests against arecanut Whitegrubs showed *H. indica* PDBC EN 13.3 to be promising besides *Steinernema feltiae* (Filipjev), *S. abbasi*, *S. carpocapsae*, *S. glaseri* and *S. tami*. LC<sub>50</sub> for *S. carpocapsae* and *H. indica* at 48 h exposure @ 11 and 8 IJs/larva, respectively, were effective against *P. xylostella*.

Shelf- life of talc based formulation in bioassay against *G. mellonella* larvae was 10 months. Sponge formulation of *S. carpocapsae* found suitable and stable.

Effect of initial inoculum of IJS on final *invivo* yields in *G. mellonella* was determined besides pathogenicity and time of emergence of *S. carpocapsae* and *H. indica*. Higher yields were obtained at 1000 IJs of *S. carpocapsae* per larva within 6 days; whereas 300 IJs of *H. indica* gave max yield within 10 days. Yield of *H. indica* was 3 times the yield of *S. carpocapsae*. Persistence of *S. carpocapsae*, *S. abbasi*, *H. indica* and *H. bacteriophora* was determined under shaded and open conditions in soil through the year. Persistence ranged from 60 days to 90 days.

Persistence of nematodes in soil and on leaf was monitored post application. In months of July and August with temperature of 22-23 and RH 89-93 *H. indica* CRS and *H. indica* 6.71, *S. carpocapsae* and *S. abbasi* were found surviving in soil. Bioefficacy of *S. carpocapsae* 11 was compared using dose response assay against *P. xylostella*, *S. litura*, *H. armigera*, *G. mellonella* in lab. The LC<sub>50</sub> and LT<sub>50</sub> were computed

*H. indica* and *S. carpocapsae* was supplied to different centers under AICRP and also other centers to conduct field trials against key pests of agricultural crops/ forest trees besides use against plant parasitic nematodes.

Higher yields of *S. carpocapsae* on Wouts medium supplemented with casein peptone were obtained from 62 to 89 lakhs per gram of the medium.

In bioefficacy tests *H. indica*, *H. bacteriophora*, *S. abbasi* and *S. carpocapsae* were compared for LD<sub>50</sub> against *H. armigera*. *S. abbasi* and *S. carpocapsae* caused mortality of *H. armigera* larvae at 12h, at 50 and 125 IJs/ larva respectively. *H. indica* and *H. bacteriophora* caused mortality at 24h. *S. abbasi* found effective against *H. armigera* with least LD<sub>50</sub> followed by *S. carpocapsae*, *H. indica* and *H. bacteriophora*.

Castor oil enhanced survival of *S. carpocapsae* and *H. bacteriophora* on cotton leaf. Concentration of 0.25-0.50 per cent was effective. UV protectant Sodium sulfate at 0.25- 1 per cent

protected *S. carpocapsae* and *H. bacteriophora* maximum compared to PABA, robin and Ujala. Kaolin formulation of *S. carpocapsae* and *H. indica* with 15% moisture level retained viable IJs for 45 days under storage compared to 12% moisture level. Gum based gel formulation was suitable for storage and transport of *S. carpocapsae*. IJs @  $40 \times 10^5$ /100 ml with viability for 30 days at 30°C. *S. carpocapsae* in gel was transported to Kanpur and tested against *H. armigera* in chickpea. Nematode sprayed leaves/pods offered to larvae resulted in 100, 75 and 45 per cent mortality soon after, 30 min and 60 min after spray respectively. *H. indica* in talc @ 5b/ha controlled *Holotrichia* in tobacco fields in Shimoga increasing establishment of transplants and the cured leaf yields.

Survey conducted in Kerala one *Steinernema* sp and 2 *Steinernema* sp in Varanasi. Sponge formulation was found very suitable for transport retaining 90% viability of *Steinernema* spp. for 3-4 months and up to 85% viability of *Heterorhabditis* spp. whereas for 2 months, powder (granular) formulation of EPN was suitable for application in soil. Out of about 15 combinations, two supported *Heterorhabditis* populations for 60 days, four combinations for 30 days, three for 45 days and two for 15 days effectively. *Steinernema* populations (60%) were supported for 30 days effectively. Cadaver formulation was emerging a viable method for application of EPN in field as the exit of progeny could be help up to 2 months by using specified formulations for *S. carpocapsae*, *S. abbasi*, and *H. indica* and more so for *H. indica*. Packing EPN formulations in 100% vacuum was not suitable compared to 75% vacuum or 75% nitrogen gas packing.

### **36. Rearing and evaluation of natural enemies with special reference to scelionid, braconid, ichneumonid and anthocorid groups (01/04/2002 to 31/03/2007) PI: Dr. Chandish R Ballal**

Under this project rearing protocols were developed for potential natural enemies belonging to families: scelionidae, braconidae, ichneumonidae and anthocoridae. The natural enemies which were amenable to rearing were evaluated under laboratory / net house / field conditions. Host rearing was also studied. Monitoring *H. armigera* laboratory cultures for three years indicated that per cent pupation was less than 50 during April to June. A modified diet using soaked kabuli channa as base ingredient could improve the larval survival and in general pupation varied between 55 to 73 per cent.

The oviposition preference studies on *H. armigera* indicated that chickpea varieties JG74, L550, JSC6, JG315, ICCV2, Black Channa and JG130 were equally preferred.

The size index *LxLxb* (greatest length (l) and breadth (b) of egg mass in millimeters and number of layers of eggs (L)) could be used to estimate approximately the number of eggs per egg mass of *S. litura*. Based on this size index, three major groups were defined viz., 15-50, 60-150 and 160 - >200 and approximate number of eggs per egg mass in each group was identified as approximately 100, 200 and more than 300, respectively. *Spodoptera litura* moths preferred to lay eggs on the three tested tobacco varieties. 32.1% of the eggs were laid on BanketA1, 26.91% eggs on K326 and 41% on Gold Streak.

Protocol was developed for lab multiplication of *S. exigua*. High humidity was required for egg laying. Butter and wax paper were observed to be good oviposition substrates for *S. exigua*, with respect to amount of egg laying, however, hatching was better in the case of eggs collected on wax paper.

The optimum parasitoid-host (*T. remus*: *S. litura* eggs) ratio for releasing *T. remus* on castor was 150 adults: 1 egg patch (of 200 eggs), which could bring about a pest population reduction of 95.48%. *T. remus* could parasitise 93.92% *S. litura* eggs on rose and 61.17% on *Bt* cotton at a parasitoid: host egg ratio of 1:2. *T. remus* preferred to parasitise *S. litura* eggs on tobacco variety - Gold Streak (74.7%), while parasitism was low on varieties BanketA1 and K326.

A shipment container was designed for the safe transport *T. remus* egg cards. The cost of 1 parasitised egg card of *T. remus* with 10 egg patches was worked out to be Rs.4/-. At the rate of 1 lakh parasitised eggs per ha, 50 such cards were required, which would cost Rs. 200/-.

Six eight-day-old parasitised eggs of *T. remus* could be stored for up to 10 days at  $15 \pm 2^\circ\text{C}$  ( $60 \pm 2\%$  RH) as 89.5% emergence occurred after 10 days of storage. If *C. chloridae* was released at parasitoid host ratio of 1:15 against *S. litura* larvae on castor, the pest population reduced by 81.10%.

Large cages measuring 1.7 x 1.7 x 2.5ft. were utilized effectively for multiplying *E. argenteopilosus* and *C. chloridae*. Use of large cages could improve the rearing of the ichneumonid

parasitoids with respect to mating, parasitism, adult emergence and sex ratio, maintenance of optimum RH of 60-70 per cent was observed to be equally important. *E. argenteopilosus* could provide about 30% parasitism of larvae artificially inoculated on *Bt*cotton. *C. chlorideae* could parasitise *S. litura* and *H. armigera* on *Bt* and non-*Bt* cotton.

The production details were evaluated for *C. chlorideae*. At 5-8 females per cage, 72 cocoons and 15 females can be obtained.

It was essential to maintain humidity between 70-80 per cent and a temperature between 26 to 28°C for production of *C. chlorideae*. The parasitoid could be successfully reared in walk-in chambers set at 26±2°C and 70±2% RH. Even during summer months, 30 to 38% parasitism could be recorded.

Kairomones (*S. litura* larval wash) could improve parasitism by *C. chlorideae*. Releases of *C. chlorideae* against *H. armigera* on chickpea, led to a reduction in the number of larvae per plant and per cent pod damage and an increase in per cent parasitised larvae in the treated plot in comparison to the control plot.

Indigenous anthocorid predator *O. tantillus* could be continuously multiplied on UV irradiated *C. cephalonica* eggs. However, the progeny production was low. *Sitotroga cerealella* (Olivier) eggs could be utilized effectively for the multiplication of *O. tantillus* as progeny production was higher in comparison to that when reared on *C. cephalonica*. All the biological parameters were superior when reared on *S. cerealella*. The temperature regimes 24 and 28°C were found to be suitable for the multiplication of *O. tantillus*. Based on fertility table parameters, 28°C was observed to be the most suitable temperature.

*O. tantillus* nymph and adult could feed on *Scirtothrips dorsalis* Hood. When *O. tantillus* nymphs were released against *S. dorsalis* infesting rose in field conditions, there was a 50 to 77% reduction in buds/flowers infested by thrips. However, the anthocorid bugs could not be recovered in significant numbers from the treated plots.

Release of *Blaptostethus pallelescens* Poppius against the Angoumois grain moth *S. cerealella* infesting rice indicated that it was a potential predator. It could move to a depth of upto 13 cm. Preliminary trials indicated that *B. pallelescens* adults/nymphs could predate well on *H. armigera* eggs and larvae. They could also feed on eggs and larvae on chickpea leaves inspite of the trichomes. Based on the data on fertility table parameters, it was evident that *B. pallelescens* can be multiplied successfully on *C. cephalonica* eggs and 28°C was identified as the optimum rearing temperature.

Net house studies indicated that *B. pallelescens* nymphs could cause a 78% reduction in the spider mite population on bhendi plants.

At Channapatna and Allalsandra, in the outskirts of Bangalore, field trials were conducted to evaluate the performance of the anthocorid predator *C. exiguus* against coconut black-headed caterpillar *O. arenosella*. These trials indicated that there was a clear reduction in the infestation level and pest population per leaflet in the treated trees. *C. exiguus* nymphs / adults were supplied for field releases at Trichur, Kerala and the trials indicated the effectiveness of *C. exiguus* against *O. arenosella*. *C. exiguus* was observed to be a potential predator of *S. cerealella* infesting rice. The ware house pirate bug *Xylocoris flavipes* (Reuter) was obtained from the jowar storage bins. *X. flavipes* could be successfully multiplied on *C. cephalonica* eggs.

### **37. Development and use of insect viruses for the management of major pest complex of cruciferous crops (01/04/2002 to 31/10/2005) PI: Dr. Kandaswamy Narayanan**

During the period under report the following host cultures were maintained under laboratory conditions *H. armigera*, *S. litura*, *C. partellus*, *G. mellonella*, *Trichoplusia ni* (Hübner), *S. exigua*, *C. binotalis*, *P. xylostella*, *Hellula undalis* (Fabricius), *C. infuscatellus*, *Chilo sacchariphagus indicus* Kapur and *Adisura atkinsoni* Moore etc.

During the period under report the following baculoviruses were isolated and reported. Nucleopolyhedro viruses of *T. ni*, *S. exigua*, *C. binotalis*, *H. undalis* (to be confirmed) from the cruciferous crop eco-system. Further NPVs were also reported from *O. arenosella*, *C. infuscatellus*, *C. carnea* and *Cadra cautella* (Walker). Further a neogregarine protozoan pathogen, *Mattesia dispar* Naville was reported from *C. cautella*

NPVs isolated from *T. ni* and *S. exigua* were test verified for their pathogenicity through leaf surface contamination technique.

To test the pathogenicity of NPV of cabbage of leaf webber *C. binotalis*, the virus was purified by differential centrifugation. The tiny neonate first instar larvae of *C. binotalis* were inoculated @  $1 \times 10^6$  POB's/ml through leaf surface contamination technique: resulted in cent percent mortality within 4-5 days after inoculation showing typical symptom of skin breaking, thus proving its pathogenicity.

Studies conducted on the cross infectivity of NPV of *C. cauttella* revealed the susceptibility of *G. mellonella*. The cross infectivity of *S. exigua* GV to *S. litura* and *A. atkinsoni* to *H. armigeraw* was noticed. *C. infuscatellus* NPV was found to cross infect *C. partellus*.

*O. arenosella* and *C. cephalonica* NPVs tested against mulberry silkworm, *B. mori* revealed their safety.

When second instar larvae of *C. binotalis* were inoculated @  $1 \times 10^6$  OB's/ml through leaf surface contamination technique, it resulted in cent per cent mortality within 6-7 days after inoculation showing typical symptom of skin breaking.

Occlusion bodies of GV of *P. brassicae* were extracted and partially purified from the diseased larvae of *P. brassicae* by differential centrifugation. A test was conducted to determine the pathogenicity against 10 second instar larvae and they were inoculated with GV isolated from *P. brassicae* larvae by leaf surface contamination technique. A similar number of same larvae, treated without virus served as control. The dead larvae were diagnosed by microscopic examination of the squashed preparations for the presence of occlusion bodies. The pathogenicity of *P. brassicae* GV revealed 100% mortality of the second instar larvae when administered through leaf surface contamination technique. The incubation period ranged from 6-10 days. The symptoms of the GV infected *P. brassicae* were generally comparable with those of most of the granuloviruses reported in other insects. Diseased larvae of *P. brassicae* showed typical sluggishness in their movement and were also less responsive to external tactile stimuli. Further, in contrast to healthy green caterpillar with many tiny spots and fine setae and with a narrow tallow line dorsally along the back, the GV infected *P. brassicae* become light white in colour and it was well pronounced on the ventral side mostly due to the accumulation of large number of occlusion bodies. Unlike that of NPV of *P. brassicae*, the skin of GV infected *P. brassicae* was not liquefied. After death, a characteristic white liquid oozed out through the skin after piercing. This fluid was of the consistency of thin cream, and contained large numbers of capsules. Samples of occlusion bodies of GV from some of the insects for which positive diagnosis had been made with dark field microscope were further checked with electron microscope.

From the suspected ascovirus infected *S. exigua*, the diseased larvae showed the characteristic symptoms of retardation in the development when compared to control and difficulty in sloughing off the skin especially in 3<sup>rd</sup> and 4<sup>th</sup> instar. On examination of the haemolymph, it was found that several refractile bodies, called vesicles were floating. On inoculation by parental means it was found that it was highly infective when compared to injection method.

When *S. litura* granulovirus was tested against first larvae of *S. litura* through diet surface contamination technique, cent per cent mortality was obtained in all the five concentrations viz.,  $1 \times 10^{12}$  to  $1 \times 10^8$  OB/ml tested. A reduction in larval weight and leaf area consumed when third instar larvae of *S. litura* was tested against  $1 \times 10^{12}$  OB/ml. However they were not statistically significant.

A preliminary study on the efficacy of *P. xylostella* GV (Px GV) under green house condition showed reduction in population from initial population of 20 to 1.3 per plant when compared to 20 larvae per plant in untreated control. Number of leaf holes with feeding marks was found to be 86% in Px GV treated in potted plants, when compared to 94% in untreated control. The yield was 236 gm in the case of Px GV treated when compared to 170 gms in untreated control.

Studies conducted on the cross infectivity of NPV of *C. binotalis* revealed the non-cross infective nature to all other pests of cruciferous crops, viz., *P. xylostella*, *H. undalis*, *T. ni*, *S. litura* and *H. armigera*.

Studies on the cross infectivity of *S. litura* GV on six lepidopteran crop pests viz., *G. mellonella*, *H. armigera*, *C. cephalonica*, *Earias vittella*, *P. xylostella* and *S. exigua* have shown that except *S. exigua* none of the insects tested was found to be susceptible. *S. litura* GV was found to be safe to mulberry silkworm, *B. mori*.

Efficacy of *C. binotalis* NPV when tested against late second instar larvae, with varying concentrations ranging from  $10^9$  to  $10^5$  POBs/ml revealed the percentage mortality which ranged from 90 to 33.3 per cent as per the dose dependant manner with an incubation period ranging from 4 to 6 days. The third and fourth instar larvae revealed 80 to 70 per cent mortality when it was administered through diet surface contamination technique. Bioassay of *C. binotalis* NPV was studied against 5 days old larvae through diet contamination surface technique at  $1 \times 10^6$  POBs/ml). The 7 day mortality data was transformed to percentages, subjected to probit analysis which revealed the regression equation =  $2.52 + 0.14 x$  with  $LC_{50}$  of  $6.70 + 0.08$ .

The safety of *C. binotalis* NPV was tested against the general predator, *C. carnea* and a ladybird beetle, *C. montrouzieri*. The artificial diet of *C. carnea* and mealy bugs, the natural prey for *C. montrouzieri* were smeared with the virus separately at  $1 \times 10^6$  POBs/ml and fed to the predators. The results of the above safety test against general predators revealed that there was no cross infection of *C. binotalis* NPV against both the predators tested. Similarly the safety of *C. binotalis* NPV was tested against second instar larvae of mulberry silkworm, *B. mori* by way of surface contaminating the mulberry leaf with  $1 \times 10^6$  POBs of CbNPV *ad libitum*. The above study revealed the safety of CbNPV against *B. mori*. During the safety study with recently isolated nucleopolyhedrovirus from *C. binotalis* against *B. mori* some of the larvae of *C. binotalis* were found dead. Examination of the smears of dead larvae of *C. binotalis* under phase-contrast microscope revealed the presence of polyhedral occlusion bodies (POBs) of typical hexagonal shape in the nucleus of cells of fat bodies of *C. binotalis* NPV which were small and polyhedral in shape. Hence, it was decided to determine the *B. mori* NPV cross infectivity or otherwise against *C. binotalis*. So, polyhedral occlusion bodies of BmNPV were purified from NPV infected diseased larvae of *B. mori* by differential centrifugation. Twenty neonate larvae of *C. binotalis* were inoculated with polyhedral suspension containing  $1 \times 10^6$  POBs/ml by way of contaminating the artificial diet. The larvae were allowed to feed the virus contaminated diet *ad libitum*. Same number of larvae treated with water contaminated diet served as check. Daily observations were taken to note the mortality of the larvae. The dead larvae were diagnosed by microscopic examination of the squashed preparations for the presence of polyhedral occlusion bodies

The macroscopical symptoms of orally infected *C. binotalis* with BmNPV showed typical sluggishness in their movement in contrast to very active movement of healthy larvae. In contrast to violaceous body of healthy *C. binotalis* larvae having brown longitudinal stripes and rows of tubercles, the BmNPV infected *C. binotalis* larvae become slender and pale whitish in colour mostly due to the accumulation of large numbers of polyhedral occlusion bodies. Further studies on the cross-infectivity of BmNPV on *C. binotalis* has revealed that one day old larvae were highly susceptible recording cent per cent mortality with an incubation period ranging from 4-5 days. Smears of diseased larvae of *C. binotalis* examined under phase contrast microscope revealed the hexagonal shaped polyhedral occlusion bodies. In order to confirm the identity of the progeny virus obtained from the *C. binotalis* larvae infected with BmNPV, one day old *B. mori* larvae were inoculated with progeny virus obtained from *C. binotalis* through leaf surface contamination technique. This resulted in cent per cent mortality of *B. mori* larvae Thus the virus retained its pathogenicity for *B. mori* even after passage through *C. binotalis*, Morphological observation of POBs harvested from *B. mori* were found to be hexagonal in shape, thereby confirming true cross infectivity nature of BmNPV against *C. binotalis*

In order to obtain maximum recovery of polyhedral occlusion bodies (POBs) of *C. binotalis* NPV, a study was conducted by way of exposing the fourth instar larvae of *C. binotalis* to three different doses of polyhedral, viz.,  $1 \times 10^6$ ,  $1 \times 10^5$  and  $1 \times 10^4$  POBs/ml through diet surface contamination technique. After death, the number of POBs per cadaver was determined using haemocytometer. The above study revealed that there was significant difference between the number of POBs recovered from 4<sup>th</sup> instar larvae of *C. binotalis* when it was inoculated with three different dosage of CbNPV, viz.,  $1 \times 10^6$ ,  $1 \times 10^5$  and  $1 \times 10^4$  POBs/ml A mean 850 million POBs was harvested from the fourth instar larvae of *C. binotalis* when inoculated at a low dose of  $1 \times 10^4$  POBs/ml compared to 720 million POBs when inoculated at a higher dose of  $1 \times 10^6$  POBs/ml.

**38. Development and evaluation of improved strains of trichogrammatids, *Cheilomenes sexmaculata* and *Chrysoperla carnea* tolerant to insecticides, temperature and high host searching ability (01/04/2002 to 31/03/2007) PI: Dr. Sushil Kumar Jalali**

The project work was carried out on the maintenance of species and strains of egg parasitoids for various hosts, Development of high temperature tolerant strains of *Trichogramma* and field evaluation of *T. chilonis* against sugarcane borers in Haryana, Vuyyuru (Andhra Pradesh), Pune (Maharashtra), Karnal and Sonipat (Haryana), and Mwana (Uttar Pradesh) and field evaluation of *T. japonicum* against sugarcane top borer in Lucknow (Uttar Pradesh) and field evaluation of *T. chilonis* against cotton bollworms in Coimbatore (Tamil Nadu), Selection of *T. chilonis* for tolerance to insecticide, evaluation of multiple insecticide tolerant strain against cotton bollworms in Punjab, Gujarat, Karnataka and Tamil Nadu, selection of high host searching ability strain of various *Trichogramma* species for use against crop pests, storage of 'Tricho' cards for field release, developing low and high temperature tolerant and insecticides tolerant strain of *C. carnea* by selection techniques. Field evaluation of high temperature and host searching strain of *T. chilonis* against cotton bollworms in Orissa and *T. chilonis* in combinations with *Tr. bactrae* against *Pectinophora gossypiella* (Saunders) in Karnataka.

Evaluation of Trichogrammatids against *P. xylostella* in the laboratory and on potted plants during winter and summer on cabbage, evaluation of Trichogrammatids against *E. vittella* in the laboratory and on potted cotton and Okra plants, field evaluation of *Trichogramma embryophagum* (Hartig) and *T. chilonis* against *O. arenosella* on coconut, effect of female age on parasitizing efficiency of *T. chilonis*, Effect of photoperiod on parasitizing efficiency of Trichogrammatids, storage studies with *C. cephalonica* eggs and *T. chilonis* parasitized eggs at low temperature, Efficacy of various release methods on parasitizing efficiency of *T. chilonis*, determination of differences between various strains through biochemical methods, comparative efficacy of adapted and non-adapted strain of *T. chilonis* to low temperature, molecular characterization of Trichogrammatids, determination of genetical mechanism in strains of *T. chilonis*, field evaluation of MITS and laboratory strain against *H. armigera* on tomato in Malur taluk (Bangalore) in farmer's field.

Development of low (18°-24°C) and high (30°-35°C) temperature and insecticides tolerant strains *C. sexmaculata*, screening of trichogrammatids against *Sesamia inferens* (Walker) and studies on *Wolbachia* infested Trichogrammatids were carried out.

### **39. Herbivore induced plant synomones and their utilization in enhancement of the efficiency of natural enemies (31/05/2002 to 30/04/2007) PI: Dr. Purshotam Lal Tandon**

Tomato genotype influenced the parasitization efficiency of the egg parasitoid, *T. chilonis* on *H. armigera* eggs. Maximum parasitization was recorded on genotype Arka Alok, followed by Pusa Ruby. *T. chilonis* responded maximum to the volatiles of leaves of Arka Alok and ArkaVikas under Y-Shaped Olfactometer. However, there was no difference in net response to fruit volatiles. Electro Antenna Gram (EAG) responses of *H. armigera* females to the volatiles of leaves of different varieties / hybrids of tomato revealed maximum mean absolute response to ArkaVikas followed by Arka Alok. Maximum mean absolute net EAG response by the parasitoid *C. chloridae* was evoked to the volatiles released by the leaves of Arka Alok. Maximum mean absolute net EAG response of *C. chloridae* to the volatiles from coriander leaves was higher (-0.979) than methi leaves (-0.672). The most common fractions in the volatiles released by leaves and fruits of different varieties / hybrids of tomato were: tetradecane, pentadecane, heptadecane, octadecane, nonadecane, eicosane, alpha-pinene, linalool, 1, 2, benzenedicarboxylic acid, cembrene and phenol, 2, 2-bis. There was clear cut difference in the fraction profile between leaves and fruits. Volatiles of healthy and damaged fruits of tomato by *H. armigera* revealed qualitative as well as quantitative changes in the fractions of volatiles. Linalool levels were found lower in the infested fruits of tomato hybrids- Suraksha and Uttav.

The most common compounds in the bolls of cotton genotypes were: alpha -terpinene, terpinolene, lamonene, phenole, 2, 4-bis, cembrene and 1, 8-cineole. Quantitative as well as qualitative differences were observed between damaged and undamaged bolls.

Volatile organic compounds (VOCs) were trapped, isolated and identified from fern, *Chenopodium* sp. and tulsi leaves (*O. sanctum*). Twenty-eight compounds were identified from fern leaves and the major fractions were: trans, trans-2, 4-decadienal, tetradecenal, tetradecanoic acid, hexadecanal, 2-methyl, heneicosane, octadecanoic acid, 9, 17-octadecadienal and muskolectone. In

*Chenopodium* sp. most common compounds were plant hydrocarbons. However, main VOCs of tulsi leaves were: citral, methyl eugenol, methyl chavicol,  $\beta$ -caryophyllene and  $\alpha$  and  $\beta$ -bisabolene. These compounds were known for their attractions.

Eleven plant volatile compounds bioassayed for their activity at six different concentrations revealed that ten of them were effective at 0.05 and 0.1 per cent concentrations. Above these, they were having negative effect. Linalool was effective only at 0.05%. At higher concentrations i.e. 0.2, 0.3, 0.4 and 0.5 per cent the insects were turning away from cue and cleaning antennae very fast and frequently. In another experiment, fifteen plant volatiles evaluated in a bioassay revealed that adults of *T.chilonis* respond positively to all the compounds evaluated at 0.05 and 0.1 per cent concentrations. Beyond this, except Hexadecane, Tricosane and Heneicosane, *T.chilonis* responded negatively to all other compounds. Mixture of four plant volatiles i.e. pentacosane + Tricosane + heptacosane + octocosane in the ratio of 1: 3:1:1 at 50% concentration was quite attractive to 4 day old *C. carnea* adults as indicated by mean Excess Proportion Index.

Response of six flowers i.e. rose, tuberose, goldenrod, *Allium* sp. (Ladies lace) and yellow flower volatiles to *C. carnea* indicated its preference to goldenrod and Ladieslace over all others.

Evaluation of suitability of chickpea for the release of *B. pallescens* against *H. armigeraw* was done. Our hypothesis was to prove that chickpea leaves which have glandular trichomes and responsible for the release of malic acid and ketones like 2-Tridecanone and 2-undecanone have negative effect on *B. pallescens*. The data revealed that when 7- day- old nymphs of *B. pallescens* were released, highest predation (90 %) took place on washed leaves with moisture. Least eggs were fed (60.00%) on unwashed leaves without moisture. In general, maximum predation was done by 10-day- old nymphs. Among all the three stages of the predator, adults fed relatively less eggs and the feeding pattern was almost same on all the three types of leaves provided. The 7-day-old nymphs of *B. pallescens* released on chickpea leaves were most susceptible, and on unwashed leaves without moisture highest mortality (83.33%) occurred. In 10-day-old nymphs and adults, the mortality was 28.6 and 16.7 per cent, respectively, which was relatively much less in comparison with 7-day- old nymphs. In washed leaves with moisture, no mortality was observed in all the stages, which suggested that release of early instar nymphs should be avoided on chickpea.

The major profile of green leaf volatiles included 13 fractions, and three compounds, i.e., Ethyl phthalate, Pentadecane and Hexadecane constituted 9.46, 8.03 and 6.76 per cent of total volatiles present. Similarly, the volatile compounds identified from okra leaves, mainly comprised of plant hydrocarbons, and the constituents were - Phthalic acid (37.33%), Pentadecane (17.99%) and Hexadecane (13.25%). In the volatile profile of these two species nothing was found having negative interaction with the predator. However, chickpea volatile revealed the presence of glandular trichomes, which released malic acid and two ketones i.e. 2-Tridecanone and 2-undecanone, which were known for their toxicity to small parasitoids on chickpea.

The volatiles identified from the cabbage leaves included six compounds, diethyl phthalate and heptadecane accounted for 30.31 and 5.07 per cent of the total volatiles. Similarly, volatile profile of castor leaves comprised of five major compounds. Ethyl phthalate (55.69%), Dodecanoic acid (3.86%), Octadecane (2.70%) and Heptadecane (2.23%) formed the major proportion of volatile profile.

The leaf volatile profile of sugarcane consisted of 8 compounds and the main compounds were Ethyl phthalate (8.46%) and Tetradecane (1.09%).

Eleven compounds were identified from *Ocimum* leaves, which constituted 99.26% of the total volatiles present. N- Pentadecane alone was 55.69% followed by N-Hexadecane (21.80%) and N-Decanal (19.89%). The other compounds, specifically, N- Octanol, Decanol, N-Decanal and Decanoic acid were important insect attractants.

#### **40. Development of interactive identification key for important families of insect parasitoids and predators (01/08/2002 to 31/07/2004) PI: Dr. Janakiraman Poorani**

In this project, an interactive identification key to important families of insect parasitoids and predators was constructed on the software LucID. The key to insect parasitoids covered 42 major families of parasitic Hymenoptera, Diptera, and Lepidoptera. The families included in the key were Ichneumonidae, Braconidae, Evaniidae, Gasteruptionidae, Trichogrammatidae, Mymaridae, Eulophidae,

Elasmidae, Encyrtidae, Eupelmidae, Aphelinidae, Tanaostigmatidae, Ormyridae, Torymidae, Eurytomidae, Pteromalidae, Perilampidae, Eucharitidae, Tetracampidae, Signiphoridae, Chalcididae, Leucospidae, Ceraphronidae, Megaspilidae, Proctotrupidae, Diapriidae, Heloridae, Scelionidae, Platygasteridae, Cynipidae, Charipidae, Eucoilidae, Figitidae, Chrysididae, Bethyidae, and Dryinidae (all Hymenoptera), Tachinidae, Sarcophagidae, Phoridae, Pipunculidae (all Diptera), Epipyropidae (Lepidoptera), and Strepsiptera.

The interactive key to important families of insect predators included 21 major families in the orders Heteroptera (Miridae, Anthocoridae, Pentatomidae, Lygaeidae, and Reduviidae), Coleoptera (Carabidae, Cicindelidae, Histeridae, Coccinellidae, Nitidulidae, Staphylinidae), Neuroptera (Chrysopidae, Hemerobiidae, Coniopterygidae), Diptera (Syrphidae, Chamaemyiidae, Cecidomyiidae, and Drosophilidae), Lepidoptera (Noctuidae, Lycaenidae, Pyralidae), Hymenoptera and minor groups of predators such as Odonata, Orthoptera, Dermaptera, and Thysanoptera.

In all these families, morphological characters were used in their identification and various character states were loaded on LucID software. The characters were scored for their presence or absence. Line drawings and photographs of various characters and character states were prepared, scanned and loaded in the software. Notes on the taxonomy, biology, and economic importance of the families included were added in the form of fact sheets to the software. The key was compiled, tested and validated.

As the software, Lucid Player, was required to be downloaded to use the key, the key was converted into a non-interactive format. The fact sheets for the families included in the key were prepared in the form of web pages using Front Page and compiled as a web site, with hyperlinks between the fact sheets. A list of determinators and their addresses (wherever available) and hyperlinks to useful websites were also provided for the included families. This CD was brought out as a priced publication of PDBC with the title, "A Pictorial Guide to Insect Natural Enemies in Biological Control".

#### **41. Evolving and testing superior strains of *Steinernema* sp. and *Heterorhabditis* sp. against *Spodoptera litura* in field (01/09/2002 to 31/08/2005) PI: Dr. Syed Shahabuddin Hussaini**

Progeny production of *H. indica* 13.3 and *H. bacteriophora* (4.7 and 4.2 lakh IJs/larva) was highest in *S. litura* larvae followed by *S. feltiae* and *S. abbasi* (3.7-3.5 lakh IJs/larva). Progeny production was high in pupa compared to pre pupa and the highest yield was obtained with *H. indica* and *H. bacteriophora* (4.98-4.7 lakh IJs/pupa and 2.57 IJs/prepupa). Among *Steinernema* spp. - *S. abbasi* and *S. feltiae* yielded maximum in both pupal and pre pupal stages of *S. litura*.

*Spodoptera* larvae of different instars were highly susceptible to nematode species tested. Nematode concentration differentially affected the mortality of larvae of particular instars. Second and third instars larvae were found more susceptible than fourth and fifth instars.

Isolates tested for thermo tolerance exhibited 100% survival at moderate temperature, 35°C for 10h exposure period. However exposure for longer period, 15h affected nematode survival. Generally *Steinernema* spp tolerated higher temperature better than *Heterorhabditis* spp. Exposure to 35°C 15 h did not adversely affect the pathogenicity or reproductive potential of nematodes. The progeny production of exposed IJs was found normal in the *G. mellonella* and *S. litura* larvae used as test insects. Pre conditioning of IJs at sub lethal temperature affected their survival at higher temperature (40°C). Preconditioning at 37°C prior to treatment at 40°C enhanced the survival rate of IJs of all isolates. Pre-conditioning followed by exposure to 40°C has lowered degree of mortality in different species of nematodes compared to nematodes directly exposed to high temperature. The survival rate was found high in *Steinernema* sp (66-70 per cent) compared to *Heterorhabditis* spp (61-25 per cent). Heat shock treatment resulted in significant increases in survival of nematode population. F1 generation of nematodes obtained from heat selection protocol exhibited enhanced thermo tolerance compared to the wild population at higher temperature, 42°C. Selection for heat tolerance did not deleteriously affect fitness attributes of EPN. Osmotic desiccation and slow dehydration enhanced overall tolerance level in nematode IJS but desiccation helped IJs to withstand high temperature better than slow dehydration. Osmotically desiccated and dehydration IJs retained pathogenicity in high temperature assay (HTA).

Different exposure time to nematode isolates significantly affected percent mortality of and progeny production in both the host insects tested. One-hour exposure to nematode isolates adversely affected of progeny production of all isolates tested. Progeny production was directly proportional to the number of invading nematodes. Results suggested that one exposure was not sufficient for the nematode isolates to invade the host in optimum numbers so as to proliferate to the maximum. In general, heterorhabditids required longer exposure to the target insect compared to steinernematids for successful colonization and proliferation.

Exposure to moderate temperatures, 35 and 37°C for 2hr, did not adversely affect the survival and pathogenicity of nematode isolates. The duration of exposure period at 37°C affected nematodes survive. Six –hour exposure affected the survival of all the species except *S. carpocapsae* isolate PDBC EN 6.11, which exhibited 100% survival during period. Among *Steinernema spp.* highest per cent survival was obtained in *S. carpocapsae* isolate PDBC EN 6.11 (100) followed by *S. abbasi* (98), *S. carpocapsae* PDBC EN 11 (96). *H. indica* and *H. bacteriophora* showed more susceptibility to temperature with higher mortality rates, 26 and 38 per cent, respectively.

Enhancement in heat tolerance was achieved after 5 rounds of selection. Enhancement in temperature resistance was slow and gradual in *Heterorhabditis spp.*, whereas in *Steinernema spp* it showed rapid enhancement. Studies carried out on F1 to F5 generations obtained from heat selection protocol indicated enhanced thermo tolerance in the subsequent generations compared to the wild population at higher temperature, 42°C. Selection strategy can be followed by enhance the beneficial trait, thermo –tolerance in EPN. Stability of the heat tolerance trait was stable in isolates *S. carpocapsae* and *S. abbasi*. Lipids were found to be the reserved energy for EPN. Quality of EPN produced *in vivo* was superior to those in *in vitro* diet.

**42. Development of improved formulations of NPV for management of *Helicoverpa armigera* and *Spodoptera litura* in tomato (01/12/2002 to 31/03/2007)  
PI: Dr. Veenakumari Kamalanathan**

Significant differences were not found in the tomato varieties tested, viz., Arka Alok, Arka Meghali and Arka Vikas with respect to their effect on susceptibility of *H. armigera* HearNPV and *S. litura* to SpltNPV.

Pot culture experiments were conducted to find the effect of different adjuvants such as starch and crude sugar in different combinations on the persistence of HearNPV and SpltNPV on tomato plants. Tomato plants raised in pots were sprayed with HearNPV for four consecutive days along with different adjuvant combinations and exposed to weather parameters such as sunlight and dew. Second instar *H. armigera* larvae were then released on these leaves, which were subjected to weather parameters for different number of days. Mortality data of the pot experiment conducted with different combinations of the adjuvants (along with HearNPV @  $1 \times 10^5$  POBs/ml) showed that 1 % starch along with 10% crude sugar gave a maximum mortality of 87.55% on the day of commencement of the experiment (zero day). This was followed by 0.5 % starch + 10% crude sugar and 10 % crude sugar only where the mortality was 75 and 73 per cent respectively. On the fourth day there was no significant difference in the larval mortality between the treatments.

Similar pot experiment was also conducted to study the effect of various combinations of the adjuvants on the persistence of SpltNPV. Mortality data of the pot experiment conducted with different combinations of the adjuvants (along with SpltNPV @  $1 \times 10^5$  POBs/ml) showed that 1 % starch along with 10% crude sugar gave a maximum mortality of 94.73% on the first day. The larval mortality was significantly higher in this treatment on first three days when compared to other treatments. This was followed by 0.5 % starch + 10% crude sugar, where the mortality was 81.71% on the day of commencement of the experiment (zero day). On the fourth day there was no significant difference in the larval mortality between the treatments.

Laboratory studies were conducted to evaluate the efficacy of different adjuvants in increasing the larval mortality in second instar *H. armigera* larvae. Several adjuvants were evaluated individually for their efficacy along with HearNPV. HearNPV along with respective adjuvant were exposed to an irradiation of  $500 \text{ W/m}^2$  for a period of 90 minutes using sun test machine which simulates the natural sun light. Bioassays were then conducted using these irradiated mixtures of adjuvant + virus and the larval mortality was recorded. Even though all the adjuvants screened

significantly increased the larval mortality, four adjuvants (soybean flour (10%), Tinopal (0.2%), molasses (5%), CSKE (10%)) along with virus resulted in more than 85 % larval mortality. This was followed by Boric acid (1.0%) and turmeric (2%) with larval mortality of 82.27 and 74.40 per cent, respectively. Starch (1%) was not effective in increasing the larval mortality.

All the adjuvant combinations used resulted in higher larval mortality which was statistically significant over the control. The mortality data revealed that the combination of SpltNPV + CSKE (62.5%) + corn oil 1.5% + crude sugar 25% + Tween (0.04%) was the most effective in increasing the mortality due to SpltNPV (95.23%). This combination of adjuvants was similar to widely used commercial adjuvant Coax (Traders Oil Mill Co., Texas, USA). This was followed by SpltNPV + soya flour (8%) + soya oil (5%) + crude sugar (5 %) + Triton (0.01%) and SpltNPV + CSKE (10%) + Tinopal (0.01%) + Tween (0.04%) resulting in a larval mortality of 89.47 and 84.38 per cent, respectively.

Studies on the efficacy of various adjuvants in increasing the persistence of HearNPV using sun test machine revealed that all the adjuvants either alone or in combination were superior over control. Among all the adjuvants tested molasses 5% + Tinopal 0.2% + lampblack 0.1% was superior to all other adjuvants tested. This was followed by crude sugar (5%) + Tinopal (0.2%). Studies conducted in the farmer's field for evaluating the formulation of HearNPV against *H. armigera* on tomato revealed that the pest could be effectively controlled with the virus. It was also observed that when adjuvants were used along with the virus, the fruit damage was reduced and the larval mortality increased. The shelf life studies of wettable powder formulation of HearNPV under both refrigerated and room temperatures revealed that both the formulations did not show any significant difference in the LC<sub>50</sub> values at refrigerated conditions. However the unformulated virus under room temperature recorded significantly higher LC<sub>50</sub> values from the seventh month onwards. The virus inactivity of the unformulated virus under room temperature was increased by 2.7 times by the end of ninth month. The wettable powder formulation packed with nitrogen under vacuum showed no significant difference in the LC<sub>50</sub> values during all the nine months of storage under room temperature. By the end of nine months (under room temperature) the LC<sub>50</sub> value of unformulated virus suspension was 2.1 times more than wettable powder formulation packed with nitrogen under vacuum. LT<sub>50</sub> values progressively increased with increase in storage time. After nine months of storage the unformulated virus recorded 129.7 hours (refrigerated condition) and 167.2 hrs (room temperature). Under room temperature wettable powder formulation packed under vacuum with nitrogen recorded least LT<sub>50</sub> value of 145.7 hrs, nine months after storage under room temperatures.

#### **43. Identification of pathogens of phytophagous mites and assessment of their potential in microbial control (01/04/2003 to 30/06/2008) PI: Dr. Sreerama Kumar Prakya**

The fungi frequently found associated with *Tetranychus* spp. were *Neozygites floridana* (Weiser & Muma), *Fusarium* spp., *Lecanicillium* spp., *Acremonium* spp., etc. Broad mite, *Polyphagotarsonemus latus* (Banks) samples yielded similar genera of hyphomycetes. For the first time, *Aceria litchii* (Keifer) was found infected with putative *Hirsutella thompsonii* Fisher. Pathogenicity of the coconut mite (*Aceria guerreronis* Keifer)-derived *H. thompsonii* to *Tetranychus urticae* C.L.Koch and *T. neocaledonicus* was proved. The number of conidia getting attached to an adult *T. urticae* was found to be directly proportional to the time of exposure of mite to the fungus.

Temperature had significant effect on the growth of *H. thompsonii*. No growth was observed at 37°C and above. Conidial production varied among temperatures. There was no effect of temperature on the micromorphology of *H. thompsonii* and *Hirsutella thompsonii* var. *synnematos* Samson, McCoy and O'Donnell.

The effect of different inoculum loads on *H. thompsonii* biomass in continuous shake culture was studied. With increasing number of inoculum mycelial discs, pellet number and biomass weight increased proportionately. In alternating shake-stationary culture, maximum colonies were observed when six oatmeal agar discs were used as inoculum.

Six isolates of *H. thompsonii* var. *synnematos* [MF(Ag)27 to 32] were multiplied on 10 different agar media and observed for synnemata. Cultures on potato dextrose agar were classified as grey portion sub-cultures and white portion sub-cultures. The maximum growth of 47.17 mm with a growth rate of 1.37 mm/day was observed in MF(Ag)31. Conidial production of *H. thompsonii* var.

*synnematos* varied significantly among the isolates multiplied from both grey and white portions. In general, conidia of all *H. thompsonii* var. *synnematos* isolates germinated normally. There were no significant differences in the micromorphology.

*H. thompsonii* exudate significantly reduced (64.6%) hatching of *T. urticae* eggs. The exudate also showed toxicity to adults and nymphs. An adult female mite feeding on exudate-treated leaf could lay only 30.7 eggs. Secondary metabolites of *H. thompsonii* caused 68.9% mortality in *T. urticae*. In four days of treatment, 64.4% nymphs were killed. Mite eggs treated with metabolites showed highly significant reduction (88%) in hatching. Eight days post treatment, *T. urticae* population decreased by 48.3% on rose plants. The metabolites had no detrimental effect on various insect pests, parasitoids, predators and predatory mites.

A new and simplified method was devised and perfected for passaging *H. thompsonii* through the *A. guerreronis*. A simple magnetic stirrer technique for faster mass production of *H. thompsonii* and *H. thompsonii* var. *synnematos* was developed. A new tomato-based liquid medium was developed for mass producing *H. thompsonii*. In an experiment on assessment of different surfactants on the biomass of *H. thompsonii*, Tween 80 was the best. In two experiments on the effect of simulated sunlight on *H. thompsonii*, irradiance for an hour reduced conidial production.

In the greenhouse, *H. thompsonii* and *Lecanicillium lecanii* Zare and Gams caused 37.8% and 26.1% mortality, respectively, in *T. neocaledonicus*. The effect of *H. thompsonii* biomass obtained from stationary liquid culture on *T. urticae* adults was studied on brinjal, okra and tomato in the greenhouse. Maximum mortality of 30.2% was recorded on okra. *H. thompsonii* could bring about more than 90% reduction in *T. urticae* population on okra plants pre-treated with 100, 200 and 400 ppm of dicofol.

The effect of *B. bassiana*, *L. lecanii* and *Lecanicillium psalliotae* (Treschew) Zare & Gams against *T. urticae* on okra was assessed in the greenhouse. More than 50% mite mortality was observed when a sticker was added. In the second experiment, the fungi caused 100% mortality after three days of spraying of sticker-treated spore suspension. *B. bassiana*, *H. thompsonii*, *L. psalliotae* and *M. anisopliae* in combination with two adjuvants decreased the population of *T. urticae* on okra. On rose, *H. thompsonii*, *B. bassiana* and *L. lecanii* killed 50% of *T. urticae*. In the greenhouse, plants treated with imidacloprid prior to *H. thompsonii* application showed decline in the number of live *T. urticae*.

In field experiments at Singanalore and Vellalore (Coimbatore district, Tamil Nadu) during September 2004 and February 2005, formulated *H. thompsonii* ('Mycohit') and unformulated *B. bassiana* and *M. anisopliae* were found effective against *T. urticae* on okra.

Pellets of *H. thompsonii* and *H. thompsonii* var. *synnematos* could be stored in sterile deionised water under refrigerated conditions without loss in viability and pathogenicity. Genomic DNA was extracted using a modification of the CTAB method from four isolates of *H. thompsonii* and compared. Two isolates (Tamil Nadu and Kerala) of the fungus were found to secrete high amounts of acaricidal proteins, as indicated by spectrophotometric analysis of broth filtrates. In growth comparison studies, the Kerala isolate [MF(Ag)66] grew faster (1.82 mm/day) than the Tamil Nadu isolate.

Nine substances were tested for their suitability as adjuvants to *H. thompsonii* [MF(Ag)66]. Glycerol induced maximum conidia ( $4.92 \times 10^4$  conidia/pellet). Glycerol-treated fungal pellets produced most conidia ( $39 \times 10^5/20$ -mm diameter mycelial mat). Conidiation of adjuvant-treated mycelial pellets occurred on various parts of the coconut palm.

A new variant of 'Mycohit' was developed for multilocation field trials against the coconut mite during 2006-07. At Huskuru (Bangalore Rural district), the Kerala isolate in combination with malt extract broth reduced the pest population by 97.2%. Application of *H. thompsonii* together with glycerol resulted in a tolerable mean nut damage grade of 1.96. In the second-round of multilocation field evaluation, another trial was taken up by PDBC at Huskuru from September 2007 to May 2008. The fungal formulations applied with or without glycerol brought about extremely significant reduction in the pre-harvest nut damage through timely killing of the post-treatment mite population. The mycelial formulation with glycerol reduced post-treatment population by 97.4%.

Formulations of *H. thompsonii*, along with the selected adjuvant, were sent to four centres since September 2007 for the multilocation trials on the coconut mite. *H. thompsonii* was also

supplied to Orissa and Goa for demonstrations against the coconut mite. It was also supplied to AICRP centres for experiments on apple mite and pests in polyhouses.

**44. Development of a data base on microbial biopesticides (01/07/2003 to 30/06/2006)  
PI: Dr. Maria Pratheepa**

Database on microbial Biocontrol agents in India was developed in web based form. The information about entomofungal pathogens, fungal antagonistic organisms and bacterial antagonistic organisms were collected from the secondary sources like CABPESTCD from 1973 to 2004/05. The information about entomofungal pathogens also were collected from Review of Agricultural Entomology from 1934 to 1972. The organisms covered in this database include *B. bassiana*, *V. lecanii*, *M. anisopliae*, *H. thompsonii*, *N. rileyi*, *Paecilomyces* spp. like *Paecilomyces farinosus* (Wize), *P. lilacinus*, *P. fumosoroseus*, *T. harzianum*, *Trichoderma viridae* Pers., *Trichoderma virens* (Persoon), *P. fluorescens*, *B. subtilis* and *Bacillus cereus* Frankland and Frankland.

The collected information was categorized under various heads like History, Taxonomy, Biology, Host range/Target pests and diseases, Epizootics, Lab Bioassay/Glass house studies, Culturing and Storage, Mass Production and Formulations/Application and dosage, Compatibility with Insecticides and Botanicals, Compatibility with other biocontrol agents, Commercial products and Field Trials. Database on biocontrol agents was developed in Hyper Text Markup Language (HTML). The index/home page for the database on biocontrol agents was developed. For each bioagent the home page was developed with various mentioned topics in HTML. For each topic separate HTML pages were developed and the links were established to go the corresponding pages from the home page. The sorted list of references would be displayed along with details while clicking the topics. By clicking the references the corresponding abstract would be displayed. The information collected for the identification keys of entomofungal pathogens and for the fungal antagonistic organisms from the scientific journals. The 'Identification keys' includes Introduction, Mycelium, Conidiophores, Monographic Treatments and Key to the species for *Beauveria alba* (Limber), *Beauveria brongniartii* (Sacc.), *Beauveria velata* Samson and Evans, *Beauveria geodes* (Gams), *Beauveria arenaria* (Petch), *Beauveria cylindrospora* (Gams), *Beauveria amorpha* (Höhn.), *Beauveria nivea* (Rostrup) Arx, *B. bassiana* and *Beauveria vermiconia* de Hoog and Rao. The identification keys of fungal antagonistic organisms like *T. harzianum*, *T. viridae* and *T. virens* were collected and HTML pages were developed to view that information in the web. This software can be given in the form of CD or on any other electronic media like Pen drive storage devices etc. The 'Database on Biocontrol agents in India', CD helps the students, researchers and farmers to get all the information in one place by a click, instead of referring journals and papers at the library.

**45. Efficient formulations of *Trichoderma* sp. and entomofungal pathogens with prolonged shelf-life (01/07/2004 to 31/03/2009) PI: Dr. Subbaraman Sriram**

The shelf life of the fungal bioagents is the crucial factor in converting a better bioagents into a formulation. The shelf life of *Trichoderma* formulations derived from liquid fermentation was shorter than that of solid state fermentation derived formulations. The shelf life of liquid fermentation derived formulations could be enhanced by (i) addition of 2% chitin in formulation, (ii) addition of 0.2% colloidal chitin in fermentation medium (iii) addition of glycerol at 3-6 per cent in production medium (iv) heat shock at 40°C for 30 min and (v) in combination of above interventions. Addition of chitin in formulation or colloidal chitin in medium extended the shelf life by additional 2 months while addition of glycerol in medium extended the shelf life upto 8 -9 months. Similarly the shelf life of solid state derived formulations can be extended upto 1.5 years or 2 years by different drying methods using silica gel drying or vacuum drying. The bioefficacy of the formulations with above interventions was assayed at different storage time and it was on par with fresh formulations.

**46. Evaluation of fungal pathogens against onion thrips (01/07/2004 to 30/06/2006)  
PI: Dr. Bonam Ramanujam**

Saprophytes like, *Aspergillus*, *Penicillium*, *Fusarium* sp. and *Cladosporium* sp. were isolated from dead samples of *Thrips tabaci* Lindeman, *S. dorsalis* and *Megaleus distalis* and these fungi were found to be non pathogenic on the thrips species.

Among the forty isolates of entomopathogenic fungi screened against *T. tabaci*, Bb-6 and Bb-5a isolates caused 32.5 and 31.93 per cent mortality of *T. tabaci* respectively. These two isolates showed 20.18 and 15.64 per cent mycosis on *T. tabaci*.

Higher per cent mortality of *T. tabaci* were observed with rice grain formulation and rice grain+ sunflower oil, 0.2% formulation of *B. bassiana* (Bb-6) (34.13 and 35.24 per cent), *M. anisopliae* (Ma-4) (24.38 and 23.12 per cent) and *V. lecanii* (Vl-7) (26.07 and 21.16 per cent mortality) compared to talc formulation.

In the field trials conducted in rabi season during 2004-05, all 12 isolates of fungi tested reduced the thrips population significantly over control (18-24 per cent reduction over control). Among the fungal pathogens tested, *B. bassiana* strains consistently reduced thrips populations in all the sprays. The fungal treated plots showed higher marketable yield of 14.9 to 19.0 t/ha compared to the yield of 14.0t/ha recorded in control.

In the field trials against *T. tabaci* conducted in late kharif season in 2004 and 2005, fungal pathogens minimized the thrips population significantly compared to control but failed to bring down the thrips population to below threshold levels. Yields of all fungal pathogens were found insignificant.

#### **47. Identification of effective entomofungal pathogens for the management of sugarcane woolly aphid (01/07/2004 to 31/03/2005) PI: Dr. Bonam Ramanujam**

Dead Sugarcane woolly aphid samples from Karnataka, Tamilnadu and Andhra Pradesh yielded only saprophytic fungi like, *Cladosporium oxysporum* Berk. and Curtis and *Fusarium* sp. *M. anisopliae* (Ma-4 isolate) caused highest per cent mycosis of *C. lanigera* (30.14%), followed by *B. bassiana* (Bb-5a) (20.46% mycosis). These two isolates (Ma-4 and Bb-5a) were also found pathogenic to *Dipha* causing 27.62 and 15.32 per cent mycosis respectively and Ma-4 isolate causing 29.14% mycosis of *Micromus*. The five isolates of *V. lecanii* tested were not pathogenic on SWA, but caused 7.62-33.48 per cent mycosis of *Micromus*. The percentage of mycosis of SWA observed with four different formulations were statistically on par with each other. Slight increase in the percentage mycosis of *C. lanigera* was observed with the re-isolated cultures after 5<sup>th</sup> passage on SWA. The isolates were sent to AICRP centres at TNAU, MPKV and UAS, Dharwad for field evaluation against sugarcane woolly aphid during Aug-Sept, 2004.

#### **48. Identification of *Trichoderma* isolates with enhanced biocontrol potential (01/10/2004 to 31/03/2008) PI: Dr. Subbaraman Sriram**

From 23 *T. harzianum* and 36 *Trichoderma asperellum* Samuels, Lieckf. and Nirenberg (earlier described as *T. viride*) and 12 *T. virens* isolates, highly efficient chitinase and glucanase producing isolates were identified by colorimetric assays and confirmed by the PCR amplification of chitinase and beta 1, 3 glucanase genes. The isolates with biocontrol potential as well as chitinase and glucanase producing ability were identified. The chitinase producing isolates can be used for targeting fungal pathogens belonging to true fungi like ascomycetes, basidiomycetes and deuteromycetes while glucanase producers can be used to target lower fungi like *Phytophthora* and *Pythium*.

#### **49. Isolation, characterization and toxicity test of indigenous *Bacillus thuringiensis* strains against lepidopteran pests (01/11/2004 to 30/11/2010) PI: Dr. Rajagopal Rangeswaran**

A total of 1064 samples including insect cadavers were screened for native *Bacillus thuringiensis* Berliner (*Bt*). 486 *Bt* colonies were purified. Eleven bipyrimal crystal expressing isolates were purified. NBAIIBTAS and NBAIIG4 isolates produced major proteins of 130 and 60kDa consistent with the *cry1* and *cry2* genes detected by PCR. *B. thuringiensis* (PDBCBT1) was highly toxic to *P. xylostella*, *H. armigera*, *C. partellus* and *S. inferens*. The toxicity of PDBCBT1 was further established through *in vitro* trials against *P. xylostella* and *H. armigera* at Assam Agricultural

University and Punjab Agricultural University under AICRP. Mass production of seed culture was standardized and highest protein concentration of 800 µg/ml with highest cfu of  $16.2 \times 10^9$  cfu/ml. PDBCBT1, PDBCBT2 and PDBCBNGT1 were tested against redgram pod borer (*H. armigera*) damage in field. The highest damage of 44.18% was in untreated control and the lowest (3.5%) was observed in 2% HD-1 spray. Low pod damage was also observed in other treatments viz., 4.25% (PDBCBT2), 4.41% (PDBCBT1) and 4.91% (BNGT1). The results showed that 2% spray of the tested *Bt* strains could significantly reduce pod damage. Field experiments conducted again in 2009 showed the highest pod damage of 28.05% in untreated control. Two new indigenous *Bt* isolates NBAIIBTAS and NBAIIBTG4 were evaluated against pigeon pea pod borer during 2010. The NBAIIBTAS treated gave 9.3% pod damage and the maximum pod damage (30.1%) was in untreated control.

#### **50. Development of novel mass production, storage, and packaging techniques for *Cryptolaemus montrouzieri* (01/03/2005 to 01/03/2009) PI: Dr. Sunil Joshi**

A small scale production technique was developed for *S. cerealella* (Olivier), which comprised of an oviposition cage and a moth collection device fabricated from locally available and inexpensive materials. Transparent plastic jars (27 cm high x 24 cm diameter) were used as oviposition cages and plastic boxes with ventilated lid (10 cm high x 25.5 cm diameter) were used for rearing the larvae. Unhusked wheat was used as a grain medium for larval rearing as that supported the maximum output. Each larval rearing box was charged with 330 g of pre-boiled and dried grains and was infested with 0.3 ml of *S. cerealella* eggs. A black paper (KG card board, smooth) was identified as the most suitable oviposition substrate as it allowed maximum egg laying and removal of eggs without damaging them. Approximately 8000 moths were released in an oviposition cage. The oviposition cage thus developed allowed feeding of the moths without opening the lid of the jar, removal of the oviposition substrate without disturbing the adults, and maximum deposition of eggs on the substrates without contaminating them with moth scales.

The moth collection device consisted of a card board box with plastic funnel attached to it at the base. This funnel was inserted directly into the adult oviposition cage. Larval rearing boxes (three in number) were kept open inside this card board box. The emerging moths were collected directly into the oviposition cage. This collection device caused the least disruption of the larval rearing regime and required least adult handling, thus leading to minimum adult mortality (less than five per cent) and a substantial reduction in manual labor (0.25 man days) involved in the rearing procedure. By using the newly devised technique, we could produce 50 to 60 ml of *S. cerealella* eggs per month. One oviposition cage containing around 4000 females was in use for six days and yielded 12 ml of eggs, on an average.

When reared on *Sitotroga* eggs the larval and pupal mortality of *Cryptolaemus* was 26-32 per cent and 15-18 per cent, respectively. The corresponding values when reared on mealybugs were 15-19 and 10-12 per cent. If started with 100 larvae we were likely to get 58-61 adults when reared on *Sitotroga* eggs while we might get 73-75 adults if mealybugs were used as host.

Life fecundity studies on *S. cerealella* using four grain media viz., wheat, paddy, barley and maize indicated that immature stages occupied 35 to 38 days and adult longevity ranged from 6.32 days (barley) to 7.87 days (maize). Oviposition period ranged from 4-5 days and more than 70% of the total eggs were laid on first three days of oviposition. Average fecundity ranged from 102.94 (barley) to 138.80 (maize) eggs per female. Net reproductive rate was highest (28.92) on maize and lowest (23.36) on barley, however generation time was maximum on maize (41.06 days) and minimum on wheat (37.80). Highest  $R_c$  and  $\lambda$  was obtained when wheat was used as grain media.

Life table studies on *S. cerealella* using unhusked wheat as host indicated that the real mortality within generation was 66.56%. Mortality to survival ratio was highest in larval stage. High mortality in larval stage (due to inability of larvae to enter kernel) was the key mortality factor also reflected with high  $k$  value (0.34). Survival rate within age interval was highest in pupal stage ( $S_x=0.91$ ). The  $e_x$  value (i.e. expectation of life for individuals of age  $x$ ) decreased with age giving type III survivorship curve.

There was 1.58 times more mortality of immature stages of *C. montrouzieri* when UV radiated eggs of *S. cerealella* were provided as against fresh eggs. Similarly in case of *B. suturalis*

total mortality of immature stages was 1.89 times more than on fresh eggs. Fecundity was 1.59 times more in case of *C. montrouzieri* and it was 2.22 times more in case of *B. suturalis* when fresh eggs of *S. cerealella* were provided for feeding as against UV radiated eggs.

**51. Isolation and characterization of plant growth promoting endophytic bacteria and development of improved formulations (01/03/2005 to 31/03/2010) PI: Dr. Rajagopal Rangeshwaran**

Studies were conducted to isolate and evaluate endophytic bacteria from healthy pigeon pea, cotton, and chickpea plants and test for their growth promoting and biological control ability. Twenty endophytic bacteria were isolated and identified. In chickpea the highest root length of 23.67cm and highest vigour index of 4116.67 was observed in *Bacillus megaterium* de Bary treatment. Seedling growth promotion studies showed that highest root length of 12.55cm and highest shoot length of 10.5cm was observed with the isolate *Cellulosimicrobium cellulans* (Metcalf and Brown) indicating that treatment with a combination of the microbes was needed. All the endophytes had positive effect on plant growth. *B. subtilis*, *Bacillus* sp. and *P. fluorescens* showed *in vitro* antagonism against *Fusarium vasinfectum* Atk., *F. solani* and *Verticillium dahlia* Kleb. Methanol extracts of *B. subtilis* and *P. fluorescens* showed that inhibition of 36-38 per cent was exhibited against cotton pathogens. When tested in greenhouse with chickpea, significantly high shoot lengths varying between 4.3 and 4.7 cm was recorded with *B. megaterium*, *B. circulans*, *Erwinia herbicola* (Löhnis) and *P. fluorescens* treatments. Endophytic bacteria treated chickpea plants recorded significantly higher phenol content when compared with control. In pigeon pea high phenol content (around 1.2 µg/g) was noticed in all endophyte treated plants on day 1 whereas in control it was only 0.417 µg/g. In cotton on day 5 maximum Phenylalanine Ammonia Lyase (PAL) activity of 0.524 nMol/min/g was seen in *C. cellulans* treated plants. Improved powder based formulations were developed for *Pseudomonas* and *Bacillus* sp. using nutrient amendments with shelf life of up to 240 days. At 240 days highest count of  $1 \times 10^{4.2}$  cfu/gm was obtained with formulations amended with 2% tryptone and 2% glycerol.

**52. Mass production and field evaluation of *Micromus* sp. (01/03/2005 to 01/03/2009) PI: Dr. Sunil Joshi**

*A. craccivora* was reared on the seedlings of cowpea sown in the polyurethane containers (9.5x9.5x4 cm). Adult females were allowed to lay young ones on the seedlings. Based on the life cycle of *A. craccivora*, the seedlings with first, second, third and fourth instars were removed from the polyurethane containers and were kept in plastic containers with ventilated lids measuring 6.25 cm (height) x 19.00 cm (diameter). The larvae were released immediately after hatching and pre-release aphid counts and post-feeding counts were taken. Feeding potential on each instar of aphid was calculated based on 30 larvae of *M. igorotus*. The dead larvae were replaced with the newly hatched larvae and thus the equal replications were maintained. Similar procedure was followed when *C. lanigera* was used as host.

Irrespective of instars of the aphid species, *A. craccivora* was fed in significantly higher number (680.35 aphids / larva) as compared to *C. lanigera* (510.35 aphids / larva). Regardless of species of the aphids, the first instar fed maximum number (688.25 aphids / larva), whereas fourth instar fed least (486.15 aphids / larva). In case of *A. craccivora*, first instar fed 30.22% times more as compared to 4<sup>th</sup> instar. Second and third instars fed 19.67 and 10.44 per cent times more, respectively than 4<sup>th</sup> instar. When different instars of *C. lanigera* were provided, first, second and third instars fed 59.18, 46.91 and 29.18 times more than 4<sup>th</sup> instar. As first instar fed in higher numbers, the predator is likely to work well at early infestation levels, a situation where first instars of aphids are expected to be more in number as compared to other instars.

Biology of *M. igorotus* was studied on four aphid species, viz., *A. gossypii*, *A. craccivora*, *P. bambusicola* and *C. lanigera*. Host plant parts infested with the aphid species, viz., *A. gossypii* (on cotton), *A. craccivora* (on cowpea), *P. bambusicola* (on bamboo) and *C. lanigera* (on sugarcane) were kept in a Perlpet container of half-liter capacity. For studying different biological parameters of *M. igorotus*, adults were released on each species of aphids in the Perlpet container. Eggs laid by using each species of aphid as adult host were kept separately for studying egg period. Larvae resulting

from these eggs were released on respective aphid species and period up to pupation was worked out as larval period. Similarly pupae were removed from the plant parts or from the base of the containers and were kept separately for adult emergence. Pupal period and adult longevity was worked out for all the four species of aphid hosts.

Egg period of *M. igorotus* varied from 3.7 to 4.00 days and it did not vary significantly when adults were fed with different species of aphids. Similarly, there was no significant difference in larval period when different aphids were provided as host. It varied from 6.7 to 7.5 days. Pupal period however varied significantly, being longer on *A. craccivora* (7.00 days) and *A. gossypii* (7.10 days) and shortest on *C. lanigera* (6.00 days).

Different material, viz., gauze swab, human hair, cotton swab and threads were used to evaluate as oviposition substrate for *M. igorotus*. It was found that the maximum eggs were obtained on cotton threads. However this treatment was on par with the treatments with cotton pad, gauze swab and human hairs. When substrates were not provided for oviposition, maximum eggs were laid on honey swab (87%) followed by sugarcane leaf (7%). Substrates which were used in other countries for rearing different species of *Micromus* were also evaluated. Tampon which was used successfully in other countries was not found to be useful for *M. igorotus*. The other substrates used for some species of *Micromus* like baize was very expensive and hence could not be used here. Instead of baize polycotton, white fur, cream fur scotch brite were used. Studies on these substrates indicated that density of hair per unit area and a great fibre abundance of low diameter providing many open ends can work very well as oviposition substrate.

Larvae of *M. igorotus* were reared on four different species of aphids, viz., *A. craccivora*, *A. gossypii*, *P. bambusicola* and *C. lanigera*. Resulting adults were used to derive eggs to be used for life table studies. Number of eggs reared were 1028, 982, 1135 and 880 on *A. gossypii*, *A. craccivora*, *P. bambusicola* and *C. lanigera*, respectively. Death at each stage, reasons for death and adult entering into next stage were recorded. Life table studies indicated that the immature stage mortality ranged from 29.18% (on *P. bambusicola*) to 59.00% (on *C. lanigera*). Immature stage mortality when *A. craccivora* was used as host was 51% while it was 54% when *A. gossypii* was used as host. A comparison of the key mortality factors showed that total survival was affected to the maximum by pupal stage on *A. gossypii*, *A. craccivora* and *C. lanigera* while by egg stage on *P. bambusicola*. Pupal mortality was highest on *C. lanigera* followed by that on *A. gossypii*. Egg mortality did not vary with the host. Least larval mortality occurred when *P. bambusicola* was used as host, whereas it was maximum on *C. lanigera* followed by *A. gossypii*. Irrespective of the species of aphid used for rearing,  $S_x$  value was maximum in case of third instar larva indicating least mortality in this stage.  $e_x$  value decreased with the age when *A. gossypii*, *A. craccivora* and *P. bambusicola* were used as host giving type III survivorship curve.

Life fecundity table studies were conducted by using *A. craccivora* as host. Ten pairs of adults were maintained separately in oviposition cage along with unlimited *A. craccivora* on cowpea seedlings, honey 50% and water on cotton swabs. Daily oviposition and mortality in each cage was recorded and the resulting data was used to construct fecundity tables.

*M. igorotus* had maximum life span of 61 days out of which immature stages occupied 14 days. Oviposition began on 19<sup>th</sup> day and maximum progeny per day was attained on the 11<sup>th</sup> day of oviposition. The first mortality occurred on the fourth day and 50% of population survived up to 32<sup>nd</sup> day. The average number of egg laid per female was 1082 and sex ratio was 1: 1.5 (male: female). The net reproductive rate ( $R_0$ ), length of generation ( $T_c$ ) and innate capacity of increase ( $R_c$ ) were 66.52, 33.89 and 0.124, respectively. The population of *M. igorotus* multiplies 1.3 times per day and doubled in 2.43 days.

By using recently designed and fabricated oviposition cage, it was possible to make a total of 13 harvests in a month on alternate days. First harvest was possible when female was seven days old and last harvest was made when female aged 42 days. It was possible to get yield of 20276 eggs per oviposition cage per month. A total of 1,19,670.95 eggs could be obtained per month in seven oviposition cages containing 20 pairs were used.

Laboratory reared *M. igorotus* was evaluated in the field in 0.25 ha sugarcane plot atDeshahalli, Maddur. Ten spots were selected randomly with heavy infestation of *C. lanigera* and 100 eggs were released on each spot. In total 1000 eggs of *M. igorotus* were released in two instalments (500 eggs/instalment) at weekly interval. First release was made in the first week of

December. The initial population density of aphid was 62.5 aphids/clump. The population of aphid came down to 45.6 aphids per clump a week after release. However it increased and reached to its initial density (60.1 aphids/clump) in the following week. Second instalment of release was made in this week. The population remained low in the following two weeks (43.2 and 38.8 aphids/ clump). However, the aphid population had its second peak in the next week when the population was 59.9 aphids/clump.

Even after release of 1000 *M. igorotus* eggs, the average population of the predator could never reach even up to one larva/clump. The major problem in establishment of the predator was soldier aphids, which was evident from the egg pads, which were covered with the soldier aphids in the week following its release. It was concluded that instead of releasing eggs of the predator, larvae might be released.

**53. Selection of superior strain of *Chrysoperla zastrowi sillemi* (*C. carnea*) and *Cryptolaemus montrouzieri* from different agro-ecosystems and their molecular characterization (10/04/2006 to 31/03/2010) PI: Dr. Thiruvengadam Venkatesan**

Larvae of *Chrysoperla zastrowi sillemi* (Esben-Petersen) from Nagpur, Delhi and Punjab were reared on *Corcyra* eggs for three generations and all the quality attributes of these populations were recorded. The survival of these populations ranged from 85.0 to 87.4 per cent. Highest fecundity was recorded on *C. zastrowi sillemi* from Nagpur (411 eggs/female). Oral and contact toxicity of field recommended dosages of pesticides viz., acephate (0.67 g/lit), metasystox (2 ml/li), karate (0.6 ml/lit), success (1.2 ml/lit) and imidochloprid (0.5 ml/lit) was tested on *C. zastrowi sillemi* populations (Punjab, Delhi and PDBC) and baseline data on their tolerance level was generated. For bioassays with monocrotophos, LC<sub>50</sub> of *C. zastrowi sillemi* collected from Sirsa was found to be maximum (15.6ml/lit). Highest percentage survival (52%) was recorded in Punjab, followed by Nagpur (51%) and Coimbatore (46.5%). Freshly emerged *C. zastrowi sillemi* collected from Delhi, Punjab and Bangalore populations of *C. zastrowi sillemi* exposed to 32-38 °C. The survival of *C. zastrowi sillemi* from Delhi and Punjab was 75.0 and 74.0 days, respectively and 60 days in lab reared. Fecundity of the predator varied from 38.0 days to 121.0 days. Maximum number of aphids (249/larva) was consumed by *C. zastrowi sillemi* from Anand and maximum mealy bugs were fed by *C. montrouzieri* (Pune-47). For acephate, LD<sub>50</sub> of *C. montrouzieri* of Delhi was maximum (0.75 ml/lit). Esterase activity in larvae and adults of *C. zastrowi sillemi* collected from different locations was studied.

**54. Selection of superior strains of certain parasitoids and their characterization (01/04/2006 to 31/03/2010) PI: Dr. Kotilingam Srinivasa Murthy**

Selection of superior strains of parasitoids based on their biological attributes, tolerance to high temperature and insecticidal resistance was studied under the project.

The biological attributes of different populations of *G. Nephantidis* (Andhra Pradesh, Karnataka, Kerala and Tamil Nadu) was studied. Temperature of 32°C was more congenial than 26°C for development. Population from Tamil Nadu was more tolerant to higher temperatures and recorded higher biological attributes than others. Genetic variability among the populations (*G. nephantidis*) was assessed using ITS-2 region and the sequences were submitted to Gen Bank.

Populations of *C. flavipes* were collected from Aurangabad, Bangalore, Chindwara, Devaganahalli, Dindigul, Hyderabad, Hoshiarpur, New Delhi and Shimla. The biological attributes of population from Hoshiarpur (Punjab), Dindigul (TN) and Devaganahalli (Karnataka) were found to be superior. The genetic variability and geographic lineage of populations assessed by RAPD indicated similarity coefficient of 66%, between Aurangabad and Devaganahalli and 63% between Delhi and Dindigul and the others were distinct (63%).

A temperature of 25°C was found to be optimum for developmental activity of the various populations of *C. flavipes*, irrespective of geographic location while the activity ceased at 10°C. The host searching efficiency of *C. flavipes* in the 1 cu. ft and 3 cu.ft cages was maximum in the population from Shimla followed by population from Dindigul. Endosymbionts in the parasitoids contributed to its fitness attributes (higher female progeny and longevity).

Among the various populations, the population from Shimla was highly resistant to fenvalerate (LC<sub>50</sub> 2.77 ppm) and population from Bangalore to endosulfan (LC<sub>50</sub> 56.24 ppm). Field evaluation of the pesticide tolerant parasitoid from Shimla had higher parasitism (40.8 cocoons/larva), when the releases were followed by insecticidal application.

**55. Taxonomic studies on lesser known Coccinellidae of the Indian Subcontinent (01/04/2006 to 31/03/2009) PI: Dr. Janakiraman Poorani**

Information on about 20 species studied under this project was added to the identification guide for common species of coccinellids commonly found in the agroecosystems of the Indian subcontinent, prepared earlier. The guide provides information on the current nomenclature, synonyms, a brief diagnostic description, geographic distribution, prey/ associated habitat, seasonal activity, and important references pertaining to taxonomy and biology/economic importance, for all the species included. Colour photographs or illustrations of the habitus were provided for all the species, along with illustrations of other diagnostic characters and genitalia, wherever possible.

The website “Coccinellidae of the Indian Subcontinent” constructed and hosted on the internet was maintained and new content in the form of photographs and other information were added periodically. URL: [www.angelfire.com/bug2/j\\_poorani/index.html](http://www.angelfire.com/bug2/j_poorani/index.html)

A new website, “Aphids of Karnataka” constructed and hosted at [www.aphidweb.com](http://www.aphidweb.com). The site provides factsheets on 67 species of aphids of Karnataka and their bioagents, including several common aphidophagous coccinellids.

**56. In-vitro cloning of NPV for genetic improvement (01/04/2006 to 31/03/2009) PI: Dr. Kotilingam Srinivasa Murthy**

Embryonic cell lines of *H. armigera* and *S. litura* were established for *in-vitro* cloning of NPV for genetic improvement. TNM-FH (*T.ni* medium-formulation Hank's) powdered medium dissolved in milli Q water @ 5.12 g/100ml with 1 N NaOH. Penicillin, G Sodium salt 50 g 1 gm gentamycin and 10 ml of Foetal bovine serum (FBS) was used as medium to culture cell lines. The sub culturing at cells was done at 80-90 per cent confluence. The embryonic cells of both the insect pests in cell culture flasks were observed after 24- 48 hours under an inverted phase contrast microscope for the morphology, growth and development. The cells were usually suspended in the medium and occasionally adhered and were heterogeneous in nature. Dense distribution was observed in both the cell lines in the early stages and these moved to surrounding areas and got fully distributed over the flask in the later stages. The embryonic cell population were of several shapes, elongated, spherical and trapezoid. The epithelial cells were predominant and constituted 60-70 per cent. The development and maintenance of cells was done at ambient temperature of 27°C in BOD. Density of both the cell lines of *H. armigera* and *S. litura* was determined at different passages, using a haemocytometer. Viability of the cells was determined by dye exclusion method using 0.4% Trypan blue solution. *In-vitro* manipulations in cell culture systems can generate clones of virus by plaque purification. Clones with high virulence and increased speed of kill can be selected to increase the virus productivity which would reduce the cost of formulation.

**57. Biological control of Alternaria leaf blight of tomato (01/07/2006 to 30/06/2010) PI: Dr. Bonam Ramanujam**

*In vitro* antagonistic effect of 48 isolates of *Trichoderma* spp. and one isolate each of *Gliocladium deliquescens* Sopp and *C. globosum* (available at PDBC germplasm collection) was tested on *Alternaria solani* Sorauer and *A. alternata* by dual culture and antibiosis tests for production of diffusible and volatile antifungal metabolites effective against *A. solani* and *A. alternata*.

In the dual culture test, *T. harzianum* (TH-7) isolate showed highest per cent inhibition of *A. solani* (72.78%) and *A. alternata* (65.76%), followed by *Trichoderma pseudokoningii* Rifai (TP-1) isolate showing 69.38% inhibition of *A. solani* and 65.84% inhibition of *A. alternata*. Other isolates like, *T. viride*, Tv-11, Tv-13, *T. harzianum*, TH-2 and TH-6 also showed 60-66 per cent inhibition of *A. solani* and *A. alternate*. In the antibiosis test for production of diffusible metabolites, *T. harzianum*

(TH-7) isolate showed highest per cent inhibition of *A. solani* (82.4%) and *A. alternata* (72.3%), followed by *T. pseudokoningii* (TP-1) isolate showing 71.3% inhibition of *A. solani* and 69.2% inhibition of *A. alternata*. In the antibiosis test for production of volatile metabolites, none of the 50 isolates tested showed production of volatile compounds effective against *A. solani* and *A. alternata*.

Twenty isolates of *Trichoderma* belonging to *T. viride* (Tv-5, Tv-11, Tv-13, Tv-23, TV-97, Tv-115), *T. harzianum* (Th-2, 4, 7, 8, 10, KSD, and P-26), *T. virens* (Tvs-KSD, Tvs-1, 9, 12, P-12), *T. pseudokoningii* (Tpk-1) and *C. globosum* which showed 50-82.4 per cent inhibition of *A. solani* and *A. alternata* in dual culture test and antibiosis test (diffusible) were identified as potential antagonistic candidates for glass house studies. Twenty eight isolates of bacteria (B-1 to B-28) were isolated from the phylloplane of tomato from Hesaraghatta, Tharabanahalli, Hosakote, Rajanukunte and Nelamangala (Karnataka), Madanapalli (AP), Karaikal, Chidambaram, Pondicherry (TN), Jorhat (Assam) and Solan (HP). Antagonistic effect of 28 isolates of phylloplane bacteria from tomato leaf (B-1 to B-28) and one isolate each of *P. fluorescens* and *B. subtilis* (available at PDBC germplasm collection) was tested on *A. solani* and *A. alternata* using Dual culture method on PDA. Tomato phylloplane bacterial isolate B-23 showed 68.2 and 59.3 per cent inhibition of *A. solani* and *A. alternata*. *P. fluorescens* (PDBC isolate) and *B. subtilis* (PDBC isolate) showed 52.32 and 51.23 per cent inhibition of *A. solani* and 43.12 and 49.7 per cent inhibition of *A. alternata* respectively.

Pot culture experiment was conducted in glass house conditions to evaluate nineteen promising isolates of *Trichoderma* sp. and an isolate of *C. globosum* against leaf blight pathogen of tomato viz., *A. solani*. TH-7 isolate showed lowest PDI with 62.6% reduction of blight disease. Other isolates like, TV-11, 23, 97, 115, Th-8, Th-10, Th-KSD, Th-P-26, Tvs-1, 9, 12, P-12 Tpk-1 have showed PDI of 20.43 to 24.01 indicating 50.18 to 56.76 per cent reduction of disease. In the Glass house conditions, ten bacterial isolates (*P. fluorescens* (Pf-19), *B. subtilis*, B-3, B-4, B-10, B-14, B-15, B-16, B-20 and B-23) were tested against *A. solani* infection in tomato. *P. fluorescens* (Pf-19) and *B. subtilis* showed 51.08 and 50.73 per cent reduction of blight disease. Oil based formulations of *Trichoderma* species: The invert-emulsion formulations were developed for the isolates of *T. viride* (4 isolates, TV-11, TV-23, TV-97 and TV-115), *T. virens* (4 isolates, TVs-1, TVs-9, TVs-12 and TVs-P-12) and *T. harzianum* (4 isolates, TH-10, TH-K and TH-P-26, Th-7). The emulsion formulations were prepared using dry conidia of *Trichoderma* isolates, different oils, emulsifiers and stabilizers. The formulations obtained were having  $2 \times 10^6$  CFUs ml<sup>-1</sup> of the formulation. The shelf-life of these formulations was studied at room temperature (20-32°C) was approximately 8 months.

In the field trial conducted at Attur Farm during 2009, 51 to 61.7 per cent reduction in diseases was observed in the plots treated with four antagonists viz., TV-115, Th-7, Th-10 and *P. fluorescens*. 32-36 per cent increase in yields was observed in the plots treated with four antagonists, viz., TV-115, Th-7, Th-10 and *P. fluorescens*.

#### **58. Database on Entomopathogenic Nematodes (01/07/2006 to 31/03/2013) PI: Dr. Maria Pratheepa**

Database on Entomopathogenic Nematodes (EPN) is necessary for the researchers, students and farmers to know the information about EPN on different aspects for the biological control of the pests. Hence, the database on EPN was developed in HTML (Hyper Text Markup Language) form so that it is easy to view the information in any of the web browser and there is no need for any special software for the installation. This database provides information about the EPN including field application which helps the farmers to know about the IPM practices to be carried out for the biological control of the pest with use of EPN. The EPN information was classified under different topics. The user can view the information about EPN by click of the topic in the home page. The topics covered in the database include Taxonomy, Systematics, diagnostic characters of Steinernematidae, Heterorhabditidae, occurrence, new Species, distribution details, biology, dispersal, virulence/host range/infectivity, temperature, survival and persistence, tritrophic effects, compatibility, genetic improvement, bioefficiency (laboratory/greenhouse/field), mass production, formulation and storage, application, abiotic and biotic factors, integration in IPM, biosafety, nontarget effects, commercial products, quality, techniques, chromosomes and associated bacteria.

**59. Biosystematics of *Trichogramma* and *Trichogrammatoidea* (Hymenoptera: Trichogrammatidae) (01/07/2006 to 31/03/2013) PI: Dr. Prashanth Mohanraj**

Collections were made from both agroecosystems and non-crop ecosystems using sweep nets, yellow pan traps, sentinel cards as well as insect eggs *in situ*. Karnataka, Tamil Nadu, Andhra Pradesh, Odisha, Kashmir, Assam, Maharashtra, Rajasthan, New Delhi, Uttar Pradesh, Andaman and Nicobar Islands and Madhya Pradesh were surveyed for *Trichogramma* and *Trichogrammatoidea*.

All species of *Trichogramma* and *Trichogrammatoidea* known from the country are in the collection at NBAII with the exception of four species of *Trichogramma* and two species of *Trichogrammatoidea* described from elsewhere. Cultures of the *Trichogramma* and *Trichogrammatoidea* collected from various places were built up from field collected material and maintained on the eggs of *C. cephalonica*. Once adequate numbers were available they were processed, slide mounted and identified. *T. embryophagum* was collected on trap / sentinel cards from a tea plantation in Assam. This forms the first record of this species from India. *T. pretiosum*, an exotic species imported and released in India in the late 1960s and early 1970s was for the first time recovered on trap cards from Anakapalli in Andhra Pradesh. Thus far *Trichogramma* had been collected only on lepidopteran hosts from India. For the first time they were collected from the eggs of Neuroptera from Chickaballapur, Karnataka. *T. chilonis* were found parasitizing these eggs. *Trichogramma rabindraii* Nagaraja and Prashanth Mohanraj, *Trichogramma danaidiphaga* Nagaraja and Prashanth Mohanraj, *Trichogramma pieridis* Nagaraja and Prashanth Mohanraj, *Trichogramma giriensis* Nagaraja and Prashanth Mohanraj, *Trichogrammatoidea ruficolorata* Nagaraja and Prashanth Mohanraj, *Trichogrammatoidea brevicaudata* Nagaraja and Prashanth Mohanraj were discovered and described as new.

A single specimen of a male of *Trichogramma* resembling *Trichogramma bistræ* (Kostadinov) was collected from Bhubaneswar, Odisha. Very few species are known from Europe, North and South America. This was the first record of this species from the Oriental region.

**60. Interaction within the natural enemy guilds of *Ceratovacuna langera* and *Maconellicoccus hirsutus* (01/09/2006 to 31/03/2008) PI: Dr. Srinivasan Ramani**

The structure of an ecological community would be determined by the dynamics of the interactions between the species. Intraspecific competition occurs when different individuals of the same species or population compete for a resource for survival and reproduction. It is important to understand the nature of that competition before any biological control programme. If the different species are capable of co-existing by means of resource partitioning, control of the pest host might be best achieved by introducing some or all of the parasitoid species. On the other hand, if the competitors limit each other's populations by competitive interactions, then maximum control of the pest species might be achieved by releasing the most efficient of the parasitoid species. Assessment of interactions between potential complexes of natural enemies is crucial. The results of the experiments conducted on the interactions revealed that the morphological changes that occur in aphids parasitized by *Encarsia flavoscutellum* Zehntner showed that a dark patch with less woolly covering near the caudal end probably indicated that the aphid was parasitised and from 70% of such suspected aphids the parasitoids emerged. These were not fed by predators. DNA was isolated from *D. aphidivora*, *M. igorotus*, *E. flavoscutellum* and *C. lanigera* to enable identification of the ITS-1 region from each of them. Only parasitoid *E. flavoscutellum*, which had about 700 bp could be identified.

**61. Biological and molecular characterization of inter and intra specific variation intrichogrammatids (01/04/2007 to 31/03/2010) PI: Dr. Sushil Kumar Jalali**

In the host preference studies, the results showed that *T. chilonis* preferred the eggs of *C. cephalonica* most (mean 84.8% parasitism), followed by *C. partellus* (55.1%) and *H. armigera* (mean 47.9% parasitism) and eri silk worm eggs were least preferred. Irrespective of collection crop, parasitoids prefer to parasitize *C. cephalonica* eggs, whereas *T. achaeae* was recorded from eggs collected from 3 crops only.

The host plant preference studies showed that except for parasitoids collected from sugarcane parasitizing more eggs on it, none of the other parasitoids showed any preference for originally collected crop. Sugarcane appears to be most preferred crop recording parasitism of 30.5%, followed by paddy (18.9%) and cotton (18.3%). Tomato was least preferred host plant.

Based on the size of the ITS-2 rDNA PCR products, base pairs varied from 500bp–900bp in the twelve trichogrammatid species used in the study. Based on this size variation, three groups could be distinguished: Group I included *Tr. armigera* and *Tr. bactrae* the size of ITS-2 product varied from 800bp to 900bp; Group II included *T. achaeae*, *T. japonicum* and *T. embryophagum* the size of ITS-2 product varied from 570 to 600bp and Group III included *T. chilonis*, *T. pretiosum*, *T. evanescens*, *T. mwanzai*, *T. pretiosum* (Th), *T. dendrolimi* and *T. brassicae*, the size of ITS-2 PCR products in these seven species varied from 500 to 550bp. These groups could be easily recognized after electrophoresis on agarose gel. Complete ITS-2 sequences of different species were deposited with NCBI GenBank.

Restriction digestion of all samples gave reproducible profiles. EcoR1 showed no sites for *T. achaeae*, *T. japonicum*, *T. pretiosum* (Th) and *T. pretiosum* (Ar). SacI showed no sites for *T. embryophagum*. MseI showed no sites for *T. chilonis*, *T. mwanzai*, *T. pretiosum* (Th) and *T. pretiosum* (Ar). Restriction digestion with MvaI resulted in a release of 75bp fragment except for *T. pretiosum* where two fragments of about 50bp and 75bp were observed. Restriction digestion with EcoRI enzyme enabled differentiation of ten species, while restriction digestion with MseI enabled differentiation of six species; SacI was useful to differentiate *T. embryophagum*. Based on restriction patterns, a dichotomous key was constructed for easy differentiation of these twelve trichogrammatids.

ITS2 region of *T. chilonis* collected from the field were amplified and sequenced. The sequences were analysed using Bioedit software and sequence identity matrix was generated. The most of the populations matched to the tune of 98.1 to 100.0 per cent amongst themselves.

*Wolbachia* amplification was achieved by using two primers, viz., *wsp* and *FtsZ*. Based on the size of the *wsp* and *FtsZ* PCR products, base pairs varied from 550bp–600bp and 700-750bp, respectively in the eight trichogrammatid species infected with *Wolbachia*. It was confirmed that all these species except for *Trichogramma cacoeciae* Marchal and *T. embryophagum* (Germany) contained *Wolbachia*.

Curing of *Wolbachia* infected species was carried out with rifampsin revealed that per cent females remained 100.0 in *T. cacoeciae* might be of different group as curing did not give any males. In other species, per cent females ranged from 23.3-98.1, 50.5-100.0, 60.5-90.0, 48.9-88.7, 63.2-98.6 and 35.6-100.0. Mean fecundity decreased in *T. cacoeciae*, *Trichogramma corbudensis* Vargas & Cabello, *T. embryophagum*, *T. evanescens* and *T. pretiosum* (France), but fecundity increased in *Trichogramma semblidis* (Aurivillius) and *T. pretiosum* (USA) after 20 generations of curing. Curing of *Wolbachia* infected species was carried out with tetracycline revealed that per cent females remained 100.0 in *T. cacoeciae*, which might be due to different group of *Wolbachia* as curing did not give any males. In other species, per cent females ranged from 78.5-100.0, 70.5-100.0, 60.5-90.0, 83.2-88.7, 59.6-98.6 and 52.1-100.0. Mean fecundity decreased in *T. cacoeciae*, *T. corbudensis*, *T. embryophagum* and *T. evanescens* but fecundity increased in *T. semblidis* and *T. pretiosum* (France and USA) after 20 generations of curing.

In the experiment, the ability to detect *Wolbachia* via PCR amplification, a very faint band was observed after 40<sup>th</sup> generation of curing with antibiotic, but there was no disappearance of the band in all treated lines of four *Trichogramma* species. Again an intense band was observed in case of withdrawal of antibiotics. This shows that incomplete removal of the *Wolbachia* during heat treatment. If *Wolbachia* was killed by the treatment, possibly no band would appear, but faint band was observed after curing, results suggesting ability of antibiotic to partially cure *Wolbachia* infection in various species. This indicated that because of lower microorganism titer, males 38 appeared during the time of treatment, but once the treatment was withdrawn, the microorganism titer increases again and resulting in prominent band again at 10<sup>th</sup> generation after withdrawal.

The experiment was carried out with trichogrammatids collected from crops in tomato ecosystem. The adults were sterilized with sodium hypochlorite and were homogenized in distilled water @ 20 adults in 1ml of water with micro pestle. This homogenate was plated in yeast media as per standard protocol. After 24h, plates were observed for development of micro-organisms. Of these, yeast were separated out and re-cultured and were centrifuged. The material thus obtained was used as

feed for laboratory reared adult to know its effect on fitness of the population. By ITS sequence analysis, yeast isolates were correctly identified to species level. A BLAST search revealed that yeast isolated from sesamum and rose had sequence similarities of 99 and 100 per cent respectively with their corresponding type strains *Pichia anomala* E.C. Hansen (GenBank Accession No. AY349442). Yeast species isolated from tomato had an ITS sequence similarity of 100% with those of the type strain of *Candida apicola* (Hajsig) (GenBank Accession Nos. EU926481). The endosymbiotic yeast associated with *T. chilonis* obtained from rose and tomato were identified by ITS sequence analysis as *P. anomala* Tcy1 strain (GenBank Accession No. FJ224365), *P. anomala* Tcy2 strain (GenBank Accession No. FJ599744) and *C. apicola* Tcy3 strain (GenBank Accession No. FJ713025), respectively. Fitness experiment with yeast was carried out with laboratory reared trichogrammatids. The per cent females in *T. chilonis*, *T. japonicum*, *T. achaeae* and *Tr. bactrae* enhanced to 45.0–80.0, 50.0–79.4, 45.0–64.5 and 56.0–9.0, respectively. The fecundity of various species also increased marginally.

**62. Attractants for natural enemies of rice pests for use in conservation of natural enemies (01/04/2007 to 30/06/2011) PI: Dr. Deepa Bhagat**

The volatiles were identified in rice varieties viz., Basmati 370, CTH-3, KMT-105, JR-20, Mandya Vijaya, MTU-1001, Jyoti Tanu, Vilirajamundi, Rasi, KMP-149, IR-30864 and Mangala. The extract of Basmati 370, CTH-3, KMT-105, JR-20, Mandya vijaya and MTU-1001 was encapsulated in alginate-chitosan (ALG-CS) nanoparticles for slow release and attraction of natural enemies.

ALG-CS nanoparticles prepared at an acidic pH with a higher ALG/CS mass ratio resulted in reduction in the mean size of NPs. Nanoparticles of the lowest mean size possible are always preferable in applications where slow release of compounds are required. We have prepared nanoparticles with ALG/CS mass ratio of 7:1 at a final pH of 5 for encapsulation of the extract of Basmati 370, CTH-3, KMT-105, JR-20, Mandya vijaya and MTU-1001. We followed the “incorporation method” for compound-loading purposes. Incorporation of extract of Basmati 370, CTH-3, KMT-105, JR-20, Mandya vijaya and MTU-1001 into ALG-CS nanoparticles was done by adding ethanol solution of extract of Basmati 370, CTH-3, KMT-105, JR-20, Mandya vijaya and MTU-1001 into calcium chloride aqueous solution. Pre-gel of calcium alginate as discrete NPs along with extract of Basmati 370, CTH-3, KMT-105, JR-20, Mandya vijaya and MTU-1001 was embedded when cross-linked with the polycationic polymer CS and a nano dispersion of extract of Basmati 370, CTH-3, KMT-105, JR-20, Mandya vijaya and MTU-1001 - loaded ALG-CS-NPs was obtained. Thus, water miscibility of ethanol and extract of Basmati 370, CTH-3, KMT-105, JR-20, Mandya vijaya and MTU-1001 behaviour in aqueous phase were the two important factors causing successful loading of extract of Basmati 370, CTH-3, KMT-105, JR-20, Mandya vijaya and MTU-1001 to hydrophilic ALG-CS NPs.

**63. Conservation of natural enemies of rice pests through habitat manipulation techniques (01/04/2007 to 30/06/2009) PI: Dr. Nandagopal Bakthavatsalam**

Survey of pests and natural enemies was conducted at the Mandya region of Karnataka in rice fields. The incidence of stem borer was more (6.05% white earhead) followed by the leaf folder, *Cnaphlocrocis medinalis* (Guenée) (5.8 leaves per m<sup>2</sup>). The incidence of natural enemies in Mandya was negligible.

Egg masses of stem borer *Scirpophaga incertulas* (Walker) were collected from Tamil Nadu and the incidence of natural enemies was recorded. The incidence of *Tetrastichus schoenobii* Ferrière was very high. However, the incidence of *T. japonicum* was negligible. From the egg masses, an average of 15.95 *T. schoenobii* emerged. The number of males was 8.57 and females were 7.40 per egg mass. The female proportion was 46.33%.

Surveys were carried out for the weed flora within and outside the rice fields during the cropping season and off season. More than 20 species of plants, including several species of grasses were identified. The incidence of pests or natural enemies was assessed by keeping them in the laboratory and observing for the emergence of pests and entomophages. However, none of the flora supported any entomophage as no natural enemies/pests emerged during the period of study.

Study was conducted to find out the tritrophic relations between the crop, pests and their natural enemies, with particular emphasis on using these cultivars as reservoir hosts. The behavioural response of the *T. japonicum* to the cultivars of rice such as TN1, Jaya, Vijitha and Triguna was studied by extracting the seedlings of different ages in hexane, acetone, chloroform or dichloromethane. Among the cultivars Triguna recorded highest response and among the different age old seedlings, 30 and 43 age old seedlings recorded maximum response from the parasitoid. Studies were also conducted to find out the attraction or repulsion effect of the common weeds on the egg parasitoid, *T. japonicum*. Among the weeds, *Melia dubia* Cav. and *Malvastrum coramandelianum* (L.) recorded maximum attraction, while on *Cleome viscosa* L. least attraction was recorded.

Around 100 analytical fractions were collected through column chromatography from the weed *M. dubia* and evaluated for the response of *T. japonicum* to the volatile fractions in an effort to identify attractants. Fraction 22 and 23 showed highest attraction recording 84.0 and 87.75 per cent parasitisation respectively in ovipositional response studies. Least attraction was noticed in fractions 25, 26, 27 (0% parasitisation). Similarly, 100 analytical fractions were collected through column chromatography from the weed *Tephrosia purpurea* (L.) and evaluated for their response to *T. japonicum* in an effort to identify attractants. Fraction 38 and 60 showed highest attraction recording 81.0 and 84.5 per cent parasitization, respectively, in ovipositional response studies.

Wind tunnel studies were conducted to find out the attraction of *T. shoenobii* to the volatiles of rice cultivars. Cultivar Triguna recorded highest attraction, though on par with all the other cultivars like TN-1, Vijitha and Jaya. Honey (reference) and rice recorded highest response, followed by extracts from *Hyptis suaveolens* (L.).

To improve the storage of kairomone formulation with tricosane, stabilizers like citric acid and acetic acid were added. The formulations with acetic acid could be stored for 15 days.

The volatile composition of rice, and several weed species was analyzed through GCMS. Important compounds such as methyl hexadecanoate, 2,-di—butyl phenol, caryophyllene and phytol were identified.

#### **64. Development of production protocols and evaluation of anthocorid and mite predators (01/04/2007 to 31/03/2012) PI: Dr. Chandish R Ballal**

The project concentrated on identifying potential anthocorid and mite predators, devising production protocols for them and evaluating them against serious pests. A new species of *Xylocoris* (*Proxylocoris*) was collected from maize and a new species of *Orius* was collected from *Calotropis*. *Physopleurella armata* Poppius was collected from coconut and it was the first record for India.

A new anthocorid predator *Montandoniola indica* Yamada, sp. nov. was recorded for the first time as a predator of *Gynaikothrips uzeli* Zimmermann infesting *Ficus retusa* L. in Karnataka. *M. indica* was also recorded as an efficient predator of gall-forming thrips, *Liothrips karnyi* Bagnall infesting black-pepper leaves in Kerala. True *Montandoniola moraguesi* (Puton) was reported to be restricted to the Mediterranean region and Africa, indicating that earlier reports of *M. moraguesi* from India could in fact be *M. indica*. This anthocorid predator was amenable to laboratory production on UV irradiated *C. cephalonica* eggs.

An insectary producing *Corcyra* eggs, can produce anthocorid, *C. exiguus* @ Rs 5.8 for 100 nymphs. Continuous lab rearing adversely affected the progeny production in *C. exiguus*. 25 and 30°C were suitable temperatures for survival and reproduction of *C. exiguus*, while 20°C was detrimental as developmental period was prolonged and progeny production was low at this temperature.

*C. exiguus* eggs can be stored for 5 days at 10°C (with 64% hatching and 64% adult emergence) and 10 days at 15°C (with 68% hatching and 68% adult emergence). By storing for 5 days at 10°C or 15°C, incubation period could be staggered to 10 days, while storage for 10 days at 15°C, could extend incubation period to 13 days. *C. exiguus* adults could be stored at 10°C for upto 5 days, beyond which it was detrimental to the biological parameters of the adults. Based on the survival, longevity and progeny production of the stored adults, *C. exiguus* adults could be stored for up to 15 days at 15°C. Thrips population and curling of the leaves in capsicum could be reduced by making three releases of *B. pallascens*, however, the anthocorid did not establish well in the released plots. *T. tabaci* on onion and garlic could be effectively reduced through four releases of *B. pallascens*. The anthocorid established them well in the ecosystem. *B. pallascens* could cause oviposition disruption in

bruchids. *B. pallescens* could be used as a potential predator of chilli broad mite *P. latus* and four releases could result in significant reduction in leaf curling and drying. Nymphs and adults of *B. pallescens* were observed to be potential predators of the cotton mealybugs. Adult release at a ratio of 1:5 provided maximum mortality, which was also on par with nymphal treatments at ratios of 1:5 and 1:10. Mature nymphs and adult *B. pallescens* could effectively predate on both young and mature cotton mealy bug crawlers, however, preference was more for younger mealybugs. Similarly the nymph of *B. pallescens* was observed to be more voracious in comparison to adult stage. It was also recorded that the anthocorid could not feed on the adult mealybugs.

Young nymphs, mature nymphs and adults of *B. pallescens* could feed on papaya mealybug. However, the longevity of *B. pallescens* when fed on papaya mealybug was significantly reduced, indicating that it was not a potential predator for targeting papaya mealybug.

Adult predatory potential and longevity of *B. pallescens* could be improved by providing cotton mealy bug crawlers right from the nymphal stage, indicating that prey available during the nymphal stage could have a clear bearing on the feeding potential of the adult stages.

Releases of *B. pallescens* against *Frankliniella schultzei* (Trybom) infesting freshno chilli (var. Supreme) in polyhouse resulted in reduction in thrips population and the yield and quality parameters of the harvested chillies from the bio-control plot was comparable with that of the chemical control plot.

Maximum egg laying in *O. tantillus* could be obtained by providing pollen and eggs and beans with stalks as oviposition substrates. *O. tantillus* could be effectively reared using frozen *H. armigera* eggs mixed with pollen. While lab rearing *O. tantillus*, there was a continuous reduction in progeny production from the XI<sup>th</sup> generation, while in the case of *O. maxidentex*, this reduction was from the VI<sup>th</sup> generation, indicating that *O. tantillus* was more amenable to continuous laboratory multiplication.

A new anthocorid, *Anthocoris muraleedharani* Yamada collected from *Bauhinia purpurea* L. infested by *F. virgata* was observed to be a potential predator of mealybugs. Five nymphal instars were observed on *A. muraleedharani*, the durations being 3 to 4, 2 to 3, 4 to 5, 4 to 5 and 6 to 7 days, respectively. The total nymphal feeding was 55 Cotton mealybug crawlers per nymph with a per day feeding of 3.23.

The fertility table parameters, viz., the reproductive rate, intrinsic and finite rates of increase, hypothetical F2 females and Weekly Multiplication rate of *X. flavipes* were higher and Doubling Time lower when reared in pairs in comparison to rearing as mated females.

Treatment with anthocorids, viz., *C. exiguus*, *B. pallescens* and *X. flavipes* led to significant reduction in population of *S. cerealella* infesting paddy and treatments with *C. exiguus* and *X. flavipes* @ 30 nymphs per container were observed to be superior treatments.

#### **65. Effect of different edaphic factors on Entomopathogenic nematode (EPN) activity and refinement of packaging for EPN formulations (01/04/2007 to 31/10/2008) PI: Dr. Syed Shahabuddin Hussaini**

Soil composition and texture are the important factors which affect the post application persistence of EPN in the soil. Among nematode species and soil types studied, *S. carpocapsae* persistence was maximum (180 days) in soil types, viz., sandy clay loam of Bapatla and Attur and Silt of Kanpur and silty of Indore whereas *H. indica* persisted for 180 days only in sandy clay loam of Bapatla and silty of Indore. Though sandy soils were suitable in terms of activity / pathogenicity for both the species, persistence was found to be least in this soil type. In general it was observed that the soil types with higher silt fraction and higher moisture holding capacity favored the persistence.

EPN activity in soil including post application persistence is regulated by the physical and chemical characteristics of soil. The P<sup>H</sup> of soil is one of the factors that determine the persistence of EPN in field. Hence an experiment was set up to study the effect of pH of soil on infectivity and survival of EPN population. Pathogenicity did not vary in any soil type 90 days after application except in clay soil. In this case percent mortality of wax moth larvae was reduced by both *S. carpocapsae* (66.6%) and *H. indica* (60%) the pH of this particular soil type was recorded as 4.8 and was the minimum included in the study. The soil pH in the experiment ranged from 4.8-8.5. The

results indicated that the pH range chosen for the study had no adverse effect on survival and pathogenicity of both the isolates tested.

Survival and persistence of EPN species was studied in different soil types with varying pH levels. *H. indica* and *S. carpocapsae* originally isolated from Bapatla (A.P.) was used in the study. The persistence of above isolates applied in soil was determined by means of soil baiting using wax moth larvae. The pathogenicity of applied nematodes was checked monthly for six months.

Of the two nematode species tested, *S. carpocapsae* persisted maximum in all the soil types tested. Among soil types studied, *S. carpocapsae* persistence was maximum in 4 soil types viz., sandy clay loam of Bapatla and Attur and silt of Kanpur and silty clay of Indore whereas maximum persistence of *H. indica* was 120 days in sandy clay loam of Bapatla and silty clay of Indore. Among the soil types studied clay soil had the minimum pH of 4.8 in which the maximum persistence observed was 90 days for both nematode species

Although soil is the natural habitat for entomopathogenic nematodes, several environmental factors, including various physical and chemical properties of soil influence nematode performance, movement and persistence in soil. However species strains differ in their specific responses to abiotic and biotic factors.

Pathogenicity varied in different soil types at different depths. Absolute mortality of *G. mellonella* larvae was observed at 10 cm at depth in sandy clay loam, sandy, silty and silty clay soil types. However infectivity of *S. feltiae*, *S. bicornutum* and *Steinernema riobrave* Cabanillas, Poinar and Raulston was lowered in clay soil and the mortality of *G. mellonella* larvae ranged from 50-66.6 per cent. This indicates the difference in suitability for nematodes activity in different soil types. Clay soil with about 40% each of sand and clay and rest silt was suited for *S. carpocapsae*, *S. abbasi* and *H. indica* than *S. feltiae*, *S. riobrave* and *S. bicornutum*.

Infectivity of nematode species at 15 cm depth was different from the results at 10 cm. infectivity of all Steinernematids tested was reduced whereas *H. indica* caused absolute mortality in all soil types except clay soil where the mortality of *G. mellonella* larvae was 66.6%. Of *Steinernema* spp., the maximum mortality of wax moth larvae was 83.3% caused by *S. feltiae* in sand and silt soils. Mortality of wax moth larvae ranged from 66.6 to 83.3 per cent in silt soil. Infectivity of *S. feltiae*, *S. bicornutum* and *S. riobrave* was adversely affected in clay soil at both depths tested. Maximum mortality observed was 50-66.6 per cent at 10cm where as no mortality was observed when tested at 50 cm depth. *S. carpocapsae*, *S. abbasi* and *H. indica* caused absolute mortality in this soil type at 10 cm depth.

EPN are known to move vertically in the natural habitat. Horizontal movement of EPN species and isolates tested was not affected adversely at 10 distances in any soil type studied. When compared to vertical movement, the response elicited only at 48h. exposure in all the treatments in horizontal movement and with respect to number of larvae infected at the both the distance, *S. carpocapsae* out competed *H. indica* isolate with maximum mortality at all exposure periods in all treatments. The difference in the mortality rate with respect to soil types and nematode species were observed up to 72h after exposure. The present results indicate that nematode species belonging to Steinernematidae and Heterorhabditidae exhibit both types of movement in soil.

Sponge based formulation was more suitable with increased shelf life Steinernematids and talc based Heterorhabditids. Irrespective of nematode species/isolates the viability and shelf life was maximum in high density sponge, 100% survival was observed in high and medium density sponges for all nematode isolates and species for 1 month.

Polypropylene with medium gauge (transparent) was found most suitable as packaging material for both talc and sponge based formulation of EPN and normal air packing, 75% nitrogen was found more suitable than vacuum packing.

## **66. Long term management of red hairy caterpillar (*Amsacta albistriga*) by creating epizootics of Nuclear polyhedrosis virus (01/05/2007 to 30/08/2008)**

**PI: Dr. Veenakumari Kamalanathan**

Surveys were conducted for the caterpillars of *A. albistriga* in different locations such as Chikmagalur district and in Tumkur district on sesamum and groundnut crops, respectively. Virosed

larvae were collected from these areas and three new isolates of AmalNPV (Kadur, P. Samudrum and B.R. Halli) were obtained by semipurification following standard procedures.

Bioassays were conducted to study the relative efficacy of these isolates of AmalNPV against first, second and third instar larvae. Studies on the relative efficacy of these isolates revealed that the Pavagada isolate was the most virulent with a  $LC_{50}$  of  $0.89 \times 10^2$ . It was 9.16, 7.88 and 5.40-fold more virulent than the B.R. Halli, P. Samudrum and Kadur isolates, respectively against the first instar larvae. Similarly,  $LT_{50}$  values were also calculated for all the four isolates using a concentration of  $1 \times 10^6$  POB/ml of AmalNPV. It was found that the least time required was by the Pavagada isolate (89.69hrs) against first instar. This was followed by P. Samudrum, B.R. Halli and Kadur isolates which had  $LT_{50}$  values of 96.35 hrs, 106.38 hrs. and 90.36 hrs. respectively. The activity of the four isolates of AmalNPV in decreasing order of virulence was Pavagada>Kadur>P.Samudrum>B.R.Halli for the first instar. It was also noticed that the late instar larvae of *A. albistriga* collected from B.R. Halli were parasitized by two species of Tachinidae (43.6%) and *Apanteles* sp. (28.6%). As the emergence of red hairy caterpillar was very poor in the endemic areas work was initiated on the other arctiid hairy caterpillar *Spilarctia obliqua* (Walker). Surveys were conducted for the caterpillars of *S. obliqua* in Kolar district and in Bangalore district on field bean, mulberry and sunflower. Virosed larvae were collected from these areas and three new isolates (Devaganahalli, Talagavara and Hoskote) were obtained by semipurification following standard procedures. Bioassay studies were conducted to study the relative efficacy of these isolates.

Studies on the relative efficacy of these isolates revealed that the D.G.Halli isolate was the most virulent with a  $LC_{50}$  of  $1.94 \times 10^3$ . It was 4.19, 2.65 and 1.20 folds more virulent than the Manipur, T. Vara and H. Kote isolates respectively against the first instar larvae. Similarly  $LT_{50}$  values were also calculated for all the four isolates using a concentration of  $1 \times 10^6$  POB/ml of SoNPV. It was found that the least time required was by the D.G. Halli isolate (4.56 days) against first instar. This was followed by H. Kote, T.Vara, and Manipur isolates which had  $LT_{50}$  values of 5.01, 5.94 and 8.34 days, respectively. The activity of the four isolates of SoNPV in decreasing order of virulence was D.G.Halli>H.Kote>T.Vara> Manipur for the first instar.

Effect of simulated sunlight on the virulence of SoNPV : SoNPV was subjected to five doses of simulated sunlight viz., 350 W/m<sup>2</sup>, 450 W/m<sup>2</sup>, 550 W/m<sup>2</sup>, 650 W/m<sup>2</sup> and 750 W/m<sup>2</sup> for one, two, three and four hours. Bioassays were conducted against second instar larvae using these irradiated viruses. Un-irradiated virus served as control. When SoNPV was exposed (for one hour) to the simulated sunlight of 450 and 550 W/m<sup>2</sup> a larval mortality of 55.57 and 53.33 per cent were obtained. When they were exposed to 350 W/m<sup>2</sup> it took three hours for obtaining around 50% mortality. When the larvae were exposed at 750 the larval mortality ranged from 39.00 to 4.54 per cent for a period of 1 hour to four hours.

Effect of various adjuvants on the efficacy of SoNPV was studied: Laboratory studies were conducted to evaluate the efficacy of different adjuvants in increasing the larval mortality in second instars *S. obliqua* larvae. SoNPV along with respective adjuvant were exposed to an irradiation of 550 W/m<sup>2</sup> for a period of 60 minutes using sun test machine, which simulates natural sunlight. Bioassays were then conducted using these irradiated mixtures of adjuvant + virus against second instar host larvae and the larval mortality were recorded.

Even though all the adjuvants screened significantly increased the larval mortality, crude sugar (5%), molasses (5%) and Tinopal (0.2%) resulted in a larval mortality of 92.33, 91.66 and 89.19 per cent respectively. This was followed by CSKE (5.0%) and starch (1%) with a larval mortality of 84.66 and 49.50 per cent, respectively.

Bioassays were conducted to study the efficacy of different combinations of adjuvants for their efficacy in increasing larval mortality of *S. obliqua*. SoNPV along with different combinations of adjuvants were exposed to simulated sunlight. All adjuvants screened significantly increased the larval mortality. However adjuvant combinations viz., molasses 5% + Tinopal 0.2%, molasses 5% + Tinopal 0.1% and crude sugar 5% + Tinopal 0.2% resulted in highest larval mortality of 89-91 per cent with a relative efficacy of 2.1 times when compared with irradiated virus. These treatments were on par with non-irradiated virus. This was followed by molasses 5% + starch 0.1% resulting 83% larval mortality.

**67. Formulations of pheromones of important borers and other crop pests and kairomones for natural enemies using nanotechnology (01/04/2008 to 31/03/2013) PI: Dr. Deepa Bhagat**

Sodium alginate nanoparticles (NPs) of linalool, myrcene and caryophyllene were synthesized. Usually the linalool release had a three-step profile: a quick initial release, a slowdown and finally an increased constant release rate. The linalool release was slower with capsule crosslinking. The release profile was more pronounced in capsules incorporating formaldehyde as compared to glutaraldehyde. Release rates were studied with a thermogravimetric analyzer, the size of NPs was measured by differential light scattering (DLS) equipment and NP morphology was studied with a scanning electron microscope. The latter showed the outer part of the NP was sodium alginate, whereas the core consisted of linalool, myrcene and carophyllene. An 8-arm olfactometer bioassay using *T. chilonis* revealed the percentage of parasitism was higher with NPs of linalool in comparison with a previous formulation of linalool with hexane loaded in septa.

Synthesized sodium alginate NPs of tricosane, pentacosane, docosane: The stability of NPs and the slow release of tricosane were studied with a thermogravimetric analyzer and a uniform loss of NP weight over time occurred. The NP's size was measured by DLS equipment every 15 days and after one month the smaller nanoparticles had formed into larger NPs. Scanning electron microscopy revealed the NP's outer shell was sodium alginate whereas the inside core consisted of tricosane, pentacosane and docosane.

Linalool gelatin nanocapsules were synthesized to enhance the parasitization efficiency of a *Trichogramma* parasitoid that naturally acts as a biological control agent against *H. armigera*. Five different nanocapsule formulations were synthesized and the sizes were found to differ. In a further study with *T. chilonis*, its parasitisation efficiency was observed with the 60 nm (GLC 3) NPs at 69%, followed with the 112 nm (GLC 4) NPs at 62%, the 312 nm (GLC 2) NPs at 27% and finally the control 48 nm (GLC 5) NPs at 7% parasitisation. Evidently there is a particle-size effect worthy of more study. Sodium alginate NPs of linalool also enhanced the parasitism efficiency of *T. chilonis* in similar studies. The encapsulation of the *H. armigera* pheromone (HAP) in alginate-chitosan (ALG-CS) composite NPs was achieved using a pH of 4.5 – 5.0, which resulted in smaller NPs. Smaller NPs release their compounds more slowly. ALG-CS NPs had a mass ratio of 6:1 at a final pH of 4.7 for the encapsulation of the *H. armigera* pheromone. The aqueous miscibility of ethanol and the *H. armigera* pheromone in the aqueous phase are two important factors allowing the loading of the hydrophobic pheromone to the hydrophilic ALG-CS NPs. NPs were synthesized with different particle sizes, zeta potentials and electrophoretic mobilities. Fortunately, ALG and CS are biodegradable.

A dispenser was made for nanoformulations of HAP loaded with ALG-CS NPs and ( $\pm$ ) linalool-loaded gelatin. LDPE and Nylon were used and the mixture of these and the NPs were molded into a circular form. The *L. orbonalis* pheromone was immobilized in chiton-alginate NPs. Their size was in the range of 200 – 600 nm and their zeta potential showed they were stable. SEM studies confirmed the NP size. Headspace-GCMS confirmed the pheromone was encapsulated. *H. armigera* pheromone was immobilized in gelatin NPs. We found the concentration of the cross linker glutaraldehyde controls the NP size, with increases in cross linker glutaraldehyde concentration decreasing the NP size. The electroantennogram response to *H. armigera* pheromone immobilized in gelatin NPs: These numerous experiments demonstrated that loading onto NPs did not adversely affect the activity of the pheromone.

Female sex pheromones of the brinjal fruit and shoot borer, *L. orbonalis* were nanoencapsulated in chitosan-alginate NPs and field trials were done. The blend of E-11-16: Ac and E-11-16: OH in a 100:1 ratio was effective for attracting males. Field trials with planted brinjal seedlings at the Attur Farm showed that plants artificially infested with 3 to 4 first instar *L. orbonalis* larvae confirmed the laboratory studies when traps were setup with NPs and pheromone blends. More confirmatory field trials are needed.

Effect of chitosan alginate NPs: blank or loaded with *H. armigera* pheromone (HAP) on a natural enemy *C. z. sillemi*. The larvae of *C. z. sillemi*, especially the 3rd instar, showed good predation potential in controlling *H. armigera* eggs and larvae in various crops. Toxicological studies were done on ALG-CS NPs, blank and loaded with HAP, on different life stages of *C. z. sillemi*. Larval, pupal and adult survival rates were all cut by the HAP blend. Again, the role of glutaraldehyde

cross linker in controlling particle size was confirmed, *i.e.*, the size of the NPs was inversely related to the glutaraldehyde concentration. EAG responses to several nanoformulations were studied using honey as a reference.

The volatile extracts from different varieties of rice were encapsulated in chitosan-alginate NPs (CANPs) to attract *S. incertulas*. The CANPs prepared with higher ALG/CS mass ratios resulted in smaller NPs, which is beneficial because smaller NPs have a slower release of the pheromone. NPs with an ALG/CS mass ratio of 7:1 were prepared for the encapsulation of the extracts of Basmati 370, CTH-3, KMT-105, JR-20, MandyaVijaya, and MTU-1001.

Polymer gel formulations for the slow release of the coffee stem borer, *Xylotrechus quadripes* Chevrolat pheromone were synthesized. Field trials confirmed the effectiveness of these NPs. The release profile at various temperatures showed the linalool release remained relatively constant (effective for many weeks) over a temperature range of 17 to 38°C.

#### **68. Nematode-derived fungi and bacteria for exploitation in agriculture (16/04/2008 to 31/03/2012) PI: Dr. Mandadi Nagesh**

Catalogued eighteen beneficial nematophagous fungal diversity in two states and developed passport database for 22 native fungal isolates from 8 states. Pure cultures of *P. lilacinus*, *P. chlamydosporia* and *A. oligospora* were supplied to 18 firms/R and D centres and AICRP centres.

Soil and nematode-infested roots from six nematode-endemic locations surveyed in Dharmapuri, Hosur and Attibele yielded three isolates of *Arthrobotrys* fungus and one *Verticillium suchlosporium* Gams & Dackman. Two new isolates of antagonistic fungi, *Dactylella oviparasitica* Stirling and Mankau and *Dreschlera* species were isolated from diseased egg masses and eggs sampled from commercial polyhouses. Isolated two new isolates of *P. lilacinus* from the infected root-knot nematode egg masses collected from soils of Puttur, Dakshina Kannada, Karnataka. Isolated one new isolate of *Arthrobotrys conoides* Drechsler from the galled and root-knot nematode infected roots and soil mix of commercial polyhouse in Nelamangala. Under *in vitro* conditions, *A. conoides* and *A. oligospora* resulted in 90-98 per cent mortality of *M. incognita* and *R. reniformis*.

Two new isolates of antagonistic fungi, *D. oviparasitica* and *Dreschlera* species were isolated from diseased egg masses and eggs samples from commercial polyhouses. They exhibited high percent infection of egg masses and eggs under *in vitro* and *in vivo* against root-knot and reniform nematodes. Database on passport information of these two isolates were developed and added to the data base. The temperature optima were 26 to 34°C for mycelial growth and 26 to 38°C for spore germination for both the fungi. Data base and performance conditions for NBAII isolates of *A. conoides* and *A. oligospora* were worked out.

Submission of isolates of fungi antagonistic to nematodes at the national collection and microbial gene bank at NBAIM, MAU, UP: Nine pure cultures of three antagonistic fungi including four isolates each of *P. lilacinus* and *P. chlamydosporia*, and one isolate of *A. oligospora* were submitted to NBAIR (NBAII) collection and repository along with the relevant passport data of each isolate.

Molecular identity of root-knot nematodes from field samples and their antagonistic fungi, *A. oligospora*, *A. conoides* and *D. oviparasitica* using ITS and beta-tubulin gene sequences were established.

Data base and performance conditions for PDBC isolates of *P. lilacinus*, *P. chlamydosporia* and *A. oligospora* were worked out and a chart was prepared.

Three NBAII isolates of *A. oligospora* and two of *A. conoides* exhibited 64-78 per cent parasitisation of infective juveniles of *M. incognita* at  $10^4$  spores/250cc soil on brinjal under glass house conditions.

Mass multiplied and developed 600 kg of talc formulations of *P. lilacinus* ( $10^8$  spores/g), 1000 kg of *P. chlamydosporia* and 100 kg of *A. oligospora* for AICRP trials. Moisture content and water activity of the formulations were maintained at 6-8 per cent and 0.68 to 0.70, respectively.

LD<sub>50</sub> for *P. lilacinus* and *P. chlamydosporia* formulations against root-knot nematodes in terms of reduction in root galls and egg masses under glasshouse conditions on tomato were 34.9 and 22.6 spores per 100cc soil at 28-30 days, respectively.

*A. conoides* and *A. oligospora* established and proliferated significantly at a soil temperature range of 22-38 ±1°C; pH of 5.6-7.9; organic carbon status of 0.18 and above. Application of 14-18 cfus/cc soil reduced nematode populations in soil by 50-54 per cent, root infection by 48-66 per cent and root-knot index to 1.2-1.8.

In a field study, *P. chlamydosporia* established and proliferated in tomato, brinjal, carrot, okra compared to radish, maize, chrysanthemum crop conditions.

Crop rotation with marigold followed by application of talc formulations of *P. chlamydosporia* in carnation polyhouses effectively controlled root-knot infection in commercial polyhouses.

Among different organics such as vermicompost, commercial organic pellets, coir pith, FYM, sheep/goat droppings and oil cakes, vermicompost, sheep droppings, FYM and processed organic pellets at 10g/250cc soil favoured better establishment and survival of fungal spores compared to untreated. Such amended soils when cultured on media recorded higher populations of microorganisms compared to untreated.

*P. lilacinus* and *P. chlamydosporia* were recovered from samples obtained from treated fields under AICRP on Nematodes and AICRP Biological control from AAU, Anand; GKVK, Bangalore; Rahuri, Maharashtra and AAU, Jorhat.

*P. chlamydosporia* and *P. lilacinus* on application to soils (type: laterite, sandy loam and loam with pH 6.4, 6.8 and 7.6, respectively, and organic carbon at 0.65, 0.82 and 0.48, respectively) exhibited spore germination and mycelia growth in 32-44 hrs at 32-33 °C and soil moisture of 58-59 per cent (W/V); 48-52 per cent root colonization and nematode eggmass infection of 38-46 per cent at 36-42 days of treatment.

Standardized scale-up production and formulation protocols for *A. oligospora* for technology transfer.

Influence of initial moisture content of solid substrates on conidial biomass of *P. lilacinus* for solid state mass production and downstream processing showed that there was a sharp decline in spore production in all the three bran at a WC of 1.25: 1.00. Increase in substrate WC recorded an increase in MC and  $a_w$  of conidiospores on all substrates but did not affect the spore viability. A combination of MC at 49-50 per cent and  $a_w$  at saturation (1.00) was ideal for conidiospore production of *P. lilacinus* on bran, while a MC of 20-23 per cent and saturated  $a_w$  was ideal on rice grain. Identification of an ideal combination of MC and  $a_w$  were more critical than an optimum level of MC or  $a_w$  for conidiospore production.

Mode of action and virulence factors of the isolates of *P. lilacinus* and *P. chlamydosporia* against root-knot nematodes: Serine protease, collagenase and chitinase enzymes responsible for virulence against root-knot nematode eggs and egg masses were detected in the isolates of *P. lilacinus* and *P. chlamydosporia* under *in vitro*.

Gene coding for serine protease, responsible for virulence against root-knot nematode eggs and egg masses, was amplified from the isolates of *P. lilacinus* and *P. chlamydosporia*.

Biological suppression of root-knot nematodes in polyhouses in combination with crop rotation (marigold): Application of 10<sup>7</sup> spores of *P. lilacinus* and *A. oligospora* /m<sup>2</sup> in carnation followed by rotation with marigold effectively controlled root-knot infection and the nematode suppression was observed to persist for one year depending on soil type and organic status.

The behavior of introduced antagonistic fungus in soil and in plant rhizosphere was studied using rapid technique developed for the antagonistic fungi.

An easy, accurate and rapid technique to detect the target antagonistic fungi (*P. chlamydosporia*, *P. lilacinus*) was developed successfully for the first time and was being validated under different conditions.

Field experiments at different AICRP Nematodes and Biocontrol centres indicated that root-knot nematode infection in pomegranate and citrus were effectively controlled in MPKV, Pune and AAU, Anand using the NBAIR (NBAIL) wettable powder formulations of *P. lilacinus* and *P. chlamydosporia*. Based on these results use of WP of these two antagonists are included in package practices in parts of Gujarat for the control of root-knot nematodes in these crops.

Similarly, the pigeon pea cyst nematode in red gram was controlled in AAU, Anand by using the NBAIR (NBAIL) wettable powder formulations of *P. lilacinus* and *P. chlamydosporia*. Based on

these results use of WP of these two antagonists are included in package practices in parts of Gujarat for the control of pigeon pea cyst nematode.

Incorporation of *P. lilacinus* or *P. chlamydosporia* talc formulations at 20 Kg/ha along with 200kg/ha of vermicompost in furrows before sowing gherkin seeds recorded 54-72 per cent infection of egg masses, reduction in the nematode populations by 22-38 per cent in soil and 38-66 per cent reduction in root-knot nematode infection in roots, in 75 days of crop growth.

Soil solarization using plastic mulches for 45-60 days during the May-July followed by the incorporation of *P. chlamydosporia* ( $2 \times 10^{10}$  spores/m<sup>2</sup>) + neem cake (1kg/m<sup>2</sup>) before sowing of tomato seeds in the nursery recorded better soil moisture retention which resulted in higher fungal cfus/g in soil after two months and better root colonization compared to the incorporation of the fungus and neem cake without mulching. Establishment of the fungus and increased population was recorded using PCR method.

Filed an application for complete patent on wettable powder formulation of *P. chlamydosporia* at Indian Patent Office, Chennai (Patent application no. 2664/CHE/2010) entitled 'Development of novel wettable powder formulation of *P. chlamydosporia* var. *chlamydosporia* as bionematicide and methods thereof for scale-up production and down-stream processing for commercial use'.

#### **69. Mass production and exploitation of entomopathogenic nematodes (EPN) against white grubs from diverse habitats (16/04/2008 to 31/03/2012) PI: Dr. Mandadi Nagesh**

DNA bar coding of NBAIR (PDBC) isolates of entomopathogenic nematodes: A comparison of the nucleotide sequences of the COI gene (DNA bar coding) revealed that the sequences of *Heterorhabditis* and *Steinernema* apparently differed between the two reproductive forms, and a high homology within each reproductive form. These genetic characterizations (DNA bar coding) strongly support the similarities and dissimilarities revealed by some morphological characters and morphometrics in correspondent isolates, making them a reliable tool to catalogue the EPN diversity and also to examine check the label claims in EPN formulations.

Scale-up production system(s) of *Steinernema* and *Heterorhabditis* species through *in vitro* cultures. Modification of diet media and growth conditions for economization of *Galleria* production under *in vivo*: Several inexpensive alternatives for diet ingredients were examined for their effect on growth and lifecycle of *Galleria* in comparison to the recommended diet. Some diet compositions were identified which did adversely affect the *Galleria* production and productivity. *In vivo* production of *H. indica* and *S. carpocapsae* on Greater wax moth larvae was enhanced by synchronizing the larval production and diet modification for the larvae. Surveys for white grubs and their associated EPN: Recorded endemicity of *Anomala bengalensis* Blanchard, *Leucopholis lepidophora* Blanchard, *Leucopholis burmeisteri* Brenske and *Cosmopolites* species in Sulya. Two new isolates of *Heterorhabditis* spp. were isolated from diseased grubs collected from Madikeri and added to collections. Recorded endemicity of *A. bengalensis*, *L. lepidophora*, and *Cosmopolites* species in Bankal. Two isolates of *Heterorhabditis* spp. from soil samples obtained from Maharashtra, and one each of *H. indica* and *Steinernema* sp. from the soils of Srinagar.

Performance conditions of NBAII isolates of EPN: Among the 3 nematodes, *H. indica* followed by *S. riobrave* and *S. carpocapsae* consistently recorded lower LD and LT values for the 3 white grub species (*A. bengalensis*, *L. lepidophora* and *L. burmeisteri*) at 3 depths (10,20 and 30cm). These observations aid us in fixing the EPN doses for field application for the three species and to estimate the probable time of grub mortality at different depths after application of the EPN.

Bioefficacy and identification of efficient strains and performance conditions of EPN against root grubs: Replicated screening of twelve EPN isolates for their bioefficacy against eggs of root grubs under *in vitro* with *Leucopholis* and *Anomala* species confirmed that there was no infection/penetration of eggs by the EPN screened. Eggs were found resistant to EPN infection.

*In vitro* studies were carried out with *H. indica* and *S. carpocapsae* obtained from *G. mellonella*, *C. cephalonica*, *H. armigera*, *S. litura*, *P. xylostella* and root grub (*L. lepidophora*) against *G. mellonella* and root grub. Results indicated that *H. indica* and *S. carpocapsae* obtained from *G. mellonella*, *C. cephalonica* and root grub exhibited better infectivity in shorter duration against *G. mellonella* and root grub, compared to the progeny obtained from *H. armigera*, *S. litura* and *P.*

*xylostella*. It indicated that the insect host from which the EPN have multiplied had an effect on infectivity of EPN.

Addition of coir pith, farm compost, vermicompost, vermiculite to talc or aqueous preparations of EPN at 10:1 proportion enhanced persistence and infectivity of EPN on test larvae of *Galleria*.

Field efficacy of EPN preparations in root grub endemic areas of arecanut in Western Ghats: Two field experiments were conducted at Sulya, Mangalore for evaluating bioefficacy of EPN preparations for the control of root grubs viz., *L. lepidophora*, *A. bengalensis* and *L. burmeisteri* in root grub endemic arecanut fields. For the first time, successfully established EPN in treated fields, recovered the EPN in large number from treated soils up to 6 months and effectively controlled root grubs of *Leucopholis*, *Anomala* and *Cosmopolites* species with field monitoring done at intervals.

A new technique to observe *in situ* the behaviour of root grubs and recover the cadavers in treated soil to confirm EPN as cause of root grub control was developed.

Effect of field soil moisture on the behaviour of root grubs, EPN persistence and infectivity was successfully studied for one season for developing guideline data. Soil moisture was recorded at 7-10, 17-20 and 27-30cm depth at monthly intervals between August and February at a predetermined EPN-treated plot in rainfed field at Sampaje. Root grubs of different sizes 1cm to 2.5cm were predominant in top 20cm from August to October with intermittent large sized grubs, while, grubs of more than 2.5cm were predominant between 10 and 30 cm from Oct to January. Soil moisture content was at saturation up to 20 cm till September which decreased from October.

**70. Establishment of *Puccinia spegazzinii* on *Mikania micrantha* (01/07/2008 to 30/06/2009)  
PI: Dr. Sreerama Kumar Prakya**

Twelve populations (AN-1 to AN-12) of the mile-a-minute weed, *Mikania micrantha* Kunth, collected in October 2005 from the Union Territory of Andaman and Nicobar Islands were continuously maintained in the PDBC greenhouse under quarantine-like conditions. For the first time, *M. micrantha* was found at the Ranganathittu Bird Sanctuary and in areas surrounding the waterbody near Mysore, Karnataka. An application was submitted to the Plant Protection Advisor to the Government of India on 21 February 2009 for obtaining a field-release permit to introduce the rust pathogen *Puccinia spegazzinii* De Toni (Trinidadian and Peruvian isolates) in and around the sanctuary.

A new methodology useful in the process of isolating endophytes from plant leaves was developed in the course of investigations on *M. micrantha*. Thirteen different sterilisation regimes were tested in an experiment to determine the best method to remove epiphytes from *M. micrantha* plant parts. The consistency of isolation of fungi and bacteria alone or both together from replicate pieces of *M. micrantha* was investigated through the four best methods of sterilisation.

In the study on general profiling of endophytic composition of different populations of *M. micrantha*, variations were observed at several levels. In general, stems and roots exhibited relatively high diversity of endophytes. Overall, non-sporulating, sterile fungal genera dominated the sporulating fungi. It was evident that, in general, older plant parts contained more endophytic fungi than their younger counterparts. The presence of suspected fungal endophytes in living tissues was demonstrated through microscopy. None of the 20 each of sporulating and non-sporulating fungi tested for their probable pathogenicity to *M. micrantha* produced any kind of disease symptoms on the inoculated plants during the one-month incubation period. It was found that the *M. micrantha* population (AN-9) from Andaman and Nicobar Islands harboured more endophytes than did the Karnataka population (KA-1). There was an assortment of fungal genera on the phylloplane of *M. micrantha* belonging to these two regions. Field-collected Karnataka population of *M. micrantha* was susceptible to foliage diseases by species of *Alternaria*, *Cercospora*, *Colletotrichum*, *Dreschlera* and *Phoma*. Continuous monitoring by scientists of the Kerala Forest Research Institute (KFRI, Peechi) revealed that *P. spegazzinii* did not get established in Kerala, indicating that the inoculum used was insufficient. No spread of the rust, therefore, was observed.

**71. Phytophagous mites as a source of microbes for harnessing in pest management (01/07/2008 to 30/06/2011) PI: Dr. Sreerama Kumar Prakya**

The diversity of microbial associates of three spider mite species (*Tetranychus ludeni* Zacher, *T. neocaledonicus* André and *T. urticae*) was discovered, investigated and documented. Both external and internal microbial associates were analysed for each species, healthy and diseased samples of which were regularly collected from fields in Bangalore Urban and Rural districts. Most of the fungal associates found exiting from the integument and sporulating externally were pathogenic genera of hyphomycetous clavicipitalean anamorphs of ascomycetes. There were no symptomatic bacterial or viral associations. Fungal pathogens of *Oligonychus coffeae* (Neitner) (ex Assam and Tamil Nadu), *Acalitus adoratus* Keifer, *Phyllocoptruta oleivora* (Ashmead), *A. litchii* (ex Assam) and *Rhombacus eucalypti* Ghosh and Chakrabarti were also studied.

A new method was developed for imitating the pre-ballooning effect of *T. urticae* on detached mulberry leaves. New methodologies were created for easier isolation and investigation of culturable and non-culturable microbial associates from populations and individuals of two representative tetranychid and eriophyid phytophagous mites with 80–90 per cent efficiency.

Bioefficacy of *Acremonium* sp., *B. bassiana*, *L. lecanii* and *L. psalliotae* was evaluated against *T. urticae* on three cucurbits (bitter gourd, bottle gourd and ridge gourd) in three greenhouse experiments. The fungal pathogens sprayed in combination with glycerol brought about extremely significant reduction in the density of *T. urticae*.

In the greenhouse, *B. bassiana*, *L. lecanii* and *L. psalliotae* in combination with a sticker brought about extremely significant reduction in the density of *T. urticae* on brinjal. Overall, *L. psalliotae* was the best among the fungal treatments with a mean reduction of 56.6% in the mite population. Both *L. lecanii* (38.8%) and *B. bassiana* (35%) performed similarly, and the results were validated in the field.

In the field study at the Biocontrol Research Farm (Yelahanka, Bangalore) on the unseasonal occurrence of *Tetranychus* spp. and their pathogens, at least 25 diseased adult mites were encountered on okra within a span of 10 days of initial crop growth. Subsequently, 36 diseased adult/ nymphal stages were encountered in the first 26 days of December 2009. During January 2010, seven diseased adult mites were confirmed to have *Neozygites* infection and five diseased adult mites were found to have multiple infections.

Bioefficacy of *Acremonium* sp. and *L. psalliotae* was evaluated against *T. urticae* on five cucurbits (ash gourd, bitter gourd, bottle gourd, cucumber and ridge gourd) in three rounds of experiments set up in the greenhouse at the Biocontrol Research Farm during 2010-11. A new concept of 'prior-weakening' of the target pest was introduced for the first time. Three weakening agents (coded PWA-1A, PWA-1B and PWA-1C) were used one after the other in the series of experiments. In the first round, *Acremonium* sp. applied subsequent to the treatment with the weakening agent (PWA-1A) was the best in terms of reduction (74.7 to 82.8 per cent) of the mite density. In the second round, wherein the weakening agent PWA-1B was tried, a trend similar to that seen in the first round was observed. However, PWA-1B was found to be better than PWA-1A in enhancing the efficacy of the fungal pathogens. The weakening agent PWA-1C was used in the third round. Once again all the fungal treatments were much superior to the controls. Among the fungal treatments, *Acremonium* sp. applied subsequent to the treatment with the weakening agent (PWA-1C) was the best with a reduction ranging from 81.1 to 93 per cent.

In the field trial on seasonal occurrence of *Tetranychus* spp. and their pathogens, a total of 85 and 32 diseased adult mites were encountered on okra and brinjal, respectively, within a span of 10 days of initial crop growth. Totally, 22 different fungal infections were observed in adult mites during the season.

Two new experimental liquid formulations, one each for *Acremonium* sp. and *L. psalliotae*, were developed during 2010-11. Both mycelial-conidial mycoacaricides were field-tested on tomato and ridge gourd.

Prevalence of *P. oleivora* and its pathogens on orange, sweet orange and acid lime was studied in Bangalore Urban and Rural districts. Overall, 12 important pathogenic genera of clavicipitaceous hyphomycetes were found in 10% of the mite samples. Fungal associates of *Eutetranychus orientalis* (Klein) and *Brevipalpus phoenicis* (Geijskes) were also recorded.

Several plant parts of orange and sweet orange were tested for their suitability as substrates for germination and conidiation of *H. thompsonii* mycelial pellets. The progress of fresh fungal growth out of the pellets was the best on the rind or fruit surface.

Field bioefficacy of two *H. thompsonii* isolates, viz., MF(Ag)205 (host-derived) and MF(Ag)66 (non-host-derived), against *P. oleivora* on orange and sweet orange was proved at the Biocontrol Research Farm. On orange, MF(Ag)205 was slightly better than MF(Ag)66 in reducing the mite population (82.1% compared with 79%). Both isolates could also significantly reduce the pest population on sweet orange. The continuous trial on field bioefficacy of *H. thompsonii* against *P. oleivora* partly served the purpose of establishing the candidate fungus in the field.

Ten fungal species that originated from phytophagous mites were assessed and found to be pathogenic to *A. craccivora* on cowpea and *Myzus persicae* (Sulzer) on brinjal.

Formulation or pure culture of *H. thompsonii* was sent to the identified research centres as per their request during 2009-11. Slants of *H. thompsonii* and *Sporothrix fungorum* de Hoog & G.A. de Vries were supplied to different firms and individuals to generate revenue. During the project period, Rs 10,000/- was generated as revenue for NBAII.

## **72. Management of bacterial wilts of Tomato and Brinjal caused by *Ralstonia solanacearum* through *Bacillus* spp. (01/01/2009 to 31/03/2013) PI: Dr. Gopalsamy Sivakumar**

Among 100 isolates of *Bacillus* spp. screened under *in vitro* ten of them were found inhibitory against *Ralstonia solanacearum* (Smith) Yabuuchi et al., which causes bacterial wilt of tomato. Six of them were identified as *B. megaterium* (NBAII 63), *B. subtilis* (NBAII 25), *B. cereus* (NBAII 7), *B. cereus* (NBAII 71), *B. cereus* (NBAII 33) and *B. megaterium* (NBAII 65) through 16s rDNA analysis. *Bacillus* isolate NBAII 63 which was isolated from the soils of reserved forest of Western Ghats of Kerala was selected as most promising one based on *in vitro* and *in vivo* screening.

Talc based formulation of *B. megaterium* can be stored upto 240 days with required viable population of  $6.20 \times 10^7$  cfu/g of the product. The bacterial wilt of tomato and brinjal could effectively be managed by *B. megaterium* NBAII 63 through different methods of applications. Although a single method of application was effective in reducing the disease under green house conditions, the most effective management was attained when the talc based formulation of *B. megaterium* NBAII 63 ( $10^8$  cfu/ml) was applied as seed treatment (4g /kg of seed), soil application (3kg/ha), seedling root dip (10g /L of water) and foliar spray (10g /L of water) in combination. Combination approach was found to be best and resulted in 60% bacterial wilt reduction in tomato and 51% wilt reduction in brinjal, under field condition as compared to single methods such as seed treatment and soil application. Among the single methods seed treatment was found to be the best and resulted in 42% wilt reduction in tomato and 41% wilt reduction in brinjal under field condition as compared to soil application which resulted in 38 % reduction of wilt in tomato and 36 % reduction of wilt in brinjal as compared to other methods and control. The chemical check streptomycin sulphate (1g/L) application resulted in 75% reduction of wilt in tomato and 71% reduction in brinjal under field condition. Highest rhizosphere population of 65.0 to  $67.0 \times 10^6$  cfu/g was recorded in tomato and brinjal crops in the field at 40 days after transplanting, when the antagonist was applied in combination of all methods ie on seed treatment, soil application, seedling dip and foliar spray. The rhizosphere population decreased slowly after 40 days of transplanting.

*B. megaterium* strain NBAII 63 was tested for its ability to induce defense related enzymes viz., peroxidase (PO), polyphenoloxidase (PPO) and total phenols against *R. solanacearum* in brinjal and tomato plants. Plants treated with bacterial antagonist *B. megaterium* challenge inoculated with *R. solanacearum* showed higher levels of defense related enzymes and phenols compared to antagonist alone, pathogen alone and untreated plants. *B. megaterium* strain NBAII 63 showed the higher activities of total phenols ( $173 - 175 \mu\text{g g}^{-1}$  of tissue compared to control 121), PO (2.75- 2.99 change in  $\text{OD min}^{-1}\text{g}^{-1}$  of tissue compared to control 0.75) and PPO activity (0.91-0.96 change in  $\text{OD min}^{-1}\text{g}^{-1}$  compared to control 0.13) in brinjal and tomato plants treated with *R. solanacearum*. The study clearly indicated that *B. megaterium* strain NBAII 63 has the ability to induce the defense related enzymes in the brinjal and tomato plants against *R. solanacearum*. The bacterial strain could effectively be utilized for the management of bacterial wilt disease of brinjal and tomato.

**73. Molecular characterization of Indian Coccinellids (01/01/2009 to 31/12/2011)  
PI: Dr. Ramasamy Gandhi Gracy**

Twenty four species of coccinellids were collected from all over India on different hosts. The morphological identification was established and species identity was confirmed by taxonomist. The protocol for isolation of genomic DNA and the quality parameters for the storage of DNA were carried out. Standardization of PCR protocol and primers for partial gene amplification of CO1 gene for the coccinellids was carried out. The partial gene sequences were obtained for 24 species of coccinellids. The sequence similarity was confirmed thrice in-order to avoid intra species variation. The sequences were edited and annotated and submitted to GenBank and accession numbers were obtained. Molecular phylogeny was constructed to know the species evolutionary pattern and to define the species delineating factors. The result showed that >3-5 per cent sequence dissimilarity was considered as species delineating factor except for the *Harmonia* species, wherein it seems to be poly phyletic. A new project with BOLD database was opened and all the meta data and trace file information were submitted at the BOLD and DNA Barcode was generated for 24 species of important Indian coccinellids.

**74. Cataloguing of insect fauna of India, with emphasis on minor orders (01/04/2009 to 31/03/2013) PI: Dr. Janakiraman Poorani**

Checklists were prepared for 20 orders, namely, Collembola, Protura, Diplura, Thysanura, Ephemeroptera, Odonata, Mantodea, Phasmatodea, Blattodea, Embioptera, Isoptera, Orthoptera, Plecoptera, Psocoptera, Pthiraptera, Neuroptera, Megaloptera, Raphidioptera, Mecoptera, and Trichoptera. The geographical scope of the checklists included India and its neighbouring countries in South Asia, namely, Pakistan, Bangladesh, Nepal, Bhutan, and Sri Lanka. For preparing the checklists, world and regional checklists and catalogues on these orders were used as the starting point and subsequent additions and changes in nomenclature were made on the basis of Zoological Record abstracts. Data mining from online resources was done for some orders for which online catalogues were available. The scientific names were validated and verified for accuracy and recency of latest scientific names and / or classification in consultation with abstracts in Zoological Record and the latest world and regional catalogues and checklists. All the checklists were uploaded on Platypus and output checklists were generated in RTF format. Checklists prepared for the 18 orders were converted into web-ready formats after validation for uploading in NBAIR's website. Web content based on these verified checklists is prepared as a reference source for names of Indian insects, along with links to major world and regional catalogues / checklists of insect names.

**75. Studies on bee pollinators in crop-ecosystems with special reference to pulses and oilseeds crops (01/04/2009 to 31/03/2012) PI: Dr. Sundararaju Dheravaraju**

Collection and identification of bee pollinators which maintain constancy in crop ecosystems: In addition to *Megachile* spp., *Ceratina (Pithitis) binghami* Cockerell was spotted as pollinators of pigeonpea at Tamil nadu. The survey taken on oilseed crops indicated that *Apis dorsata* Fabricius and *Apis cerana indica* Fabricius were the common visitors on gingelly at Tamil Nadu.

Collection and identification of flora which support native bees: In the nontraditional pigeonpea area of Karnataka, Singapore cherry, *Muntingia calabura* L. supported all species of honey bees. *Centrosema pubescens* Benth was confirmed to be visited only by carpenter bees. *Spermacoce hispida* L. was found to conserve all *Apis* spp.

Enhancement of population of bee pollinators through faunal/crop biodiversity: Dry bamboo culms, as a nesting source for carpenter bees within the bamboo bushes were confirmed.

Identification of attractant chemicals of floral biodiversity responsible for attracting native bees through GCMS/LC: The profile of volatiles of flowers of *Blumea lacera* (Burm.f.) DC and cashew (var: Bhaskara) was identified. The hexane extract and poropak desorbed volatiles consistently revealed the presence of beta-caryophyllene and trans-caryophyllene (sesquiterpenes) with maximum of 98% matching in the GC-MS analysis. These volatiles were reported to be common volatiles from flowers of many plant species. Whereas in cashew, the poropak desorbed volatiles

having more than 90.0% matching consisted of compounds of monoterpenes, sesquiterpenes and fatty acid derivatives.

**76. Standardization of solid state fermentation conditions and development of prototypes with semi-automation for the mass production of *Trichoderma* spp. (01/04/2009 to 31/03/2012) PI: Dr. Subbaraman Sriram**

Among the substrates tested sorghum and ragi grains were found to be suitable substrates and provided nutrition base for solid state fermentation. Earlier reports recommended overnight soaking of substrates. However soaking for four hours was found sufficient to get the optimum spore production during solid state fermentation. Suitability of sponges and sugarcane baggase were tested as inert-support culture in solid state fermentation (SSF) for the mass production of *T. harzianum* and sugarcane baggase was found the most suitable. Optimum temperature was determined for ragi and sugarcane baggase as substrates during SSF in the mass production of *Trichoderma*. Temperature range of 28 to 30 C was found to be the most optimum. More than the moisture content, water activity was very crucial in the determination of spore production. The optimum conditions for the SSF in mass production of *M. anisopliae* and *B. bassiana* were also standardized. Design of semi-automated units of tray-bed type of bioreactor for solid state fermentation was prepared and provisional patent was filed for the same (2271/CHE/2011).

**77. Influence of elevated levels of carbon dioxide on the tritrophic interactions in some crops (01/07/2009 to 31/03/2014) PI: Dr. Nandagopal Bakthavatsalam**

Pigeon pea plants were grown in the open top carbondioxide chamber (OTC) at 500 ppm of carbondioxide with ambient temperature and 2°C above ambient and compared with those grown at ambient conditions CO<sub>2</sub> (380 ppm). The pigeon pea plants grown under 500 ppm showed profuse vegetative growth, delayed flowering than those grown at ambient conditions. The ovipositional preference studies conducted at the laboratory revealed that the plants grown at 500 ppm preferred by *H. armigera*. Volatile profile of plants indicated the presence of compounds like  $\alpha$  copaene in plants grown at 500ppm, which might be attributed to the more attraction to *H. armigera*. Biological studies indicated higher larval length, pupation and adult emergence in plants grown at 500 ppm. The incidence of various pests on eggplant, and cabbage were documented along with their variation in the volatile profiles when grown under different levels of carbondioxide.

**78. Polymorphism in pheromone reception in *Helicoverpa armigera* (01/07/2009 to 31/03/2013) PI: Dr. Nandagopal Bakthavatsalam**

Different geographical populations of *H. armigera* were collected from Guntur, Raichur, Bangalore and Coimbatore. Besides, three populations were received from Central Institute of Cotton Research, Nagpur and these were reared on the soaked kabuligram. Morphological studies on the pheromone glands did not reveals much of variation in the structures. In field studies conducted at three locations the response was noticed to other blends 85:15 or 91:9 besides the commercially available blend 97:3. The response of males to different pheromone gland extract studied in the laboratory indicated greater response in the synthetic pheromone than the gland extract. GC-EAD studies indicated that some populations respond to 97:3.

**79. Isolation identification and characterization of endosymbionts of trichogrammatids and their role on the fitness attributes (01/04/2010 to 31/03/2013) PI: Dr. Sushil Kumar Jalali**

Isolation of endosymbionts from different species and populations of *Trichogramma* collected from different locations and ecosystem: White colonies developed on these plates gave indication of yeast colonies, which was purified by single colony isolation for three times. The identified yeast and bacterial colonies were *P. anomala*, *Pichia guilliermondii*, *C. apicola*, *Candida pimensis*, *Metschnikowia reukaufii*, *Hanseniaspora uvarum*, *Wickerhamomyces anomalus*, *Zygosaccharomyces rouxii* and *B. cereus*.

Mapping, documentation and characterization of endosymbiont biodiversity from *Trichogramma* eggs collected from different agro-climatic zones such as north (Punjab), west (Gujarat), east (Bhubaneswar) and south (Andhra Pradesh, Tamil Nadu and Karnataka): The identified yeast and bacterial colonies were – *P. anomala*, *Pichia ohmeri*, *C. apicola*, *C. pimensis*, *M. reukaufii*, *H. uvarum*, *W. anomalus*, *Z. rouxii* and *B. subtilis*

Visualization of yeast and bacteria under microscope: A loopful of the culture isolated was spread on a glass slide, stained with cotton blue, covered with the cover slip and viewed under 40x eye piece. Individual cells were viewed and determined presence of yeast or bacteria present in the individual culture. The bacterial endosymbionts observed were further subjected for Gram staining and observed under oil immersion objective 100x for determination of Gram +ve and Gram –ve rods. Based on Gram staining, the bacteria isolated from *T. embryophagum* and *T. danauciphaga* were identified as Gram –ve and Gram +ve rods, respectively.

Maintenance of different cultures of endosymbiotic yeasts and bacteria: Yeast and bacterial endosymbionts isolated from *Trichogramma*, viz., *P. anomala*, *P. guillermondii*, *C. apicola*, *C. pimensis*, *M. reukaufii*, *H. uvarum*, *W. anomalus*, *Z. rouxii* and *B. cereus* were sub-cultured on to YEPDA slants and refrigerated. The slants were sub-cultured at regular intervals and were used for further studies.

Biochemical identification of yeast endosymbionts: The kit used was a standardized colorimetric identification system utilizing twelve conventional biochemical tests based on the principle of pH change and substrate utilization. On incubation, the organism underwent metabolic changes which were indicated by a spontaneous colour change in the media.

Based on carbohydrate fermentation test, yeast isolated from Gurdarapur showed utilization of sugars, viz., maltose, sucrose, galactose, cellobiose, xylose and raffinose and was found to be very close to *Candida lyopolitica*. Yeasts isolated from Srinagar showed utilization of sugars, viz., maltose, sucrose, galactose, xylose and raffinose and was found to be very close to *Candida guillermondii*.

DNA isolation, amplification, sequencing and identification: A total of 20 strains including two *Candida* species and 10 *Pichia* species were obtained. By ITS sequence analysis, the yeast isolates were identified to species level. A BLAST search revealed that yeast strains Tcy1 and Tcy2 had an ITS1-5.8S-ITS-2 sequence similarities of 99 and 100 per cent, respectively, with their corresponding type strain *P. anomala* isolate P13 (GenBank Accession No.AY349442). The output of the BLAST search of ITS sequence of Tcy3 strain showed 100% sequence identity with the respective yeast sequences in GenBank. Likewise, all the endosymbiotic yeasts and bacteria associated with *Trichogramma* obtained from different locations were identified by ITS sequencing analysis as *P. anomala*, *Candida* cf. *apicola*, *W. anomalus*, *M. reukaufii*, *H. uvarum*, *C. pimensis*, *P. guillermondii*, *Z. rouxii*, and bacteria as *B. cereus* and *B. subtilis*.

The ITS sequences and 16S rDNA sequences identified in this study were deposited in GenBank with the accession numbers. The registered sequences were complete ITS sequences containing flanking sequences of 5.8S and 28S rDNA for yeast and 16s rDNA sequences for bacteria.

Phylogenetics analysis of endosymbionts cultured from different populations of *Trichogramma*: The Phylogenetics tree was constructed by the neighbor joining method using the distance matrix from the alignment. The pair-wise alignment was done by DNASTAR Lasergene 8 software. The sequences were uploaded to SeqBuilder. The pair-wise alignment was done by MegAlign of DNASTAR. The results were confirmed by clustering *P. anomala* as one group, *Candida* as another and other related ones joining with each other.

Phylogenetics analysis of endosymbionts cultured from different populations of *Trichogramma*: The results confirmed by clustering that *P. anomala*, *P. ohmeri*, *C. apicola*, *C. pimensis*, *M. reukaufii*, *H. uvarum*, *W. anomalus* and *Z. rouxii* forming different clusters, and other related ones joining with each other, thus indicating them to be different from each other.

Determination of role of endosymbionts in fitness attributes of laboratory reared *Trichogramma* spp.: The results indicated that per cent parasitism ranged from 50.0 – 97.0, per cent females from 63.0 – 85.0 and fecundity of 40.0 – 68.0 / female in various generations and in different yeast symbionts feeding compared to 30.0 – 40.0 per cent, 23.0 – 49.0 per cent and 28.0 – 38.0 / female, respectively, in control. Studies indicated that these symbionts had definite role in enhancing biological fitness of laboratory population *T. japonicum*.

The results for *T. chilonis* indicated that per cent parasitism ranged from 73.0 – 90.0, per cent females from 73.0 – 90.0 and fecundity of 70.0 – 80.0 / female in various symbionts feeding compared to 38.0% parasitism, 40.0% females and 28.0 / female fecundity, respectively, in control. Studies indicated that these symbionts had definite role in enhancing biological fitness of laboratory population.

Development of symbiont based product and determination of shelf life of the formulated products: After 90 days of storage the colonies recorded ranged from 83.0 – 177 in cellulose and 109 – 189 in casein. The colony survival was low in *P. ohmeri* compared to other four symbionts.

Determination of shelf life of the endosymbionts formulated products: The shelf life of formulated products from endosymbionts indicated that up to 90 days of storage, colonies ranged from 83-177 and 109-189 in cellulose and casein based products. Casein was found to be more viable media for product. After 120 days of storage, the shelf life declined drastically and no growth was recorded after 180 days of storage. The studies, therefore, suggested that products can be used for 90 days for utilization purpose.

Preliminary study on insecticide degradation ability of symbionts: The endosymbionts – *W. anomolus* isolated from the insecticide (endosulfan) resistant strain of *T. chilonis* was subjected to the detoxification assay using agar well diffusion method using minimal media. Pesticide degradation was assessed based on lush growth of the endosymbionts after 24-48 hrs of incubation indicating ability of symbiont to detoxify the insecticide.

Insecticide degradation studies with symbionts: The indicated differential response of five different endosymbionts for their ability to degrade imidacloprid insecticide in a minimal media through spectrophotometer at 650nm. After 96h of observation, *W. anomolus* was able to grow in insecticide media by 3.39 times compared to control followed by *P. ohmeri* 2.13 times, *C. apicola* 1.80 times and *M. reukaufii* 1.59 times, however, *Z. rouxii* was not able to degrade imidacloprid, indicating that all symbionts do not have similar role.

The results for response of five different endosymbionts for their ability to degrade indoxacarb insecticide in a minimal media through spectrophotometer at 650nm indicated that after 96h of observation, *W. anomolus* was able to grow in insecticide media by 1.97 times compared to control followed by *Z. rouxii* 1.77 times, however, *P. ohmeri*, *C. apicola* and *M. reukaufii* were not able to degrade indoxacarb, indicating that all symbionts do not have similar role.

Determination of role of endosymbionts in fitness attributes of laboratory reared *Trichogramma* spp.: The results indicated that per cent parasitism ranged from 33.0-97.0, per cent females from 49.0-82.0 and fecundity from 32.0-57.0 / female in F<sub>5</sub> generation in different yeast symbionts and after feeding these symbionts for 30 generations per cent parasitism ranged from 88.0-91.0, per cent females from 63.0-85.0 and fecundity from 45.0-58.0 / female compared to 58.0% parasitism, 55.0% females and fecundity of 36.0 / female, respectively. Studies indicated that these symbionts had definite role in enhancing biological fitness of laboratory population of *T. japonicum*.

The results for feeding laboratory population of *T. chilonis* indicated that per cent parasitism ranged from 38.0– 90.0, per cent females from 40.0-80.0 and fecundity of 28.0-61.0 / female in various symbionts in F<sub>5</sub> generation. After feeding symbionts for 30 generations, per cent parasitism ranged from 84.0-97.0, per cent females from 61.0-75.0 and fecundity from 50.0-63.0 / female compared to 72.0% parasitism, 53.0% females and fecundity of 42.0 / female, respectively. Studies indicated that these symbionts had definite role in enhancing biological fitness of laboratory population.

Role of symbionts in thermal tolerance: Endosymbionts are beneficial organisms in parasitoids, which help them to tolerate the abiotic stress. In order to find out the role of yeasts in fitness attributes, yeasts fed were (*M. reukaufii*, *P. ohmeri*, *W. anomolus*, *C. apicola*, *Z. rouxii* and *P. anamola*) to the laboratory population of *T. chilonis* for five generations and was compared with the population fed with 50% honey solution. The result indicated that *Z. rouxii*, *P. anamola* and *W. anomolus* had higher role in imparting thermal tolerance, therefore can be utilised for their role. In general study suggested that symbionts have role in thermal tolerance.

Formulation of concoction of symbiotic products and testing their shelf life and viability: A concoction of five endosymbionts - *W. anomolus*, *P.ohmeri*, *C. apicola*, *M. reukaufii* and *Z. rouxii* was made in a product. However, their shelf life was found to be for 90 days and was similar to results obtained with individual symbionts.

**80. Molecular characterization and identification of endosymbionts of chrysopid predators and their functional role on the biological attributes (01/04/2010 to 31/03/2013) PI: Dr. Thiruvengadam Venkatesan**

Thirty yeast and bacteria isolated from different populations of *C. zastrowi sillemi* were maintained. The amplicons of various yeasts, isolated from different populations of *C. zastrowi sillemi* were sequenced and obtained GenBank Acc. Nos. The yeast isolates were found to be *W. anomalus* (strain CZS-1 and 5), *P. anomala* (CZS-2, 8 and 15), *Candida blankii* (CZS-3), *C. apicola* (CZS-2), *Torulaspora delbrueckii* (CZS-4), *Z. rouxii* (CZS-7), *Kodamea ohmeri* (CZS-9 and 16), *C. pimensis* (CZS-10). *W. anomalus* was found in most of the populations of the predator (CZS-1, CZS-2, CZS-5 and CZS-8). Phylogenetic tree of 18S rRNA region of the yeast isolates constructed from NJ classified all organisms into two major clades representing that the first clade contains 17 species (*W. anomalus* to *C. pimensis*) further divided into three sub clades. All *W. anomalus* species except two were closely related to each other with an average of 70% bootstrap support. The rest two *W. anomalus* species showed similarity to *C. apicola* and *C. pimensis* with 56% bootstrap support. *T. delbrueckii* showed similarity to *Z. rouxii* by sharing common inter node. *K. ohmeri* and *C. blankii* present close to each other with 94% bootstrap support. Based on the 16S rRNA, the bacterial communities were identified as *Enterobacter*, *Enterobacter hormaechei*, *Enterobacter cloacae*, *Enterobacter asburiae*, *Pantoea dispersa*, *Bacillus*, *B. subtilis*, *B. cereus*, *Enterococcus faecalis*, *Bacillus pumilus*, *Enterococcus faecium*, *Empedobacter* sp, *Agrobacterium tumefaciens*, *Lactococcus garvieae*, *Enterococcus gallinarum* submitted in GenBank and Acc. Nos. were obtained.

**81. Studies on *Trichogramma brassicae* and *Cotesia plutellae* interaction with their host in cabbage ecosystem (01/04/2010 to 31/03/2014) PI: Dr. Kotilingam Srinivasa Murthy**

The populations of egg parasitoid *Tr. bactrae* and endolarval parasitoid *C. vestalis* (*plutellae*) were collected from various geographic locations of the country. Both the parasitoids were assayed for the presence of gut microflora (endosymbionts), which were reported to play role in the fitness attributes of the parasitoids. The gut microflora were isolated, identified and characterized and gene sequences were submitted to Genbank and accession numbers were obtained.

Evolutionary relationship among the endosymbionts from the different geographical populations of the parasitoids was established. *Wolbachia*, a symbiont reported to alter the reproductive physiology and sex ratio was detected in the parasitoid populations from different regions. The significance of symbionts in the fitness attributes of the parasitoid was studied and the role of *Wolbachia* in feminization was determined. Further, the role of the symbiont in pesticide degradation was studied. Degradation of insecticides by the bacterial endosymbionts, *Bacillus* sp and *Enterobacter cancerogenus* was established through minimal media and LCMS studies. Heat shock proteins (*Hsps*) that contribute to the sustenance of the parasitoid under stressed conditions were also detected in the populations from Bhubaneswar, Oddanchatram, Rajahmundry, Tirupathi and Varanasi. The *Hsp70* was detected at abrupt temperature of 28, 30 and 32°C and ramping temperature from 28-34°C.

The associated endosymbionts in the parasitoid differed and contributed to variations in the parasitoid populations of *C. vestalis*. Symbionts contribute to the fitness attributes of the parasitoid (nutrition, enhanced parasitism, adult longevity and sex ratio) and play role in degradation of insecticides contributing to resistance. Exploitation of the symbionts and their horizontal transmission would contribute to more effective biocontrol programmes.

**82. *In situ* conservation of natural enemies and pollinators in pigeon pea and sunflower ecosystem (01/06/2010 to 31/05/2013) PI: Dr. Timalapur Maharudrappa Shivalingaswamy**

A weed flora associated with pigeon pea, *S. hispidus* was confirmed as a useful plant in supporting pollinators which were common to pigeon pea and sunflower. The relative abundance of

pollinators with respect to *Megachile* spp. was highest followed by *Xylocopa* spp. in pigeon pea whereas in Sunflower, *A. dorsata* was dominant followed by *Megachile* spp and *Xylocopa* spp. The results indicated Pigeon pea- marigold intercropping was effective compared to others significantly in terms of infestation- less in covered plants and more in uncovered and more seed weight in uncovered because of some amount of cross pollination by pollinators which was not possible in covered plants though pigeon pea is self pollinated crop. The results indicated that pigeon pea+marigold intercrop was found to be effective in enhancing the yield compared to pigeon pea +sunflower but both intercrops were better than the sole crop. The test weight of pigeon pea increased from 11.8g to 13.8 (pigeon pea +sunflower) and 15.7g (pigeon pea+marigold) indicating the role of pollinators in enhancing the yield. In sunflower, increase in seed set by 3.7% and 1.04g increase in test weight of seeds in open pollinated condition compared to control (pollinator exclusion) indicated the role of pollinators in yield enhancement.

**83. Semiochemicals for the management of coleopteran pests (01/11/2010 to 31/03/2015)  
PI: Dr. Nandagopal Bakthavatsalam**

GCEAD studies were conducted to identify the EAG response of male *Rhynchophorus ferrugineus* (Olivier) to the volatiles of coconut palm. Several peaks were identified which elicited good response in the antenna. During the volatile analysis of coconut stem using GCMS, the compounds identified included 1, 3 butanediene, tetradecenal, benzopyran etc. Electroantennogram studies on male and female of cashew stem and root borer, *Plocaederus ferrugineus* Gahan was done for the volatiles collected from the males and females through entrapment method. The extracts from females of *P. ferrugineus* elicited better response than the male extracts.

The volatiles entrapped were eluted in hexane and the extract was concentrated using a vacuum concentrator and injected in the GCMS as per the standard program. The males elicited several peaks than the females. Compounds such as tetradecenal, an active ingredient in several pheromones were identified using mass spectral data. However, the other minor peaks were identified and confirmed using the standards. Through GCMS/GCEAD studies, several compounds were identified as aggregation pheromone from thoracic gland extracts.

*Alcidodes affaber* Auriv. was a serious pest on cotton and okra in North Karnataka. The grubs usually tunnel the stem and damage the plants. An aggregation pheromone and volatiles from okra were identified through GCEAD & GCMS for the attraction of *A. affaber*. Volatiles from the gland extracts of *A. affaber* were identified as dodecanoic acid, 2, propenoic acid dodecyl ester, tetradecanoic acid, hexadecanoic acid, hexadecanoic acid and oleic acid.

The plant volatiles from different sweet potato varieties were identified using GCMS & GCEAD for the attraction of *Cylas formicarius* (Fabricius) (collaboration with CTCRI).

The volatiles from different varieties of banana were identified for *Odoiphorus longicollis* (Olivier) using GCEAD & GCMS.

The male gland extracts of *Lepidiota mansuata* Burmeister were analysed and compound like cis-9-hexadecenal, cis-9-hexadecanoic acid, octadec-9-enoic acid and 1-hexacosene were identified as important volatiles. Several volatiles were identified from the susceptible cultivars of sweet potato weevil, *C. formicarius*. The volatiles from susceptible banana variety were identified through GCEAD & GCMS for *O. longicollis*. Several compounds were identified. Three formulations with the selected compounds were developed and evaluated at NRC Banana for its efficacy. One combination of compound was found to be very attractive with 70% adults responded in wind tunnel studies. Further, refining of the compound elicited attraction in 80% of adults.

**84. Evaluation of fungal pathogens on *Aphis craccivora* in cowpea and *Bemisia tabaci* in tomato and capsicum (01/10/2010 to 31/03/2014) PI: Dr. Bonam Ramanujam**

Biocontrol based technology using entomofungal pathogens, *L. Lecanii* V1-8 isolate, *M. anisopliae* Ma-6 isolate and *B. bassiana*, Bb-5a isolate for management of cowpea aphid (*A. craccivora*) in cowpea (Variety KBC-2) was developed, which showed 72.7-75.7 per cent reduction of aphid population during the field trials conducted in kharif in 2010, 2012 and 2013 at NBAII Farm

Attur. The entomofungal treated plots also showed significantly higher yields (32.85, 32.75 and 32.70 g/plant) compared to control (25.79 g/plant).

Evaluation of entomofungal pathogens on *Bemisia tabaci* (Gennadius) infestation in tomato (variety, NS501) and capsicum (var. Indria) was carried out in the polyhouse at NBAII Farm, Attur with ten isolates of entomopathogenic fungi (*B. bassiana* Bb-5a, Bb-36, Bb-68, Bb-9, *M. anisopliae* Ma-42, Ma-41, Ma-6, *L. lecanii* VI-8, VI-12 and VI-32) during 2012 (July-September) and 2013 (February-May). Pooled data analysis for 2 years indicated that *B. bassiana* (Bb-9 isolate), *L. lecanii* (VI-8 isolate) and *B. bassiana* (Bb-5a isolate) showed 59.5-66.6 per cent reduction of whiteflies in tomato and 63.1-73.1 per cent reduction in capsicum. In tomato, significantly higher yields were recorded in the plots treated with VI-8 and Bb-9 isolates (4.77 and 4.73 kg/plant) compared to the yield in control plants (3.29 kg/plant). In capsicum, statistically significant differences in the yield were not observed in entomofungal pathogen treated plants compared to the yield in control plants, although VI-8 Bb-9 and Bb-5a treated plants showed higher yield of 2.46, 2.3 and 2.25 kg/plant respectively compared to the yield of 1.63 kg/plant in untreated control.

The field trial for evaluation of entomofungal pathogens on cabbage aphid (*Brevicoryne brassicae*) in cabbage (var. Saint) was carried out at NBAII Farm Attur with nine isolates of entomopathogenic fungi (*B. bassiana* Bb-5a, Bb-9, Bb-68, *M. anisopliae* Ma-42, Ma-41, Ma-6 and *L. lecanii* VI-8, VI-12 and VI-32) during kharif season July-November, 2013. Isolates of Bb-5a, Ma-6 and VI-8 showed 60.0-68.25 per cent reduction in aphid population. With regard to yield, statistically significant differences in the yield were not observed in entomofungal pathogen treated plants and untreated control plants, although Bb-5a, Ma-6 and VI-8 treated plants showed higher yields compared to the yield of untreated control.

Laboratory bioassays on the infectivity of 11 isolates of fungal pathogens, *B. bassiana* (Bb-5a, Bb-9, Bb-36 and Bb-68 isolates), *M. anisopliae* (Ma-4, Ma-6, Ma-41 and Ma-42 isolates) and *L. lecanii* (VI-8, VI-12 and VI-32 isolates) indicated no pathogenicity on *Micromus timidus* and very low infectivity (0.3-2.9 per cent mycosis) on *C. sexmaculata*. In the field trials, no significant difference in the natural population of coccinellids in cowpea plants treated with fungal pathogens and untreated control plants was observed, indicating the safety of fungal pathogens to the natural enemies (coccinellids) of cowpea aphid.

## **85. Interactions of microbial control agents in diverse soil types (01/10/2010 to 30/09/2013)** **PI: Dr. Sreerama Kumar Prakya**

Basic soil samples originated from 14 villages under eight panchayats within Chikkarasinakere hobli in Maddur taluk, Karnataka. Structural and textural analyses indicated that there were just two structural categories among the soil samples, i.e. granular and subangular blocky. On the other hand, the soils fell under one of the three textural categories, viz., loamy sand, sandy clay loam or sandy loam. There was a significant variation in the pH of the samples, with values ranging from 5.6 (moderate acid) to 8.5 (strongly alkaline).

Fast-growing, mesophilic bacteria were abundant in most of the samples. The land-use pattern seemed to influence the bacterial population, with paddy soils comprising high numbers. Non-sporulating genera dominated (39%) the fungal populations. The identity of one of the *Trichoderma* cultures from the Chittoor forest soils was confirmed through sequencing the ITS1 and ITS4 regions of its rDNA. The Sample was found to be closest to *Hypocrea lixii* (= *T. harzianum*) isolate ZQ3101 (NCBI Acc. No.: HQ259306.1).

In the polyhouse, resident soil fungi and bacteria, as well as root-associated, culturable microbes dominated the experimentally applied antagonist up to 97% within the tomato rhizosphere, especially in the periphery. The antagonist, whether applied in the form of a formulation or in an unformulated manner, could be established in the root zone of tomato. Soil-applied *T. harzianum* grew readily along with the developing root system of treated tomato plants. Both unsterilised and surfaced-sterilised tomato root segments yielded the antagonist when plated. In the field, at least five known phytopathogenic fungal genera, one antagonistic fungal genus and two antagonistic bacterial genera were found active in the rhizospheres of tomato as well as five cucurbit species separately grown in the vicinity.

Though *M. anisopliae* could tolerate a temperature of 39°C, but not beyond, *B. bassiana* was unable to stand more than 37°C. An elevated carbon dioxide level of 0.1% neither affected the growth nor suppressed the conidiation of *B. bassiana* and *M. anisopliae* incorporated in soil and incubated at 32°C for 72 h. *M. anisopliae* incorporated in selected soil samples with individual pH values of 5.6 (sandy clay loam), 6.6 (sandy loam) and 7.6 (sandy clay loam) was unaffected even after incubation at 25°C for 30 days. At least three different modes of action of antagonistic fungi were observed against entomopathogenic fungi in a range of soil types. Both *T. harzianum* and *T. virens* exhibited similar mechanisms of antagonism against at least two entomopathogenic fungi, viz., *B. bassiana* and *M. anisopliae*.

Native entomopathogenic fungi belonging to 10 genera, including *Beauveria* and *Metarhizium*, could coexist in three representative soil types for at least six months along with two antagonists and other resident soil microflora. Twelve potential and proven entomopathogenic fungi, viz., *Acremonium* sp., *Aschersonia* sp., *Aspergillus* sp., *B. bassiana*, *F. pallidroseum*, *H. thompsonii*, *M. anisopliae*, *N. rileyi*, *L. lecanii*, *L. psalliotae*, *P. fumosoroseus* and *P. lilacinus*, were affected by *T. harzianum* as well as *T. virens* in concurrent plating. Out of the five inorganic soil amendments tested, vermiculite was the best in providing optimal moisture (10%) for the sustenance of an antagonist (*T. harzianum*) and an entomopathogenic fungus (*M. anisopliae*). Conidia of two antagonistic fungi and two entomopathogens were able to adhere to soil particles of various sizes though all conidia did not show hydrophilic nature. Two external applications of *H. lixii*, in a span of a week, resulted in the suppression of five potential entomopathogenic fungi to an extent of 30%.

A new method for simple retrieval of antagonistic as well as entomopathogenic fungi from soil was developed and validated. Ultrastructural interactions among entomogenous and entomopathogenic fungal species were well pronounced in the presence of insect cadavers. Despite pressure from the three different modes of action by antagonistic fungi, entomogenous and entomopathogenic fungi could form interrelationships among themselves. Only antibiosis and mild mycoparasitism were observed between the antagonists and insect fungi. There was conidial affinity between antagonistic fungi and two species of *Lecanicillium* as well as *B. bassiana*. Entomopathogenic fungi producing dry spores (e.g., *M. anisopliae*) did not show affinity towards both *T. harzianum* and *T. virens*. Chlamydospores were the most resistant propagules in 10 sandy clay loam (subangular blocky structure) soils with less than 5% moisture level after storage for a year. *B. bassiana* and *L. lecanii* showed no antagonistic effect against each other under *in vitro* conditions and exhibited probable synergistic action through normal germination and conidiation when used together. A reciprocal effect in survival promotion of the interacting partners, viz., *B. bassiana* and *L. lecanii*, was observed in two soil types disturbed at frequent intervals.

#### **86. Bio-intensive management of root-knot nematode and /*Fusarium* disease complex in tomato and okra using PGPR (22/11/2010 to 22/11/2013) PI: Dr. Rajkumar Manikappa Gond**

Fortynine soil samples of infected crop roots of tomato, brinjal, okra and chilliwere collected from Malur, Kolar, Mandya, GKVK campus (Bangalore) and Attur farm of NBAIR (NBAII), Bangalore. Root-knot nematode was maintained on tomato crop in glasshouse. Around 245 soil samples of root-knot nematodes were surveyed to identify prominent occurrence of root-knot nematodes and to identify conducive agro-ecological conditions from Karnataka and Andhra Pradesh. Results revealed that maximum occurrence of root-knot nematodes was noticed in Malur region of Kolar district (85.71%) followed by Bangalore (69.00%). In Kadapa district of Andhra Pradesh, root-knot nematodes were observed on crossandra (42.85%) and tomato (37.5%) crops. Two *Meloidogyne* species namely *M. incognita* and *M. javanica* were identified based on perineal pattern characteristics. Out of these two, *M. incognita* was more frequent in tomato, brinjal and okra at Bangalore and Mandya regions of Karnataka. It was found either singly or inconcomitantly with *M. javanica* in tomato of Kolar region of Karnataka.

Fifteen rhizobacteria were isolated from infected egg masses of root knot nematode infested in brinjal and tomato crop of Attur farm of NBAII and Malur village of Hoskote (Tq). Eleven isolated rhizobacteria were screened against second stage juveniles of root knot nematode under *in-vitro* condition. Four isolates of rhizobacteria were found to have antagonistic effect on second stage

juveniles of root knot nematode. Nematode mortality was 57 to 64 per cent. For short time storage rhizobacteria were maintained in slant and water and for long term storage, they were maintained in glycerol water (1:1) in refrigerator. Total of 48 *Pseudomonas* isolates were isolated from above soil samples and 34 from egg masses of root-knot nematodes and were morphologically identified as Gram negative bacteria (small rod shaped *Pseudomonas* sp.), in which 12 isolates collected from crop rhizosphere and 15 isolates collected from egg masses along with four isolates of NBAIR (NBAIL) collections were morphologically characterized. *Pseudomonas* isolates collected from crop rhizosphere (CRS) were tested for efficacy against egg hatching and mortality of second stage juveniles of *M. incognita*, in which 12 isolates performed better, among which five isolates were found prominent in causing mortality of *M. incognita* juveniles and inhibition of egg hatching. The percent mortality was recorded between 14-60 and inhibition of egg hatching recorded between 7.33-45.33 per cent. The results revealed significant nematode mortality to varying degrees compared to distilled water. Per cent larval mortality increased with increase in the exposure period and there was difference in reactions among isolates to egg hatching and juveniles mortality and the isolates which were prominent against killing of juveniles were not so effective in inhibition of egg hatching and vice versa. A total of 34 *Pseudomonas* isolates collected from egg masses of root-knot nematodes were documented and tested for efficacy against mortality of second stage juveniles of *M. incognita*. Among them, 15 isolates performed better and two isolates were found prominent in mortality of *M. incognita* juveniles. The percent mortality was recorded between 69.44-83.33 per cent. The results revealed significant nematode mortality to varying degrees compared to distilled water. Per cent larval mortality increased with increase in the exposure period and increase in concentration of culture filtrates. Twenty *Pseudomonas* isolates of NBAIL were tested against collected soil borne fungal pathogen *F. oxysporum*, in which 2 isolates of *Pseudomonas* showed better growth inhibition of *F. oxysporum*. In case of *F. oxysporum* maximum inhibition was observed in case of CRSRPF7 and CRSGR3ARS3 exhibiting 58%. Maintained pure cultures of root-knot nematodes in glass house on tomato for studies. Maintained isolated rhizobacteria in slants and in water for short time storage and for long time storage isolated rhizobacteria were maintained in glycerol water (1:1) in refrigerators.

**87. Studies on Thrips components influencing the epidemiology of Tospoviruses (01/04/2012 to 11/12/2012) PI: Dr. Subbaraman Sriram**

Sap transmission of WBNV and GBNV from watermelon and tomato to cowpea cv.152 was carried out to maintain virus culture. *Thrips palmi* and *F. schultzei* were tested for their potential to transmit WBNV. Only *T. palmi* transmitted WBNV while transmission by *F. schultzei* could not be confirmed. The identity of Thrips species and WBNV was confirmed using molecular tools. The epidemiology of the spread of GBNV in tomato and its correlation with thrips population was studied in a field trial and the analysis was made using Spatio-Temporal Class analysis that revealed the possible role of weed hosts and vectors.

**88. Genetic diversity, biology and utilization of entomopathogenic nematodes (EPN) against cryptic pests (01/04/2012 to 10/11/2014) PI: Dr. Mandadi Nagesh**

Developed a novel WP formulation with *H. bacteriophora* with twelve months' shelf-life and effective against scarabeid and curculionid grubs in arecanut, sugarcane, sweet potato, fodder grass and groundnut. WP formulations developed at NBAIL were suitable for delivering EPN to Crop rhizosphere through drip irrigation for the management of *Myloccerus subfasciatus* Guerin-Meneville. This could be successfully replicated in other crops and pests.

Through the studies on genomics coupled with transcriptomics on trophic relationships of EPN-bacteria on insects, thirteen functional genes involved in pathogenesis, virulence and mutualism of EPN- associated bacteria were established and identified.

Four applications of EPN at 21 days interval from May-August reduced thrips and whiteflies in capsicum, carnation, gerbera and roses in polyhouses and were found compatible with pesticides and synergistic against *Holotrichia serrata* Fabricius, *Anomala ruficapilla* Burmeister, *M. subfasciatus* and *C. formicarius*.

Field level demonstration with EPN formulations for management of brinjal ash weevil (0.5 acre in Somanahalli; 0.7 acre in Pachappakonda, Pullalapatti; 0.8 acres in Karimpatti) under KVK, Dharmapuri, Tamil Nadu and at Doddaballapur (5ha); arecanut white grubs in Heggodu, Sagar, Shimoga, Sringeri; ash weevil in Doddaballapur, Hosur (16 ha).

Identity of twenty different geographical isolates of *S. abbasi*, *S. feltiae*, *S. carpocapsae*, *S. glaseri*, *Steinernema* spp., *Heterorhabditis* spp., *H. indica*, and *H. bacteriophora* were validated and confirmed using COI, ITS and SSU RNA gene sequences and RFLP studies were carried out. DNA barcoding for eighteen *S. abbasi*, *S. feltiae*, *S. carpocapsae*, *S. glaseri*, *Steinernema* spp., *Heterorhabditis* spp., *H. indica*, and *H. bacteriophora* were generated for the first time from India for NBAII isolates using COI gene.

Predominant white grub species in sugarcane of Kolhapur region (Maharashtra) were *Phyllophaga calciata*, *L. lepidophora* and *Anamola* species. LD<sub>50</sub> and LT<sub>50</sub> values for *H. indica*, *H. bacteriophora*, *S. abbasi*, *S. carpocapsae*, *S. tami* and *S. glaseri* were worked out against *Phyllophaga*. Grub populations of *Lepidiota mansueta* Burmeister from Majauli, Assam, were susceptible to EPN isolates. NBAII isolates of *H. indica*, *S. abbasi* and *S. glaseri* were effective at 2.5x10<sup>9</sup> IJs/ha causing a mortality of 80-96% in soil column assay in seven days.

Demonstrated efficacy of EPN formulations against *L. burmeisteri*, *Phyllophaga* sp., and *Phyllognathus dionycius* in sugarcane (3 acres each) in three villages in Malkapur area of Kolhapur district of Maharashtra. WP formulations of *H. indica* NBAII HI01, *S. abbasi* NBAII SA01, *S. carpocapsae* NBAII SC04, and *S. glaseri* SG01 recovered tillering, cane length, reduced grubs by 48-64% & persisted for 240 days in western UP, Maharashtra and Belgaum.

Field evaluation of combinations of EPN and *B. bassiana* and *M. anisopliae* against brinjal ash weevil grubs indicated that *H. indica* in combination with *M. anisopliae* gave 73% control of grubs in soil observed at 90 and 120 days of crop growth. Synergistic effects were recorded in EPN+EPF combinations against white grubs.

For the first time genomic and transcriptome sequencing and analysis from NBAII strains of symbiotic bacteria of Indian biodiversity accomplished. EPN & PC technologies transferred to Dr. Abdul Rauf Agri - Research Foundation, Sirsi; Ambrosia vegetables Doddaballapura; Ponalab, Bengaluru; revenue generated for Rs. 10.5 lakhs.

## **89. Insect vector components influencing phytoplasma diseases (01/04/2012 to 31/03/2015)** **PI: Dr. Sreerama Kumar Prakya**

Leafhoppers (Cicadellidae) and other prospective insect vectors, mostly planthoppers (Fulgoroidea), belonging to Hemiptera were collected from several states during 2012-15. Species of all the three sub-orders of Hemiptera (Auchenorrhyncha, Sternorrhyncha and Heteroptera) were also documented on plant species with potential phytoplasma-associated diseases. Leafhoppers belonging to 10 tribes under five subfamilies were the most dominant.

Simultaneously, global information was collated on 97 confirmed insect vector species belonging to seven families of Hemiptera, transmitting more than 700 phytoplasma diseases.

Commonly found phytoplasma-associated diseases were recorded in several plant species (Bermuda grass, chilli, parthenium, sunn hemp, the four o'clock flower, etc). Identities of the associated phytoplasmas were confirmed through molecular studies. The phytoplasma associated with a disease in *Richardia scabra* (Florida pusley) was characterised and the nucleotide sequence submitted to GenBank (Accession no. KF709193).

Natural incidence of brinjal (cvs Black Star, MEBH-9 and MEBH-11) little-leaf phytoplasma and its putative insect vectors was studied in two field experiments in 2012. *Amrasca biguttula biguttula* (Ishida), the leafhopper not implicated as a vector, was more abundant than *Hishimonus phycitis* (Distant), with negligible disease incidence. During 2012-13, two field experiments were conducted on sesame (cv. YLM-17) to study the natural incidence of phytoplasma and its putative insect vectors. Observations on phyllody indicated that its incidence could go up to 30% in a single sub-plot of 80 plants and that the distribution of the disease could be even across the crop. *H. phycitis* outnumbered *Orosius albicinctus* Distant. The disease progressed rapidly in the field and was severe.

Sweep-net samples of all the dominant captured species were taken from the canopies of both crops and weeds in 2013-14. Out of the 15 species of leafhoppers belonging to five subfamilies of

Cicadellidae tested, only *Batracomorphus angustatus* Osborn, *Cicadulina bipunctata* (Melichar), *Exitianus indicus* Distant, *Hecalus* sp., *H. phycitis*, *Nirvana pallid* Melichar and *O. albicinctus* were found to be viruliferous based on symptom production in brinjal, sesame and/or periwinkle. Though other leafhoppers were absolutely nonviruliferous, *Austroagallia sinuata* (Mulsant & Rey) showed inconsistent transmission. Three planthoppers, including a *Stenocranus* sp., did not carry phytoplasmas.

In the greenhouse, *O. albicinctus* fed on infected sesame could transmit phyllody pathogen to 78% of healthy sesame plants. On the other hand, *H. phycitis* could also transmit the same phytoplasma to 75% of the plants. Additionally, the same phytoplasma could be transmitted to 52% of healthy brinjal plants by *H. phycitis*. Continual monitoring indicated the potential vectoring role of species of *Empoasca* and *Balclutha*. Four other suspected vectors, including *Mukaria* and *Hecalus* sp., were also identified. During 2013-14, the two field experiments on sesame indicated that phyllody incidence could go up to 35% in a single sub-plot and that the distribution of the disease could be even across the crop. *H. phycitis* outnumbered *O. albicinctus* throughout the crop period. In a non-experimental crop of brinjal (cv. MEBH-11), *H. phycitis*, though not predominantly present, was the principal vector of the phytoplasma disease. At least five other leafhoppers, including *B. angustatus* did not have vectoring ability.

Out of the 10 plant combinations tried, a mix of flowering brinjal and vegetative sesame supported all instars of *H. phycitis* in a cage system. A large-scale rearing methodology for *H. phycitis* was optimised to produce and maintain over 2,000 adults at any given time in a single bay of the greenhouse. In a rearing cage, not less than 100 adults could be maintained continuously with plant replacement once a month. At least six insect pests and two mite species caused damage to brinjal and interfere with the rearing of *H. phycitis*. In the field, three fungal pathogens (*Beauveria* and *Lecanicillium* spp.), a parasitic mite, two coccinellid predators and two predaceous spider species were found to be hostile to *H. phycitis* on brinjal. For the first time, a methodology was standardised to mass rear *H. phycitis* on Madagascan periwinkle in the greenhouse.

During 2014-15, totally 960 leafhoppers were captured from various plants for identification and documentation. Members of Delphacidae (65 planthoppers), Psyllidae (20 jumping plant lice), Cercopidae (30 froghoppers) and Membracidae (30 treehoppers) were collected from both crop plants and weeds for basic transmission studies. In the field experiment on sesame initiated in June 2014, *H. phycitis* (11.9 and 16.9 adults/infected plant in August and September, respectively) outnumbered *O. albicinctus* (4.6 and 7.0 adults/infected plant) throughout. Similar results were obtained in the second field experiment as well. In the greenhouse, directly field-collected *H. phycitis* induced symptoms in 65% brinjal plants, irrespective of the crop from which the insects originated. *O. albicinctus* could transmit the pathogen to only sesame at 50%. Ten other genera of leafhoppers were found to be aviruliferous when caged on periwinkle. Adult *H. phycitis* was able to transmit the phytoplasma associated with a disease in periwinkle to sesame (88%), brinjal (84%) and sunn hemp (45%) in the greenhouse. Primary PCR followed by nested PCR indicated the association of the same phytoplasma with symptomatic plant species. Studies on plant parameters in 10 herbaceous species indicated that surface texture (topology, trichomes, etc.) of the abaxial side of the leaf played the most important role in harbouring or keeping away a potential vector. On 15 potential plant hosts of phytoplasmas, exploratory probing of *H. phycitis* was more pronounced than that of 10 other leafhopper species. Both mouthparts and salivary glands of 25 representative samples of *H. phycitis* (ex brinjal and sesame) and *O. albicinctus* (ex sesame) resembled that of other deltocephaline leafhoppers. The salivary flanges or feeding marks left by *H. phycitis* on both sesame and brinjal were similar.

Molecular diagnosis through primary PCR followed by secondary/ nested PCR revealed that, in general, potato purple-top phytoplasma (98% identity) was the causative agent of little-leaf disease in brinjal (cv. MEBH-11). For the first time, phytoplasma was detected in *N. pallida* through real-time PCR (qPCR). DNA barcoding was completed for *N. pallida*, *H. phycitis* and *O. Albicinctus* and the nucleotide sequences were submitted to GenBank (KJ465911, KJ465912 and KJ465913).

**90. Mechanism of insecticide resistance in certain mealybugs (01/04/2013 to 31/05/2013)  
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Field surveys were conducted and different mealybugs were collected and maintained in the laboratory. There were different bioassay techniques followed for determining baseline toxicity for different insecticides. Susceptibility to contact insecticides (e.g. buprofezin, chlorpyrifos) that were applied on foliar was assessed using an established Petri-dish technique and systemic uptake technique for systemic insecticide like imidacloprid. The project was discontinued based on the recommendations of IRC and it was suggested to take a project on DNA barcoding of parasitoids and predators, keeping in view, the mandate of the institute.