

# Biological and Molecular Approaches in Pest Management

Balwinder Singh  
Ramesh Arora  
S. S. Gosal

# Biological and Molecular Approaches in Pest Management

*Editors*

***Balwinder Singh***

Director of Research

***Ramesh Arora***

Senior Entomologist

Department of Entomology

***S S Gosal***

Director of Research (Retd)

**Punjab Agricultural University**

**Ludhiana – 141 004, India**



**SCIENTIFIC  
PUBLISHERS (INDIA)**

*Published by:*

Scientific Publishers (India)  
5 A, New Pali Road, P.O. Box 91  
Jodhpur 342 001 (India)

E-mail: [info@scientificpub.com](mailto:info@scientificpub.com)  
Website: [www.scientificpub.com](http://www.scientificpub.com)

*Branch Office*

Scientific Publishers (India)  
4806/24, Ansari Road, Daryaganj  
New Delhi - 110 002 (India)

© 2015, Editors

All rights reserved. No part of this publication or the information contained herein may be reproduced, adapted, abridged, translated, stored in a retrieval system, computer system, photographic or other systems or transmitted in any form or by any means, electronic, mechanical, by photocopying, recording or otherwise, without written prior permission from the editors.

Disclaimer: Whereas every effort has been made to avoid errors and omissions, this publication is being sold on the understanding that neither the editors (or authors of chapters in edited volume) nor the publishers nor the printers would be liable in any manner to any person either for an error or for an omission in this publication, or for any action to be taken on the basis of this work. Any inadvertent discrepancy noted may be brought to the attention of the publishers, for rectifying it in future editions, if published.

ISBN: 978-81-7233-923-4

eISBN: 978-93-86102-27-0

Printed in India

## About the Editors

**Dr Balwinder Singh** is Director of Research at Punjab Agricultural University, Ludhiana. He did his Ph.D. in Entomology with specialization in Insect Toxicology in 1984 from PAU, Ludhiana. He was Commonwealth Fellow at Rothamsted Research, Harpenden, U.K. for six months. He has more than 28 years experience in teaching and research in the field of pesticide residues. He has handled many research projects funded by various international and national agencies. He has more than 200 publications to his credit. He has also edited three books and contributed 11 chapters in different books. He is acting as a reviewer for number of International Journals related to field pesticide residues. He is associated with a number of professional societies at the National Level and is the President of the Indian Society for Advancement of Insect Science.

**Dr Ramesh Arora** is Senior Entomologist in the Department of Entomology, Punjab Agricultural University, Ludhiana. He obtained his M.Sc. and Ph.D. in Entomology from the same institute in 1978 and 1990, respectively. He has been working on development and dissemination of IPM technologies for the last more than 35 years. His current research interests include climate resilient IPM and management of insect pests in vegetable crops. He has more than 250 publications including 80 research articles in refereed journals, 15 books and 40 book chapters to his credit. He is associated with a member of professional societies and served as Editor-in-Chief of Indian Journal of Ecology (1996-2003). He is Vice President, Society of Pesticide Science India, General Secretary, Indian Society for the Advancement of Insect Science, and Editor, Entomology Reporter.

**Dr S S Gosal** served as Director of Research (2010-2014) at Punjab Agricultural University, Ludhiana. He obtained his M.Sc. and Ph.D. degrees in the discipline of Plant Breeding from the same university. He joined PAU as Assistant Breeder in 1984 and contributed towards strengthening the education and research in the area of plant biotechnology. During 1993, he was awarded a unique Biotechnology Career Fellowship by the Rockefeller Foundation, USA for 6 years, to conduct research in the area of transgenesis at University of Nottingham, England and John Innes Centre Norwich, UK for three months each year. He is elected Member/Fellow of three professional societies and member of several other scientific societies. Dr Gosal is founder Director of School of Agricultural Biotechnology at PAU, Ludhiana. To put his ideas into motion he has competitive research grants for more than 20 adhoc research projects. Besides, he has served as a member of Board of Assessors, Australian Research Council, Canberra. He has undergone a rigorous training on environment risk assessment of GM crops at Danforth Centre for Plant Science Research, St. Louis; APHIS, EPA (USDA) and USTDA, Washington DC, USA. His contributions in the field of plant biotechnology can be gauged from the volume of over 155 original research papers in refereed Indian and foreign journals. He has been the examiner, member of the advisory board and selection committees in more than 15 Universities and Institutes. Dr Gosal has co-authored a text book, two dozen chapters in books, many Practical Manuals and has supervised thesis research as major/co-major advisor/member of advisory committee of more than 70 post graduate students.

## Preface

Despite great advances in agricultural productivity and economic well-being in much of the world over the past 50 years, food insecurity and poverty continue to be serious issues in many regions of the world. Since most of the cultivable land is already under cultivation, future increases in food, feed and fibre production have to be achieved with increased productivity and improved crop protection. Ironically, more than a third of the global agricultural production is lost to the activity of animal pests and diseases. Further, the losses are significantly higher in the tropical areas where the food shortages are already serious. The strategy of exclusive reliance on chemical pesticides for minimizing crop losses caused by pests has led to human health and safety hazards and caused a number of ecological and economic problems. The concept of integrated pest management was propounded to minimize these side-effects. But in spite of its inspirational value, most IPM programmes still include economic threshold level based application of chemical insecticides as a major input. In this context, the wide range of biological and molecular approaches discussed in 15 Chapters in this book offer a plethora of environmentally benign alternatives to these chemical insecticides.

The introductory Chapter outlines the role of molecular techniques in improving the efficacy of a diverse range of pest management options including genetically engineered plants, insects and microorganisms as well as host plant resistance and chemical insecticides. The second Chapter discusses the role the biotechnological approaches have played in development of rice genotypes resistant to planthoppers. The third Chapter highlights the importance of molecular taxonomy in studies on fruit flies. The RNAi has emerged as a powerful gene-silencing technique and its potential for utilization in pest management is revealed in Chapter 4. The persistence of pesticide residues in the environment poses significant ecological risks. Bioremediation is emerging as the method-of-choice for dealing with such residues and its potential is explored in Chapter 5.

Insects, like other organisms, are prone to diseases caused by a diverse range of microorganisms. The exploitation of these microbes as a tool in pest management offers immense possibilities which are highlighted in Chapter 6. The gall midge, a major pest of rice, is notorious for evolving new biotypes to overcome plant resistance genes. Chapter 7 explains how an understanding of the molecular basis of these interactions can help in development of durable gall midge resistant genotypes. The wide range of molecular markers and their applications in entomological research are concised in Chapter 8. The success story of *Bt* cotton which has transformed India from a net importer to a major exporter of 'white gold' is highlighted in Chapter 9. The potential of biotechnological techniques in developing pest-resistant fruit plants is outlined in Chapter 10.

Insects being highly versatile organisms have the ability to develop resistance to any pest management tactic which exerts sufficient selection pressure on their populations. Chapter 11 focuses on the strategies for management of resistance in insects to microbial control agents. Chapters 12 and 13 highlight the role of biological and molecular approaches in management of mites and *Conogethes* spp., respectively. Bee diseases and pests present a serious challenge to commercial apiculture. The utilization of molecular techniques for precise identification of these organisms is explained in Chapter 14. There is now a wide array of techniques available to replace the use of conventional insecticides in IPM programmes. But it is essential that all the non-chemical approaches are combined within the framework of IPM. The emergence of biointensive IPM as the preferred alternative to conventional IPM is explained in the concluding Chapter of the book.

It is hoped that the book will fill the wide gap in literature on utilization of biotechnological approaches in biointensive IPM as an alternative to chemical insecticide based IPM for sustainable insect pest management in future. We trust that you, the reader, will find the subject matter interesting and informative. We hope that this compilation answers questions you might have, and serves to stimulate further development of this fascinating science of pest management.

**Balwinder Singh**  
**Ramesh Arora**  
**S S Gosal**

## Contributors

### **P K Arora**

PAU Regional Research Station  
Abohar-152 116, India

### **Ramesh Arora**

Department of Entomology  
Punjab Agricultural University  
Ludhiana 141 004, India

### **J S Bentur**

Directorate of Rice Research  
Rajendranagar,  
Hyderabad 500 030, India

### **Manmeet B Bhullar**

Department of Entomology  
Punjab Agricultural University  
Ludhiana – 141 001, India

### **D S Brar**

School of Agricultural Biotechnology  
Punjab Agricultural University  
Ludhiana – 141 004, India  
(Former Head, Plant Breeding,  
Genetics & Biotechnology Division  
International Rice Research Institute,  
Manila, Philippines)

### **P S Burange**

Department of Entomology  
Punjab Agricultural University  
Ludhiana 141 004, India

### **A K Chakravorthy**

Department of Entomology  
University of Agricultural Sciences  
Bangalore-560 065, India

### **Chandrashekharaiiah**

Department of Entomology  
University of Agricultural Sciences  
Bangalore-560 065, India

### **Pardeep K Chhuneja**

Department of Entomology  
Punjab Agricultural University  
Ludhiana 141 004, India

### **A K Dhawan**

Department of Entomology  
Punjab Agricultural University  
Ludhiana-141 004, India

### **B Doddabasappa**

Department of Entomology  
University of Agricultural Sciences  
Bangalore-560 065, India

### **D Fujita**

Plant Breeding, Genetics &  
Biotechnology Division  
International Rice Research Institute  
Manila, Philippines

### **Arshdeep K Gill**

Department of Entomology  
Punjab Agricultural University  
Ludhiana – 141 001, India

### **P D Kamala Jayanthi**

Division of Entomology and Nematology  
Indian Institute of Horticultural  
Research  
Bangalore -560 089, India

### **K K Jena**

Plant Breeding, Genetics &  
Biotechnology Division  
International Rice Research Institute  
Manila, Philippines

### **S B Kandakoor**

Department of Entomology  
University of Agricultural Sciences  
Bangalore-560 065, India

**Uma Kanta**

Department of Entomology  
Punjab Agricultural University  
Ludhiana 141 004, India

**Paramjit Kaur**

Department of Entomology  
Punjab Agricultural University  
Ludhiana 141 004, India

**Krishan Kumar**

PAU Regional Research Station  
Abohar-152 116, India

**Vijay Kumar**

Department of Entomology  
Punjab Agricultural University  
Ludhiana 141 004, India

**Sujan Mamidi**

Genomics and Bioinformatics Program  
North Dakota State University  
Fargo, ND 58102, U.S.A.

**Kousik Mandal**

Department of Entomology  
Punjab Agricultural University  
Ludhiana-141 004, India

**Prashant Mohanpuria**

School of Agricultural Biotechnology  
Department of Entomology  
Punjab Agricultural University  
Ludhiana-141 004, India

**G Nagalakshmi**

Division of Entomology and Nematology  
Indian Institute of Horticultural  
Research  
Bangalore -560 089, India

**P C Pathania**

Department of Entomology  
Punjab Agricultural University  
Ludhiana 141 004, India

**M A Rashmi**

Department of Entomology  
University of Agricultural Sciences  
GKVK, Bangalore-560 065, India

**Surinder K Sandhu**

Department of Plant Breeding &  
Genetics  
Punjab Agricultural University  
Ludhiana-141 004, India

**P S Sarao**

Department of Plant Breeding and  
Genetics  
Punjab Agricultural University  
Ludhiana – 141 004, India

**H C Sharma**

International Crop Research Institute  
for the Semi-Arid Tropics (ICRISAT)  
Patancheru - 502 324, India

**P R Shashank**

Department of Entomology  
University of Agricultural Sciences  
Bangalore-560 065, India

**P S Shera**

Department of Entomology  
Punjab Agricultural University  
Ludhiana 141 004, India

**Balwinder Singh**

Department of Entomology  
Punjab Agricultural University  
Ludhiana-141 004, India

**Kuldeep Singh**

School of Agricultural Biotechnology  
Punjab Agricultural University  
Ludhiana – 141 004, India

**Abraham Verghese**

National Bureau of Agriculturally  
Important Insects  
Hebbal, Bangalore – 560 024, India

# Contents

<i>About the Editors</i>	iii
<i>Preface</i>	v
<i>Contributors</i>	vii
<i>Contents</i>	ix
<b>1. Application of Modern Tools of Biotechnology for Pest Management - Prospects and Limitations</b>	<b>1-12</b>
<i>H C Sharma</i>	
1.1. Introduction	1
1.2. Genetic Transformation of Crop Plants for Insect Resistance	1
1.3. Genetic Improvement of Natural Enemies	3
1.4. Genetic Improvement of Biopesticides	3
1.5. Molecular Marker-Assisted Selection for Insect Resistance	4
1.6. Understanding Gene Sequence and Function	4
1.7. Metabolic Pathways	5
1.8. Inducible Resistance	5
1.9. Use of Molecular Markers for Diagnosis of Insect Pests and Their Natural Enemies	5
1.10. Development of New Insecticide Molecules	6
1.11. Dominant Repressible Lethal Genetic System to Produce Sterile Insects	6
1.12. Prospects and Limitations	7
1.13. Conclusions	9
References	9
<b>2. Biotechnological Approaches for Enhancing Resistance to Planthoppers in Rice</b>	<b>13-38</b>
<i>D S Brar, P S Sarao, Kuldeep Singh, K K Jena and D Fujita</i>	
2.1. Introduction	13
2.2. Sources of Resistance to Hoppers in Rice	14
2.3. Breeding Strategy to Enhance Hopper Resistance	17
2.3.1. Phenotyping for Hopper Resistance	17
2.3.2. Genetics of hopper resistance	18

2.4. Enhancement of Resistance in Rice to Hoppers through Genetic Engineering	29
2.4.1. RNAi-mediated gene silencing	30
2.5. Conclusion	31
References	31
<b>3. Molecular Studies in Biosystematics, Phylogeny and Distribution of Tephritid Fruit flies: A Global Perspective</b>	<b>39-51</b>
<i>Abraham Verghese, M A Rashmi, A K Chakravarthy, P D Kamala Jayanthi and G Nagalakshmi</i>	
3.1. Introduction	39
3.2. Molecular Taxonomy	40
3.3. Molecular Tools in Analyzing Invasion History	44
3.4. Molecular Methodologies in Detection of Gut Bacteria	45
3.5. Transcriptome Analysis	46
3.6. Pest Management	46
3.7. Conclusion	47
References	47
<b>4. RNA Interference Research: Current Status and Future Outlook for Utilization in Insect Pest Management</b>	<b>52-72</b>
<i>Prashant Mohanpuria, Surinder K Sandhu and Ramesh Arora</i>	
4.1. Introduction	52
4.2. RNAi Vector Construction Strategies	56
4.2.1. HairpinRNA construct	56
4.2.2. Fusion PCR	56
4.3. Advantages of Utilizing RNAi in Pest Management	57
4.4. Requirements for Utilizing RNAi for Pest Management	58
4.4.1. Identification of suitable target in pest-insects	59
4.4.2. dsRNA delivery methods	59
4.4.3. Mechanisms of uptake of dsRNA/siRNA by cells and spread of silencing signals in plants and insects	63
4.4.4. Persistence of silencing effect and life stages of target insects	66
4.5. Conclusions	67
References	68
<b>5. Bioremediation of Pesticides in the Environment</b>	<b>73-96</b>
<i>Balwinder Singh and Kousik Mandal</i>	
5.1. Introduction	73

<i>Contents</i>	<i>xi</i>
5.2. Biodegradation and Bioremediation	75
5.3. Strategies for Bioremediation	76
5.3.1. <i>In situ</i> bioremediation	77
5.3.2. <i>Ex situ</i> bioremediation	78
5.4. Role of Microbes	80
5.5. Factors Affecting Bioremediation of Pesticides	82
5.6. Microorganisms in Bioremediation	85
5.7. Biotechnology in Bioremediation	86
5.8. Phytoremediation	87
5.9. Advantages of Bioremediation	88
5.10. Disadvantages of Bioremediation	89
5.11. Conclusions	89
References	90
<b>6. Microbial Control in Insect Pest Management: Achievements and Challenges</b>	<b>97-152</b>
<i>Ramesh Arora</i>	
6.1. Introduction	97
6.2. Viral Entomopathogens	98
6.2.1. Baculoviruses	100
6.3. Bacterial Entomopathogens	108
6.3.1. Genus <i>Bacillus</i>	108
6.3.2. Genus <i>Lysinibacillus</i>	117
6.3.3. Genus <i>Paenibacillus</i>	118
6.3.4. Genus <i>Brevibacillus</i>	119
6.3.5. Gram – Negative Bacteria	120
6.4. Fungal Entomopathogens	123
6.4.1. Structure and Reproduction	124
6.4.2. Host Range	124
6.4.3. The Infection Process	125
6.4.4. Mycotoxins	125
6.4.5. Role in Pest Management	126
6.4.6. Genetic Improvement	129
6.5. Microsporidian Entomopathogens	129
6.6. Entomopathogenic Nematodes (EPNs)	130
6.6.1. Important Entomopathogenic Groups	131
6.6.2. Role in Pest Management	132

6.7. Status of Microbial Control in Punjab, India	133
6.7.1. Indigenous Entomopathogens Detected	133
6.7.2. Laboratory Studies	135
6.7.3. Field Trials	137
6.7.4. Safety Evaluation	139
6.8. Conclusions	140
References	140
<b>7. Towards Durable Gall Midge Resistance in Rice</b>	<b>153-160</b>
<i>J S Bentur</i>	
7.1. Introduction	153
7.2. Plant Resistance and Biotypes	153
7.3. Tagging and Mapping Gall Midge Resistance Genes in Rice	154
7.4. Gene Pyramiding for Durable Resistance	155
7.5. Virulence Monitoring in Gall Midge Populations	156
7.6. Molecular Basis of Resistance	156
7.7. Insect Virulence Genes	158
7.8. Conclusions	158
References	158
<b>8. Molecular Markers in Entomological Research</b>	<b>161-201</b>
<i>P S Burange, Sujana Mamidi, P C Pathania and Uma Kanta</i>	
8.1. Introduction	161
8.2. Types of Molecular Markers	162
8.2.1. Restriction digestion based markers	162
8.2.2. PCR based markers	166
8.2.3. Restriction and PCR based markers	169
8.2.4. DNA sequencing	170
8.2.5 Next generation markers	175
8.3. Applications of Molecular Markers in Entomology	180
8.4. Conclusions	184
References	185
<b>9. Transgenic Cotton in India: Ten Years and Beyond</b>	<b>202-227</b>
<i>Vijay Kumar, A K Dhawan and P S Shera</i>	
9.1. Introduction	202
9.2. Transgenic BT Cotton	203
9.3. Introduction of BT Cotton in India	204

9.4. Approved Events and BT Cotton Hybrids in India	204
9.4.1. Approved Events of Bt Cotton	206
9.4.2. New Events yet to be Commercialised	207
9.5. Adoption and Impact Analysis of Bt Cotton	209
9.5.1. Increase in Area	210
9.5.2. Increase in Production and Productivity	212
9.5.3. Pest Scenario on Bt Cotton	212
9.5.4. Reduction in Insecticide Usage	217
9.5.5. Economic Benefits	219
9.5.6. Impact of IPM Strategies in Bt Cotton	221
9.6. Conclusions	223
References	224
<b>10. A Status Update on the Use of Biotechnological Techniques for Combating Insect Pests of Fruit Crops</b>	<b>228-248</b>
<i>Krishan Kumar, P K Arora and Kuldeep Singh</i>	
10.1. Introduction	228
10.2. Markers Assisted Breeding	229
10.2.1. Linkage Map	229
10.2.2. Gene/QTL Mapping	232
10.2.3. High Resolution Mapping	232
10.3. Transgenics	234
10.3.1. Gene Construct	234
10.3.2. Methods of Gene Introduction	237
10.3.3. Selection, Regeneration and Testing of Transgenics	237
10.4. RNA Interference	238
10.4.1. Mechanism of RNAi	238
10.4.2. Status and Factors Affecting the Success of RNAi	238
10.5. Conclusions	242
References	243
<b>11. Pest- Insects Resistance to Microbial Control Agents: Current Status and Management Strategies</b>	<b>249-311</b>
<i>Arshdeep K Gill and Ramesh Arora</i>	
11.1. Introduction	249
11.2. Status of Insect Resistance to Microbial Control Agents	250
11.2.1. <i>Bacillus thuringiensis</i>	250
11.2.2. Resistance to Dipteran - specific <i>Bacillus</i> Toxins	263

11.2.3. Entomopathogenic Viruses	265
11.3. Cross Resistance among Toxins	266
11.3.1. Cross Resistance Between Cry Proteins	266
11.3.2. Cross Resistance to <i>B. thuringiensis</i> subsp. <i>israelensis</i>	267
11.3.3. Cross-Resistance to <i>Lysinibacillus sphaericus</i>	268
11.4. Basis of Insect Resistance to MCAs	268
11.4.1. Morphological and Behavioral Basis of Resistance	268
11.4.2. Biochemical and Physiological Basis of Resistance	269
11.4.3. Genetic and Molecular Basis of Resistance	271
11.5. Stability of Bioinsecticide Resistance	276
11.6. Fitness Costs of Bioinsecticide Resistance	277
11.7. Management of Insect Resistance to MCAs	279
11.7.1. Management of Resistance to <i>B. thuringiensis</i> and its Toxins	279
11.7.2. Management of Resistance to Bacterial Insecticides in Mosquito Populations	290
11.7.3. Virulence management of <i>Cydia pomonella</i> granulovirus	292
11.8. Conclusions	293
References	293
<b>12. Biological and Molecular Approaches in Management of Mite Pests</b>	<b>312-328</b>
<i>Paramjit Kaur and Manmeet B Bhullar</i>	
12.1. Introduction	312
12.2. Integrated Mite management	313
12.2.1. Biological control	314
12.2.2. Molecular approaches	319
12.3. Conclusions	323
References	323
<b>13. Biosystematics, Molecular Characterization and Management of Shoot and Fruit Borer <i>Conogethes</i> spp. (Crambidae: Lepidoptera)</b>	<b>329-343</b>
<i>A K Chakravarthy, P R Shashank, B Doddabasappa, S B Kandakoor and Chandrashekharaiiah</i>	
13.1. Introduction	329
13.2. Biosystematics	330
13.3. Molecular Characterisation	331
13.4. <i>Conogethes pinicolalis</i> Inoue and Yamanaka, 2006: An Evolved Species	333
13.5. Pheromones	333
13.6. Insect Host Plant Interactions	334

13.7. Bioecology	335
13.8. Feeding Behavior	337
13.9. Alternate Host Plants	337
13.10. Crop Losses	338
13.11. Management	338
13.12. Conclusion	338
References	339
<b>14. Molecular Techniques as Precision Diagnostics for Diseases and Mites of Honey Bees</b>	<b>344-372</b>
<i>Pardeep K Chhuneja and Kuldeep Singh</i>	
14.1. Introduction	344
14.2. Beekeeping in India	345
14.3. <i>A. mellifera</i> Introduction by PAU: Safeguard against Introduction of Diseases and Enemies	346
14.4. Bee Diseases and Mites	347
14.5. Occurrence of Bee Diseases and Mites in India	348
14.6. Diagnosis of Bee Diseases and Pests	348
14.7. Molecular Techniques as Diagnostics	349
14.7.1. Polymerase chain reaction	349
14.7.2. Multiplex polymerase chain reaction	349
14.7.3. Real-time polymerase chain reaction	349
14.8. Molecular Markers	350
14.9. Steps in Molecular Diagnosis	354
14.9.1. Sample collection	354
14.9.2. RNA/ DNA extraction	354
14.9.3. Designing pathogen specific primers/probes	354
14.9.4. PCR-amplification	354
14.10. Diagnostics Developments for Bee Pathogenic Taxa	355
14.10.1. Bacterial diseases	357
14.10.2. Viral diseases	358
14.10.3. Fungal diseases	362
14.10.4. Microsporidian diseases	363
14.10.5. Differentiation and identification of acarines	364
14.11. Advantages of Molecular Techniques	364
14.12. Conclusions	364
References	364

<b>15. Biointensive Integrated Pest Management for Sustainable Agriculture</b>	<b>373-429</b>
<i>P S Shera and Ramesh Arora</i>	
15.1. Introduction	373
15.2. Strategies in Biointensive IPM	374
15.2.1. Physical, cultural and mechanical control	375
15.2.2. Host plant resistance	376
15.2.3. Biological control agents	379
15.2.4. Biorationals	391
15.2.5. Biotechnological approaches	397
15.3. Area Wide – IPM	408
15.4. Conclusions	411
References	412