# PESTICIDE RESIDUES IN SELECT COMPONENTS OF AN AGROECOSYSTEM ADOPTING ORGANIC AND CONVENTIONAL FARMING IN PADAYETTI VILLAGE, PALAKKAD DISTRICT, KERALA

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**BHARATHIAR UNIVERSITY**

**Coimbatore**

**for the award of Degree of Doctor of Philosophy in**

**ENVIRONMENTAL SCIENCES**

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*(Aided by the Ministry of Environment, Forests and Climate Change, Govt. of India)*

**Coimbatore - 641 108, INDIA**

**NOVEMBER 2014**

# CERTIFICATE

## CERTIFICATE

This is to certify that the thesis, titled "**Pesticide residues in select components of an agroecosystem adopting organic and conventional farming in Padayetti village, Palakkad district, Kerala**" submitted to the Bharathiar University, in partial fulfillment of the requirements for the award of the Degree of Doctor of Philosophy in **Environmental Sciences** is a record of original work done by **Mr. K. Ganesan** during **April 2010 to November 2014** in the Department of **Ecotoxicology** at **Sálim Ali Centre for Ornithology and Natural History (SACON), Anaikatty, Coimbatore - 641 108** under my supervision and guidance, and the thesis has not formed the basis for the award of any Degree/ Diploma/ Associateship/ Fellowship or any other similar title of any candidate of any University.

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# DECLARATION

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I, **K. Ganesan** hereby declare that the thesis, titled "**Pesticide residues in select components of an agroecosystem adopting organic and conventional farming in Padayetti village, Palakkad district, Kerala**" submitted to the Bharathiar University, in partial fulfillment of the requirements for the award of the Degree of Doctor of Philosophy in **Environmental Sciences** is a record of original work done by me during **April 2010 to November 2014** under the guidance of **Dr. S. Muralidharan,** Principal Scientist, Department of **Ecotoxicology** at **Sálim Ali Centre for Ornithology and Natural History (SACON), Anaikatty, Coimbatore - 641 108,** and it has not formed the basis for the award of any Degree/ Diploma/ Associateship/ Fellowship or any other similar title of any candidate of any University.

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*Ganesan K*

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# CONTENTS

| CONTENTS | P. Nos. |
|---|---|
| *Executive summary* | i-v |
| **1. INTRODUCTION** | **1-9** |
| 1.1. Impacts of pesticides: National and International perspective | 5 |
| **1.2. OBJECTIVES** | 9 |
| **2. LITERATURE REVIEW** | **10-28** |
| 2.1. Status of Organochlorine Pesticides in India | 14 |
| 2.2. Surface and groundwater contamination | 15 |
| 2.3. Soil/ sediment contamination | 16 |
| 2.4. Direct impact on humans | 19 |
| 2.5. Effect on non-targeted organisms | 22 |
| **3. MATERIALS AND METHODS** | **29-41** |
| 3.1. Study area | 29 |
| 3.2. Methodology | 32 |
| 3.2.1. Field survey | 32 |
| 3.2.2. Sample collection | 32 |
| 3.2.3. Pesticides chosen for this study | 35 |
| 3.2.4. Sample processing | 38 |
| 3.2.4a. Sediments | 38 |
| 3.2.4b. Animal tissues | 38 |
| 3.2.4c. Paddy and straw | 39 |
| 3.2.4d. Cow-milk | 39 |
| 3.2.5. Chemical analysis | 40 |
| 3.2.6. Data analysis | 41 |

| CONTENTS | P. Nos. |
|---|---|
| **4. RESULTS AND DISCUSSION** | **46-125** |
| **4.1. Pesticide residues in sediments in the agroecosystem in Padayetti village, Palakkad district** | **53-64** |
| 4.1.1. Residues of major isomers/ metabolites of organochlorine pesticides in sediments in Padayetti village, Palakkad district | 55 |
| 4.1.1a. HCH and its isomers | 55 |
| 4.1.1b. DDT and its metabolites | 59 |
| 4.1.1c. Cyclodiene insecticides | 59 |
| 4.1.2. Residues of organophosphate pesticides in sediments in Padayetti village, Palakkad district | 59 |
| 4.1.3. Residues of synthetic pyrethroid pesticides in sediments in Padayetti village, Palakkad district | 62 |
| 4.1.4. Residues of carbamate pesticides in sediments in Padayetti village, Palakkad district | 63 |
| **4.2. Pesticide residues in crabs in the agroecosystem in Padayetti village, Palakkad district** | **64-80** |
| 4.2.1. Residues of major isomers/ metabolites of organochlorine pesticides in crabs in Padayetti village, Palakkad district | 67 |
| 4.2.1a. HCH and its isomers | 67 |
| 4.2.1b. DDT and its metabolites | 69 |
| 4.2.1c. Cyclodiene insecticides | 70 |
| 4.2.2. Residues of organophosphate pesticides in crabs in Padayetti village, Palakkad district | 73 |
| 4.2.3. Residues of synthetic pyrethroid pesticides in crabs in Padayetti village, Palakkad district | 77 |
| 4.2.4. Residues of carbamate pesticides in crabs in Padayetti village, Palakkad district | 80 |
| **4.3. Pesticide residues in snails in the agroecosystem in Padayetti village, Palakkad district** | **80-92** |
| 4.3.1. Residues of major isomers/ metabolites of organochlorine pesticides in snails in Padayetti village, Palakkad district | 83 |
| 4.3.1a. HCH and its isomers | 83 |
| 4.3.1b. DDT and its metabolites | 85 |
| 4.3.1c. Cyclodiene insecticides | 86 |

| CONTENTS | P. Nos. |
|---|---|
| 4.3.2. Residues of organophosphate pesticides in snails in Padayetti village, Palakkad district | 88 |
| 4.3.3. Residues of synthetic pyrethroid pesticides in snails in Padayetti village, Palakkad district | 90 |
| 4.3.4. Residues of carbamate pesticides in snails in Padayetti village, Palakkad district | 91 |
| **4.4. Pesticide residues in fishes in the agroecosystem in Padayetti village, Palakkad district** | **92-99** |
| 4.4.1. Residues of major isomers/ metabolites of organochlorine pesticides in fishes in Padayetti village, Palakkad district | 93 |
| 4.4.2. Residues of organophosphate pesticides in fishes in Padayetti village, Palakkad district | 94 |
| 4.4.3. Residues of synthetic pyrethroid pesticides in fishes in Padayetti village, Palakkad district | 96 |
| 4.4.4. Residues of carbamate pesticides in fishes in Padayetti village, Palakkad district | 98 |
| 4.4.5. Dietary intake of pesticide residues to man | 98 |
| **4.5. Pesticide residues in frogs in the agroecosystem in Padayetti village, Palakkad district** | **100-108** |
| 4.5.1. Residues of major isomers/ metabolites of organochlorine pesticides in frogs in Padayetti village, Palakkad district | 102 |
| 4.5.1a. HCH and its isomers | 103 |
| 4.5.1b. DDT metabolites and Cyclodiene insecticides | 104 |
| 4.5.2. Residues of organophosphate pesticides in frogs in Padayetti village, Palakkad district | 104 |
| 4.5.3. Residues of synthetic pyrethroid pesticides in frogs in Padayetti village, Palakkad district | 105 |
| 4.5.4. Residues of carbamate pesticides in frogs in Padayetti village, Palakkad district | 107 |
| **4.6. Pesticide residues in paddy and fodder in the agroecosystem in Padayetti village, Palakkad district** | **108-122** |
| 4.6.1. Residues of major isomers/ metabolites of organochlorine pesticides in paddy and fodder in Padayetti village, Palakkad district | 108 |
| 4.6.2. Residues of organophosphate pesticides in paddy and fodder in Padayetti village, Palakkad district | 113 |
| 4.6.3. Residues of synthetic pyrethroid pesticides in paddy and fodder in Padayetti village, Palakkad district | 117 |
| 4.6.4. Residues of carbamate pesticides in paddy and fodder in Padayetti village, Palakkad district | 122 |
| **4.7. Pesticide residues in cow-milk in the agroecosystem in Padayetti village, Palakkad district** | **122-125** |
| 4.7.1. Residues of OP, SP and Cbs in cow-milk in Padayetti village, Palakkad district | 123 |
| **5. SUMMARY AND CONCLUSION** | **126-134** |
| **6. BIBLIOGRAPHY** | **I-XLIV** |
| **Publication** | |

| LIST OF FIGURES | P. Nos. |
|---|---|
| **Fig. 3.1.** Digital map of the study area: Padayetti village, Kerala, India | 31 |
| **Fig. 3.2a-n.** List of components included for the multiresidue analysis | 33-34 |
| **Fig. 3.3a.** Standard chromatogram of 40 pesticides calibrated in GC-MS | 42 |
| **Fig. 3.3b.** Quantification report of 40 calibrated pesticides quantified and qualified by GC-MS | 43 |
| **Fig. 3.3c.** Conventional farm crab sample chromatogram of pesticides quantified and qualified by GC-MS | 44 |
| **Fig. 3.3d.** Quantification report of conventional farm crab sample quantified and qualified by GC-MS | 45 |
| **Fig. 4.1.** Bionutrients supplied by Kerala State Biodiversity Board to farmers | 52 |
| **Fig. 4.1a.** Mean ( ± SE) levels of OC, OP and SP residues in sediments (n=60; ng/g wet wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12) | 55 |
| **Fig. 4.1b.** Mean ( ± SE) levels of organophosphate residues in sediments in the agroecosystem (n=60; ng/g dry wt) in Padayetti village, Palakkad district (2009-12) | 61 |
| **Fig. 4.2a.** Mean (± SE) levels of OC, OP and SP pesticide residues in *Geothelphusa dehaani* (n=74; ng/g wet wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12) | 66 |
| **Fig. 4.2b.** Variation (season, year and farming practice wise) in organochlorine pesticide residues in crabs in the agroecosystem in Padayetti village, Palakkad district (Mean ± SE; n=74 ng/g wet wt; 2009-12) | 71 |
| **Fig. 4.2c.** Variation (season, year and farming practice wise) in organophosphate pesticide residues in crabs in the agroecosystem in Padayetti village, Palakkad district | 74 |
| **Fig. 4.2d.** Variation (season, year and farming practice wise) in synthetic pyrethroid pesticide residues in crabs in the agroecosystem in Padayetti village, Palakkad district | 78 |
| **Fig. 4.2e.** Dead crab, *Geothelphusa dehaani* in the conventional farm, Padayetti village (Inset: Empty pesticide sachet disposed off in the paddy field) | 79 |
| **Fig. 4.3a.** Mean (± SE) levels of OC, OP and SP residues in snails (n=64; ng/g wet wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12) | 82 |
| **Fig. 4.3b.** Variation (season, year and farming practice wise) in OC residues in snails in the agroecosystem in Padayetti village, Palakkad district (Mean ± SE; n=64; ng/g wet wt) | 88 |
| **Fig. 4.5a.** Mean (± SE) levels of OC, OP, SP and Cb pesticide residues in frogs (n=74; ng/g wet wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12) | 102 |
| **Fig. 4.5b.** Mean (± SE) levels of HCH isomers and Heptachlor residues in frogs (n=74; ng/g wet wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12) | 103 |
| **Fig. 4.5c.** *Hoplobatrachus crassus* - Incomplete limb with missing lower portion | 106 |
| **Fig. 4.6a.** Mean (± SE) levels of organophosphate residues in fodder (n=60; ng/g dry wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12) | 115 |
| **Fig. 4.6b.** Mean (± SE) levels of synthetic pyrethroid residues in paddy (n=60; ng/g dry wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12) | 118 |
| **Fig. 4.6c.** One of the old and popular varieties of rice in Kerala, *Thavalakannan* | 120 |

| LIST OF TABLES | P. Nos. |
|---|---|
| **Table 1.1.** List of banned/ restricted organochlorines included in the present study | 5 |
| **Table 3.1.** Data sheet for survey on pesticide consumption | 36 |
| **Table 3.2.** Details of samples analysed by GC-MS from organic and conventional farms between 2009-2012 | 37 |
| **Table 4.1.** List of pesticides currently-used in conventional paddy cultivation in Padayetti village, and their relevant details | 52 |
| **Table 4.2.** List of biopesticides and bionutrients used in organic paddy cultivation in Padayetti village, and their relevant details | 53 |
| **Table 4.7a.** Mean ( ± SE) levels of individual OP, SP and Cb residues in cow-milk (n=70; ng/mL wet wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12) | 123 |

## TERMS AND ABBREVIATIONS USED

| | | |
|---|---|---|
| µg/kg | - | microgram per kilogram |
| µg/L | | - microgram per litre |
| µl/ml | - | microgram per millilitre |
| 2,4,5 T | - | 2,4,5-Trichlorophenoxyacetic acid |
| 2,4-D | - | 2,4-Dichlorophenoxyacetic acid |
| AChE | - | Acetylcholinesterase |
| ACP | - | Advisory Committee on Pesticides |
| ADI | - | Acceptable Dietary Intake |
| ANOVA | - | Analysis of Variance |
| APEDA | - | Agricultural and Processed Food Products Export Development Authority |
| APRB | - | Agricultural Biodiversity Restoration Programme |
| ARfD | - | Acceptable Reference Dose |
| BDL | - | Below Detection Limit |
| BIS | - | Bureau of Indian Standards |
| Cb | - | Carbamate |
| CDI | - | Calculated Dietary Intake |
| ChE | - | Cholinesterase |
| CIBRC | - | Central Insecticides Board and Registration Committee |
| CNS | - | Central Nervous System |
| CSE | - | Centre for Science and Environment |
| DDT | - | Dichlorodiphenyltrichloroethane |
| DoECC | - | Department of Environment and Climate Change |
| DP | - | Dustable Powder |
| EC | - | Emulsifying Concentration |
| EC | - | European Commission |
| ECG | - | Electrocardiography |
| EW | - | Emulsion in Water |
| FAO | - | Food and Agriculture Organization of the United Nations |
| FDA | - | Food and Drug Administration |
| FSSAI | - | Food Safety and Standards Authority of India |
| G | - | Granule |
| g/ha | - | gram per hectare |
| GAP | - | Good Agricultural Practice |
| GC-MS | - | Gas Chromatography-Mass Spectrometry |
| GM crop | - | Genetically Modified crop |
| ha | - | Hectare |
| HCB | - | Hexachlorobenzene |
| HCH | - | Hexachlorocyclohexane |
| ICAR | - | Indian Council of Agricultural Research |
| IPL | - | Indian Pesticide Limited |
| IPM | - | Integrated Pest Management |
| IUCN | - | International Union for Conservation of Nature |
| JMPR | - | Joint FAO/WHO Meetings on Pesticide Residues |
| KSBB | - | Kerala State Biodiversity Board |
| $LC_{50}$ | - | Lethal Concentration |
| LDH | - | Lactate Dehydrogenase |
| LED | - | Light Emitting Diode |

| | | |
|---|---|---|
| LOD | - | Limit of Detection |
| LOQ | - | Limit of Quantification |
| ME | - | Microemulsion |
| mg/L | - | milligram per litre |
| MIC | - | Methyl Isocyanate |
| MoH | - | Ministry of Health |
| MoSPI | - | Ministry of Statistics and Programme Implementation |
| MPC | - | Maximum Permissible Concentration |
| MRL | - | Maximum Residue Limit |
| MT | - | Metric Ton |
| ng/L | - | nanogram per litre |
| NGO | - | Non-governmental Organization |
| Non-IPM | - | Non- Integrated Pest Management |
| o,p'-DDD | - | o,p'-Dichlorodiphenyldichloroethane |
| o,p'-DDE | - | o,p'-Dichlorodiphenyldichloroethylene |
| o,p'-DDT | - | o,p'-Dichlorodiphenyltrichloroethane |
| OC | - | Organochlorine |
| OP | - | Organophosphate |
| p,p'-DDD | - | p,p'-Dichlorodiphenyldichloroethane |
| p,p'-DDE | - | p,p'-Dichlorodiphenyldichloroethylene |
| p,p'-DDT | - | p,p'- Dichlorodiphenyltrichloroethane |
| PAH | - | Polycyclic Aromatic Hydrocarbon |
| PAN | - | Pesticide Action Network |
| PCBs | - | Polychlorinated biphenyls |
| PCK | - | Plantation Corporation of Kerala |
| POPs | - | Persistent Organic Pollutants |
| ppb | - | parts per billion |
| ppm | - | parts per million |
| Rs | - | Rupees |
| SC | - | Suspension Concentrate |
| SL | - | Soluble Liquid |
| SP | - | Synthetic Pyrethroid |
| SRI | - | System for Rice Intensification |
| $Tb_{1/2}$ | - | Time of 50% loss |
| TP | - | Transformation Products |
| UNEP | - | United Nations Environment Programme |
| USDA | - | United States Department of Agriculture |
| USEPA | - | United States Environmental Protection Agency |
| VES | - | Visual Encounter Survey |
| w/w | - | weight by weight |
| WHO | - | World Health Organization |
| WP | - | Wettable Powder |
| Wt | - | Weight |
| α-HCH | - | alpha-Hexachlorocyclohexane |
| β-HCH | - | beta-Hexachlorocyclohexane |
| γ-HCH | - | gamma-Hexachlorocyclohexane or lindane |
| δ-HCH | - | delta-Hexachlorocyclohexane |
| λ | - | Lambda |
| ∑ | - | Summation |

## EXECUTIVE SUMMARY

*Intensive agriculture to meet the ever increasing food demand by growing human population, has led to increasing concern on the impacts, including loss of soil fertility, diminishing soil biodiversity and of course, lack of sustainability in the entire process. Challenged by these concerns there have been attempts, world over, to develop alternate methods of farming which are environment-friendly and economically viable. At this juncture, Kerala State Biodiversity Board through Department of Environment and Climate Change launched a demonstration project known as "Granary of Kerala" in Palakkad district in 2009 to conserve biodiversity associated with agroecosystems by totally avoiding chemical inputs. As part of this project, the present study was carried out to monitor the pesticide residues in organic and conventional paddy agroecosystem in Padayetti village, Palakkad district of Kerala, India. Samples of components, namely sediment, crab, snail, fish, frog, paddy, fodder (straw) and cow-milk were collected from organic and conventional farms on seasonal basis and processed for multiresidue estimation. Quantitative and qualitative analyses of a set of historical (20 OCs) and currently-used-pesticides (12 OPs, 6 SPs and 2 Cbs) were performed by GC-MS. Data have been compiled to check the overall load of pesticides and variation in residues between the farming practices, among seasons (Kharif, Rabi and summer), years and trophic levels. Further, within the group, levels of individual pesticides and their isomers or metabolites have also been compiled wherever applicable. This could help understand the implications with reference to potential health risks associated with the exposure to these pesticides, usage pattern or policy of the government in existence at national (FSSAI, India) and International (USEPA; European Commission; Health Canada; FAO/WHO Codex Alimentarius) level.*

*Questionnaire survey was conducted among farmers to know the cropping pattern, common crop pests, chemical pesticides and fertilizers used in the village. Information on pesticide application, source, frequency of application, chemical group, quantity, mode of application were collected and compiled. In addition to documenting current use of pesticides, information on historic use was also gathered. Forty nine out of 69 households in Padayetti village are engaged in paddy cultivation. Around 30 farmers practice organic farming while the rest use chemical fertilizers and pesticides. A few farmers adopted convenient farming. Most of them have kitchen gardens and cultivate vegetables without any chemical input. Their experience in farming ranges between 20 and 40 years. Organic farmers use farm yard manure, vermicompost, biogas slurry, neem, castor and groundnut oil cakes, biopesticides, plant extracts such as garlic, ginger, chilli and N, P, K bionutrients. As part of the farmers' field school programme, all the farmers were trained by the government on system of rice intensification (SRI) technique, various biological pest control measures, organic manure and vegetable bed preparations in the fields with help of experts.*

*Pesticide residues in different components in the agroecosystem in Padayetti village, Palakkad district*

*a) Residues of organochlorine (OC) pesticides*

*Residues of persistent OCs were detected in 16.67% of samples analysed with the highest (HCH: 30.90 ± 9.83 ng/g) in sediments of conventional farms collected during Kharif season (2009-10) and lowest (2.34 ng/g) during summer (2010-11). Levels of Σ-OCs were below detection limit (BDL) in sediments from organic farms. Among the OC residues detected in different biotic components, Σ-HCH was higher in the tissues of freshwater snail Pila globosa (83.91 ± 4.17 ng/g; 2009-10 Kharif) followed by freshwater paddy field crab Geothelphusa dehaani (72.30 ng/g; 2011-12 summer) and Indian rice frog Fejervarya limnocharis (33.22 ± 16.48 ng/g); 2009-10 Rabi) collected from conventional farm than organic farm. Presence of trace amounts of Σ-DDT, Dieldrin and Heptachlor in few samples of crab, snail and frog collected from both the farms could be due to past usage in this region. Residues of γ-HCH in snails varied significantly among the seasons ($p < 0.05$; $F = 8.03$). However, the variation between the farms was not quite significant ($p > 0.05$; $F = 4.11$). Further, due to less detectable levels of OCs in other components collected from both the farms, no statistical test was performed. Levels of Aldrin, Endrin, Chlordane and Mirex were at BDL in all the components studied from both the farms.*

*b) Residues of Organophosphate (OP) Pesticides*

*Among a set of recently used OPs analysed, residues of Chlorpyrifos (22.07 ± 4.80 ng/g; 2009-10 Rabi season) and Triazophos (10.47 ± 7.24 ng/g; 2009-10 summer season) were slightly higher in conventional farm sediments than organic farm. Among the individual OPs tested in crabs, Chlorpyrifos was detected in 10.81% of samples collected from conventional farms, while Methyl Parathion, Parathion Ethyl, Phosphamidon and Phenthoate were detected in less than 4% of the samples. Both Phorate and Triazophos were detected at 5.18 and 9.96 ng/g respectively in snails collected from conventional farms during Rabi and Kharif seasons (2009-10). In fishes, Methyl Parathion and Phenthoate were found at 3.23 and 2.78 ng/g in Channa punctatus and Puntius sophore collected from conventional farms during summer 2009-10 respectively. Phosphamidon (2.98 ng/g) was detected in Cirrhinus mrigala collected during Rabi season of the same year. Concentrations of Quinalphos and Phorate were 2.62 ± 1.02 ng/g and 1.02 ± 0.29 ng/g respectively, in Fejervarya limnocharis collected during Kharif (2010-11). Parathion Ethyl (1.02 ng/g) was detected in Hoplobatrachus crassus in the same season and year. Trace amounts of Chlorpyrifos (1.83 ng/g) was detected in Thavalakannan variety of paddy collected from conventional farm during Rabi (2009-10). But in fodder, Σ-OP residues were detected in 40% of the freshly harvested paddy straw collected from conventional farms ranging between 2.02 ng/g (Aishwarya; Rabi, 2011-12) and 14.62 ± 1.63 ng/g in the same variety and season during 2009-10. Among all the OP compounds, Methyl Parathion (30.09 ng/g;*

*2011-12 summer) was detected in milk samples collected from cows which were fed with feeds available in the market and fodder grown in conventional farms than in milk collected from cows fed with only feeds grown in organic (22.64 ng/g; 2010-11 summer) farms. It may be noted that pesticide residues in food such as fodder grass, readily available feeds, grain mixtures, cotton seeds and groundnut cakes are the major source of contamination in the milk. The levels of Pirimiphos Ethyl and Ethion were BDL in all the components. The statistical analyses of the OP residues in various components showed that Σ-OP residues varied significantly between organic and conventional farm sediments ($p < 0.05$; $F = 0.79$) as well as crab ($p < 0.05$; $F = 9.5$) and fodder ($p < 0.05$; $F = 4.15$).*

### *c) Residues of Synthetic Pyrethroid (SP) Pesticides*

*Levels of SP, Σ-Fenvalerate were fairly high in only one of the sediment collected from conventional farms (62.80 ng/g) during Rabi season (2011-12). Among the other components, crab Geothelphusa dehaani (59.58 ng/g; Kharif 2011-12), frogs F. limnocharis (26.91 ± 9.19 ng/g) and H. crassus (42.15 ng/g) during 2009-10 Kharif and Rabi seasons, snail Pila globosa (20.33 ng/g; 2009-10 Kharif) and freshly harvested paddy straw (Jothi matta: 6732.55 ng/g; 2009-10 Rabi), collected from conventional farms had high levels of Σ-Fenvalerate. Fenvalerate-II residues in crabs varied significantly between the seasons ($p < 0.05$; $F = 6.80$). It was observed that out of 109 frogs collected, seven had deformities (6.42%). Interestingly, all the deformed frogs were collected from conventional farm. Species with deformity were F. limnocharis (n=6) and H. crassus (n=1). High concentrations of Fenvalerate in deformed F. limnocharis (26.91 ± 9.19 ng/g) and H. crassus (42.15 ng/g) might have contributed to the deformity. However, not all the frogs collected from conventional farms showed external deformities. High concentration of these contaminants is of great concern, especially when the increasing accumulation potential of these compounds in the food chain is considered. Thus, to avoid the negative effects of agricultural pesticides on faunal diversity, in the paddy agroecosystem, we urge the farmers to at least minimize in the use of these fast degrading pesticides, and in case, if the use is unavoidable, at least use only the correct chemical and dosage.*

### *d) Residues of Carbamate (Cb) Pesticides*

*Commonly used Carbamate pesticides, namely Carbaryl and Carbofuran were analysed. Among all the components collected during the years, one of the dead crabs collected from the conventional farm recorded 3501.91 ng/g of Carbaryl (Rabi 2011-12) suggestive of poisoning. Trace levels of these residues (5.23 ± 1.5 ng/mL) were also detected in two of conventional farm milk samples analysed during summer (2009-10). Residues of Carbofuran (2.09 ng/g) were detected in one of the F. limnocharis collected from conventional farm during Rabi (2011-12). This reflects the use of Carbaryl and Carbofuran in the conventional farming*

*practice. Although we have no data on the total application of both Carbaryl and Carbofuran in the study sites, farmers of the area spray up to 250 and 750 g/ha respectively. It may be worth mentioning that around 225 and 500 MTs of Carbaryl and Carbofuran respectively is being used in India on various crops. The elimination of Carbaryl is rapid and takes place mainly through the urine, feces and milk of dairy animals. However, even minute quantities of its residues in food chains may produce toxicological effects following their consumption in man and animals. Even though Food Safety and Standards Authority of India (FSSAI) has not established any limit for Carbaryl in milk, the residue level was lower than the MRLs (50 ng/mL) fixed by FAO/WHO.*

*e) Conclusion and Recommendations*

*Due to lack of historical information on the residue levels of OCs in the study area, data generated in the present study could not be compared to understand any trend on a time scale. However, presence of HCH, DDT and Cyclodiene isomers and metabolites in both the farms clearly indicates that these insecticides are still in the environment. Due to their persistence, it is difficult to eliminate the OCPs by simply avoiding their use, as many years are required for them to disappear from the environment.*

*Residues of OC, OP, SP and Cbs in different components recorded in the paddy agroecosystem in the present investigation are found to be mostly below stipulated MRL, but their presence in conventional farms is of great concern. Most agricultural produces meet legal limits every year, but legal does not always mean safe. While the risks of a pesticide depends on how much of it is consumed and how toxic that particular pesticide is, it is hard to say exactly how much is safe. Moreover levels that are safe for adults may be harmful to children.*

*Adoption of IPM strategies involving a variety of biological and physical methods to control pests has resulted in varying degrees of success. We have noticed that the overall usage of pesticides has come down in the Padayetti agroecosystem and even a few farmers adopting conventional farming applied only fast degrading chemicals based on need. However, IPM strategies for farmers of conventional farm in Padayetti village have not been implemented effectively. This could be attributed to the farmers' lack of understanding of pest management concepts. It is recommended that effective IPM programs must be adopted based on the need of local farmers. For the long-term perspectives, paddy farms with IPM practices provide a sustainable alternative in terms of both economic as well as an ecological point of view.*

*To keep the paddy farming more sustainable, ancient practices such as mixed cropping, crop rotation and growing of legume plants should be tried. Use of organic manure and effective biological pest control measures should be promoted. Data generated through this study provides reference points for future assessment of OC, OP, SP and Cb residue levels in organic and conventional farm produces. Extensive*

*awareness programme to dissuade farmers using for pesticides through contact programmes and media should be organized. Perceivable changes can happen only when the farmer is convinced about efficacy of organic farming. Therefore, agricultural scientists must, in accordance with changing circumstances of climate, water and soil, help the farmers. To meet the challenge of taking scientific research from lab to land young educated, progressive farmers and agricultural research scholars could together form a talent pool in all districts in the state.*

*The concept of healthy food and organic farming is catching up very fast the world over. Consumers in the developed countries are prepared to pay a premium for the organic food. The price of organic paddy should be 40% higher than paddy from the conventional farm. The premium price of organic rice is an important factor to induce farmers to convert from conventional to organic rice production. It is also advisable to carryout similar studies in other parts of the state/ country. In general, our findings call for urgent action to reduce the level of OP, SP and Cb exposure and their effects on wildlife and human health. Additionally, the following recommendations are made.*

*Some of the currently-used pesticides such as OPs, SPs, and Cbs are highly toxic across the food chain including man. These classes of pesticides have relatively short half-lives in tissues, but nonetheless can still evoke toxicity. Hence, regulatory agencies in India should consider imposing restrictions on the use of these pesticides. In India although submission of toxicity data on birds and mammals are necessary for registration of a new molecule, for amphibians till date, no reference is made in the registration process. Our results indicate that existing risk assessment procedures for pesticide formulation need to be strengthened to protect also the amphibians. We hope that our work will encourage further investigations on the role of agrochemicals on frog declines, given that to-date there has been relatively very little research on contaminants in India. Farmers have to be provided with safe alternatives to handle pests without compromising on productivity. Agricultural subsidy offered to farmers towards preserving biodiversity and promoting organic farming need to be more flexible to fit different levels of farmers. Production of organic fertilizers (neem and castor oil cakes), biopesticides, bionutrients and green manures should meet the demands. More organic farm produce outlets need to be operated across the state to help farmers sell their produce at reasonable rates.*

# INTRODUCTION

# 1. INTRODUCTION

'Growth' and 'progress' are among the key words in every national vocabulary. But modern man now carries Strontium 90 in his bones.... DDT in his fat.... asbestos in his lungs.... A little more of this 'progress' and 'growth,' and this man will be dead -Morris K. "Mo" Udall (1996).

In the recent past, pesticide related issues have been extensively highlighted in the media including research journals and attracted worldwide debate and sharp focus among the interested groups in India (Prabhavathy *et al.,* 2011). If the credits of pesticides include enhanced economic potential in terms of increased production of food and fibre, and amelioration of vector-borne diseases, then their debits have resulted in serious health implications to man and his environment. There are now overwhelming evidences that some of these chemicals do pose potential risks to humans and other life forms, and unwanted side effects to the environment (Forget, 1993; Igbedioh, 1991). Ideally a pesticide must be lethal to the targeted pests, but not to the non-target species, including man. Unfortunately, this is not so (Lari *et al.,* 2014). No segment of the population is completely protected against exposure to pesticides and potentially the serious health effects, although a disproportionate burden is shouldered by the people of developing countries and by high risk groups in each country (WHO, 2000). The controversy of use and abuse or benefits and ill effects of pesticides has been an ongoing debate. Nevertheless, the rampant use of these chemicals, as the adage goes, *"if little is good, a lot more will be better"* has played havoc with all life forms (Aktar *et al.,* 2009).

Growing awareness about health and environmental issues associated with indiscriminate use of chemicals has led to interest in alternate forms of agriculture in the world (Ramesh *et al.,* 2010). Ideally, an alternative for economic crop production without the use of agrochemicals should be developed. One alternative to synthetic pesticide use ...ming (Naik and Prasad, 2006). Organic agriculture is one among the broad ...ethods that are supportive of the environment. According to ... 1999), 'organic agriculture is a holistic production ...motes and enhances agroecosystem health, including ... and soil biological activity. It emphasizes the use of

management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. This is accomplished by using, where possible, agronomic, biological and mechanical methods, as opposed to using synthetic materials, to fulfill any specific function within the system'. The key principles and practices of organic farming aims to encourage and enhance biological cycles within farming systems to maintain and increase longtime fertility of soil, to minimize all forms of pollution caused by fertilizers and pesticides and to produce food of high quality in sufficient quantity (Naik and Prasad, 2006).

Organic farming is gaining gradual momentum across the world. Based on the global survey on organic farming carried out in 2007-2008 (Willer *et al.,* 2009) by the Research Institute of Organic Agriculture (Switzerland) and the International Federation of Organic Agriculture Movements and Foundation Ecology and Agriculture (Germany), organic agriculture is now practiced in more than 130 countries with a total area of 30.4 million ha in 0.7 million number of organic farms. Global demand for organic products remains robust, with sales increasing by over US$ 5 billion a year (Ramesh *et al.,* 2010). Organic monitor estimates International sales to have reached US$ 38.6 billion in 2006, double that of 2000, when sales was at US$ 18 billion. Consumer demand for organic products is concentrated in North America and Europe; these two regions comprise 97% of global revenues. Asia, Latin America and Australasia are important producers and exporters of organic foods (Sahota, 2009). The maximum number of organic farms in Europe is in Italy followed by Austria, Spain and Germany (Ecobank, 2000). In India, about 5,28,171 ha area is under organic farming (this includes certified and area under organic conversion) with 44,926 number of certified organic farms (Ramesh *et al.,* 2010). This accounts for about 0.3% of total agricultural land. Indian organic farming industry is estimated at US$ 78 million and is almost entirely export oriented. According to Agricultural and Processed Food Products Export Development Authority (APEDA, www.apeda.com/organic.html retrieved on 7$^{th}$ July 2010), a nodal agency involved in promoting Indian organic agriculture, about 5,85,970 tons of organic products worth Rs 301 million are being exported from India. Growing awareness, increasing market demand, increasing inclination of farmers to go organic and growing institutional support have resulted in more than 200% growth in certified area (Ramesh *et al.,* 2010).

Of late, due to a greater awareness among the public, the market for organically produced foods is on the rise. In India, organic farming was practiced in 4800 ha in 2003 with a net export value of just Rs 89 crores. This accounts for only 0.8% of the current global organic produce market. Some of the major organically produced agricultural crops in India include plantation crops, spices, pulses, fruits, vegetables and oil seeds (Prasad, 2005). In recent years, increased interest in organic farming methods has been evidenced by both farmers and the government. A number of research institutions and NGOs have helped promote sustainable methods of farming (Ramesh *et al.,* 2010).

The commonly used term 'conventional agriculture' refers to the standard, dominant farming approaches promoted and researched by most government and agribusiness groups and practiced by farmers and growers throughout the world. Usually, conventional agriculture imposes no restrictions on management and encourages using chemical fertilizers, pesticides, etc. for yield maximization under a given set of farming conditions (Ramesh *et al.,* 2010). The presence of multiple pesticide residues in food crops has raised public concern and has triggered look at the potential effects of pesticide mixtures (USEPA, 2002). During the era of green revolution (late sixties), introduction of high yielding varieties, expansion of irrigated areas, increased use of nitrogen, phosphorous and potassium fertilizers; chemical pesticides and higher cropping intensity drove India towards self-sufficiency in food production (Naik and Prasad, 2006). This has reduced the productivity of the soil by deteriorating soil health in terms of soil fertility and biological activity. Use of chemical pesticides to control various insects, pests and diseases over the years destroyed many naturally occurring effective biological agents. Despite obvious benefits, the indiscriminate use of such chemicals has resulted in over intoxication of animals and accumulation of residues in crops and foods causing series of environmental impacts as reported by several researchers (Bruhl *et al.,* 2013; Hou *et al.,* 2013; Kaushik *et al.,* 2011; Mukherjee and Arora, 2011; Dhananjayan and Muralidharan, 2010; Bakore *et al.,* 2004; Kannan *et al.,* 1992). Thus it is imperative to monitor pesticide residues in food commodities so as to assure the consumers that the Maximum Residue Limits (MRLs) are not exceeded (Ravikumar *et al.,* 2013; Nag and Raikwar, 2008).

Pesticide residues may comprise many substances, including any specified derivatives such as degradation products, metabolites and impurities that are considered to be of toxicologically significant. Several studies have shown that pesticides could cause health problems such as birth defects, nerve damage and cancer in human and other life forms (Aktar *et al.,* 2009). Pesticides can be divided into many classes, of which the organochlorines (OCs) were the first to be invented. Organochlorine pesticides (OCPs) are non-polar, lipophilic, very toxic and highly persistent compounds. These synthetic chemical pesticides reach the aquatic environment via soil percolation, air drift or surface runoff, leaching and disposal of empty containers. Further, these OCs end up in groundwater and their transformation products may remain for years (Mudiam *et al.,* 2012). Pesticides are well characterized by their toxicity, resistance to biodegradation, relatively high volatility as well as their capacity to interfere with cell biochemistry when accumulated in tissues (Dhananjayan and Muralidharan 2010; Alle *et al.,* 2009; Borga *et al.,* 2001; Tanabe *et al.,* 1998). As a result, they can biomagnify through food chains and produce harmful effects at every level (Muralidharan *et al.,* 2009).

Although OCs are also successful in controlling a number of diseases, such as malaria and typhus, due to established ill effects they are banned or restricted in most of the developed countries. But in some developing countries, they are still being used due to their low cost and high effectiveness. Pesticides, namely DDT and HCH were used extensively in India until recently both for agricultural and vector control. HCH use was banned by developed nations in 1970s followed by many developing countries in 1980s (Li, 1999). Use of HCH continued unabated until it was banned in 1997 in India (Tomar and Parmar, 1998), but restricted use of γ-HCH was allowed till 2013 (CIBRC, 2014).

According to Li (1999), historically the most polluted continent by technical HCH is Asia, where the three most polluted countries, namely China, India and Japan are located. DDT was one among the 12 persistent organic pollutants (POPs) identified during the Stockholm Convention on POPs in 2001. India, China and Russia opted for exemption from total ban on DDT (Dash *et al.,* 2007). Subsequently India banned agricultural use of DDT in 1989 (Anonymous, 1991) with a ceiling to use a maximum of 10,000 tons per annum for the control of malaria and kala-azar (Dash *et al.,* 2007). During the 9$^{th}$ Five

Year Plan of India (1997-1998 to 2001-2002), the National Anti-Malaria Programme sprayed 42,200 metric tons of DDT (50% WP; including 11,600 metric tons for kala-azar disease control) (Sharma, 2003). Due to their persistence, it is difficult to eliminate the OCPs by simply avoiding their use, as many years are required for them to disappear from the environment (Kaushik *et al.*, 2011; Juhler *et al.*, 1999). However, restriction and ban of OCP compounds have resulted in reduction of their residues in the environment (Jayanthi, 2012; Kaushik *et al.*, 2011; Ramesh *et al.*, 2010). The status of OCPs (restricted/ banned) presently investigated is listed in table 1.1.

**Table 1.1. List of banned/ restricted organochlorines included in the present study**

| Pesticide | Status in India | Month/ Year |
|---|---|---|
| Aldrin | Banned | September 1996 |
| Chlordane | Banned | September 1996 |
| DDT | Restricted use | September 1996 (Banned for agriculture since 1989) |
| Dieldrin | Banned | July 2003 |
| Endrin | Banned | May 1990 |
| Endosulfan | Banned | May, 2011 |
| HCH | Banned | April 1997; γ HCH (Banned since March 2013) |
| Heptachlor | Banned | September 1996 |
| Mirex | Banned | May 2004 |

Source: http://cibrc.nic.in/; retrieved on 20th October 2014

On the other hand organophosphates (OPs), synthetic pyrethroids (SPs) and carbamates (Cbs) are more toxic but less persistent lasting only for days, weeks or months in the environment (WHO, 2000; Wania and Mackay, 1996). Introduction of these synthetic insecticides, OPs in the 1960s, Cbs in 1970s and SPs in 1980s and the introduction of herbicides and fungicides in 1970s-80s contributed greatly in pest control and agricultural output. Hence, they have almost replaced OCs except a few.

### 1.1 Impacts of pesticides: National and International perspective

Use of pesticides in agriculture commenced in India around 1948-1949 (Gupta, 2004). However, production started in 1952 with the establishment of a plant for Benzene Hexa Chloride (BHC; now HCH) in Kolkata, and now India is ranked as the 2nd largest manufacturer of pesticides in Asia preceded by China and it has been placed at the 10th

position globally as its total consumption amounts to about 500 million tons (Lari *et al.*, 2014). The worldwide consumption of pesticides is about 2 million metric tons (MTs) per year, of which 24% is consumed in USA alone, 45% in Europe and 25% in the rest of the world. In this context, the average usage of pesticides in India is about 0.6 kg/ha (Abhilash and Singh, 2009) which is a matter of concern. Annual consumption of pesticides mainly for agricultural in India (2010-11) has crossed 55,540 MT from a mere 5,000 MT during 1960s (http://www.indiastat.com retrieved on 12$^{th}$ July 2014). Kerala, one of the smaller states in the country consumed about 631 MT during 2009-10 (www.indiastat.com retrieved on 7$^{th}$ September 2014). Since the population in India is growing at a steady rate and the availability of cultivable land is limited, consumption of pesticides is expected to increase in the future (Arora *et al.*, 2011).

Consumption of pesticides in India varies with the cropping pattern, magnitude of infestations and agro-ecological regions. Pesticide use is particularly high in regions with good irrigation and also in those areas where commercial crops are grown. The major use of pesticides in India is for cotton crops (50-55%), followed by paddy and wheat (>40%) (Arora *et al.*, 2011). Chemicals belonging to groups such as OPs, SPs and Cbs are indiscriminately used unaware of their impacts on the ecosystem and inhabitants.

Although pesticide consumption per hectare in India is low (around 600 gms/ha) when compared to the developed countries (3000 gms/ha), problems in our country resulting from reckless use are quite alarming. The ill effects of pesticides on wildlife, particularly birds have been an ongoing concern. In India several species of birds have either almost disappeared in totality or their population crashed drastically. Spot-billed Pelican *Pelecanus phillippensis* whose population plummeted from 2000 to 330 within a span of 30 years in Karnataka (Kannan and Manakadan, 2005); declining breeding population of Sarus Crane *Grus antigone* from 27 pairs during 1973 (Walkinshaw, 1973) to six pairs during 1990 (Vijayan, 1991) and two pairs during 2012 (Randhir Singh pers. comm. 2012) in Keoladeo National Park, Bharatpur, Rajasthan; unsuccessful breeding of Himalayan Grey-headed Fish Eagle *Ichthyophaga nana* at Corbett National Park in Uttarkhand (Naoroji, 1997) and catastrophic population decline in *Gyps* vultures across south Asia (Prakash *et al.*, 2007) are a few examples. In recent times mass mortality of birds such as

Spot-billed Pelican *Pelecanus philippensis*, Painted Stork *Mycteria leucocephala*, Eurasian Spoonbill *Platalea leucorodia*, Little Egret *Egretta garzetta*, Black-headed Ibis *Threskiornis melanocephalus*, Black-crowned Night-heron *Nycticorax nycticorax* at Anna Zoological Park, Chennai due to Phosphamidon poisoning; Red-crested Pochard *Netta rufina* and Common Moorhen *Gallinula chloropus* in Sitarganj forest range, Uttarkhand due to Chlorpyrifos poisoning; Demoiselle Crane *Anthropoides virgo* in Amreli and Surendranagar district in Gujarat due to Phorate and rodenticide poisoning; Cattle Egret *Bubulcus ibis* in Amreli district of Gujarat, unintentional poisoning due to Phorate and Carbofuran in Indian Peafowl *Pavo cristatus* in Dindigul, and deliberate poisoning of fish-eating birds such as Black-crowned Night-heron *Nycticorax nycticorax* and Purple Heron *Ardea purpurea* using Carbofuran in Viruthunagar, Tamil Nadu have been recorded (Muralidharan *et al.*, 2013).

Many OP, SP and Cb insecticides are extremely hazardous to fish and other wildlife (Smith, 1987). Fish kills involving these insecticides have been documented. OP insecticides can bioconcentrate in fish, frog and tadpoles to levels that pose hazards to their predators. The contamination of fish pond with as little as 5 ng/mL of Chlorpyrifos can significantly reduce the abundance of zooplankton. Cb insecticide such as Carbofuran is also extremely toxic to wildlife and fish (Smith, 1987). The birds appear to be more sensitive than other vertebrates to the toxic effects of both OP and carbamate pesticides. Both OP and Cb compounds are used throughout the world as insecticides, herbicides, nematicides, acaricides, fungicides, rodenticides, avicides, and bird repellents (Laetz *et al.*, 2009; Smith, 1987) and they are the ones which are reported to be responsible for mortality of many species of waterfowl, passerines and raptors.

Pyrethroids are derived from natural pyrethrins with some modifications to enhance their environmental stability or alter their insecticidal activity. They are also synthesized. Since they guarantee as effective agents against a broad range of pests and stable under field conditions, they are used as insecticides in agriculture worldwide (Mak *et al.*, 2005; Shan *et al.*, 1999). Many products such as Raid found commonly in retail stores for home use (to eliminate household pests such as ants and spiders), contain pyrethroid such as Permethrin, (De Pasquale, 2010). Pyrethroids are reported to be present in the

environment due to their extensive usage in agriculture and households. Several studies have proved that pyrethroids are highly toxic to a number of non-target organisms such as honeybees, freshwater fishes and aquatic arthropods even at very low concentrations mainly through their action on voltage-sensitive sodium channels (Palmquist *et al.*, 2011; Tomlin, 2006; Kim *et al.*, 2005).

Pesticide usage for the cultivation of food crops among different states of India shows a mixed pattern. Particularly in Kerala although use of OCs has come down, quantity used is still considerable. As per figures available from the Toxicological Atlas of India prepared by Industrial Toxicology Research Centre/ Council of Scientific and Industrial Research (Gupta, 2004) Kerala uses 0.4 to 0.6 kg/ ha for plant protection.

Perhaps no other crop is cultivated more widely in the world than paddy. Paddy is cultivated in more than 113 countries and it provides livelihoods for nearly 250 million people. It is the staple food for over half of the world's population and provides 27% of the dietary energy supply and 20% of the dietary protein intake in the developing world. India is the second largest producer of rice in the world after China and is staple food of 65% of the Indian population. Rice accounts for nearly 46 and 42% of the cereal and total food grain production respectively, in India. Along with the other staple crop wheat, paddy has been one of the few crops that has contributed to the staggering intensification of agricultural practices, including the use of artificial fertilizers and pesticides and more recently in the use of genetically modified crops. With the intensification unlikely to abate due to increased demand by a growing population, there has been an increasing concern of the impacts, including loss of soil fertility, diminishing soil biodiversity and of course, lack of sustainability in the entire process.

Challenged by many environmental concerns, there have been attempts world over, to develop alternate methods of farming which are environment-friendly and economically viable. At this juncture, Kerala State Biodiversity Board through Directorate of Environment and Climate Change launched a demonstration project known as "*Granary of Kerala*" in Palakkad district in 2009 to conserve biodiversity associated with agroecosystems. Perhaps in no other Indian state such effects were made till-date.

Moreover, studies on the impacts of historically and currently-used-pesticides on agroecosystem components are scarce in Kerala. Hence, present study was carried out with the following objectives;

### 1.2 Objectives

1. Document residues of major group of pesticides such as organochlorines, organophosphates, synthetic pyrethroids and carbamates in select components, namely sediment, crab, snail, fish, frog, paddy, fodder and cow-milk of an agroecosystem adopting organic and conventional farming in Padayetti village, Palakkad district of Kerala, India.

2. Understand the variations in pesticide residue levels among select biotic and abiotic components, seasons and years in farms adopting organic and conventional farming practice.

3. Compare the residue levels of individual pesticides and their metabolites or isomers with International and national threshold or reference values to understand the ecological implications.

# LITERATURE REVIEW

# 2. LITERATURE REVIEW

India with 2.4% of the world's geographical area, is sustaining about 16% of the global human population. Notwithstanding high population pressure, the country has emerged from a food-deficit to a self-sufficient status, with three-fold increase in food grain production over a period of 30 years (Joshi, 2009; Naik and Prasad, 2006). Enormous quantities of man-made chemicals are being used in modern agriculture for producing food commodities as efficiently as possible to meet worldwide ever-increasing food demands (Clarke *et al.*, 2013; Ma *et al.*, 2007; Bhatnagar, 2001). These chemicals include fertilizers, pesticides and essential synthetic nutrients. Among these groups of chemicals, pesticides have proved to be the most notorious due to their residues in all the environmental components. Although pesticides must be lethal to pests, unfortunately they do not discriminate non-targeted organisms, including man.

While studies on assessing the impact of legendary pesticides on agroecosystem components are scattered in India, there are only a few studies available on the residue levels of currently used pesticides in a select agroecosystem components (Arora *et al.*, 2014, 2008; Kittusamy *et al.*, 2014; Muralidharan *et al.*, 2014; Sharma *et al.*, 2011; Gurushankara *et al.*, 2007; Naik and Prasad, 2006). An effort has been made to bring together the relevant information available in India and only pertinent studies in the International context have been referred.

In India the first report of poisoning due to pesticides was from Kerala in 1958, where over 100 people died after consuming wheat flour contaminated with Parathion (Karunakaran, 1958). This prompted the government to form a Special Committee on Harmful Effects of Pesticides under the Indian Council of Agricultural Research (ICAR) to investigate problem (Wadhawani and Lall, 1972).

Organochlorine compounds such as PCBs, DDTs and cyclodiene groups are among the most widely known contaminants because of their ubiquity, potential for magnification in the food chain and harmful biological effects. Many researchers have reported elevated

levels of OCPs in air, soil, surface and groundwater, benthic macro-invertebrates, fishes, crabs, snails, frogs, birds, fruits, vegetables, food-grains, fodder, milk, milk-products, eggs, honey and meat in India (Ravikumar *et al.,* 2013; Srivastava *et al.,* 2013, 2011; Kaushik *et al.,* 2011; Muralidharan *et al.,* 2011, 2009; Kumar *et al.,* 2006; Babu *et al.,* 2003; Monirith *et al.,* 2003; Muralidharan, 2000, 1993; Raina and Padmaja, 2000; Kannan *et al.,* 1997a,b, 1992; Agnihotri *et al.,* 1996; Ramesh *et al.,* 1991; Kaushik *et al.,* 1987).

Among chlorinated pesticides, DDT is the most important one, being effective in both agricultural pest and disease control until the appearance of resistant lineages. DDT's effects on reproduction were due to its estrogen agonistic and neurotoxic activities. Dry commodities such as tea, chilli and condiments have also been reported with pesticide residues (Greenpeace India, 2014; Kottiappan *et al.,* 2013; Seenivasan and Muraleedharan, 2011). The OCPs due to then estrogenic properties, increase risk of cancer, inhibit testicular synthesis of androgens, cause congenital birth defects, reproductive health problems, loss of immunity, neurological and neurobehavioral problems and also alter sex ratios. Impacts on male reproductive health include reduced sperm quality and count, testicular damage and delayed sexual maturity. Some laboratory studies proved that OCPs affect the red blood cells, thyroid, kidneys and the developing foetus in animal models. They are hepatotoxic, genotoxic, mutagenic, inhibitors of immune function, inducers of malignant neoplasms and lymphosarcomas in rats (Khan and Sinha, 1996).

Varying levels of Endosulfan residues were recorded in environmental components (soil/ and water) from affected villages of Kasargod district, Kerala, where state-owned Plantation Corporation of Kerala aerially had sprayed Endosulfan to control tea-bug in cashew plantations spreading across 15 panchayats between 1978 and 2001 (CSE, 2011).

Organophosphorus compounds are one of the most widely used pesticides in the world replacing the OCs. OP pesticides have a common mechanism of action even though each of their chemical structure varies in nature. (Chandrasekara and Pathiratne, 2005). Responses to OP insecticides by aquatic organisms are broad ranged depending on the compound, exposure time, water quality, sex, age and the species (Narra *et al.,* 2011).

Chlorpyrifos is a broad spectrum OP pesticide used extensively in India for the management of domestic and agricultural pests. Chlorpyrifos was commercially used for more than a decade to control foliar insects that affect agricultural crops, and subterranean termites (Narra *et al.,* 2011). Chlorpyrifos is the second largest selling insecticide in India. Surprisingly it was found that 4.8 ng/g of Chlorpyrifos residues were reported to be present in soft drinks which are 47 times higher than the permissible limits as per Bureau of Indian Standards (CSE, 2006). Its release into the aquatic environment leads to toxic effects to non-target aquatic species (Narra *et al.,* 2011). It is a highly toxic pesticide for aquatic animals even at low concentrations (Humphrey *et al.,* 2004). A number of workers reported on the toxicity of Chlorpyrifos on different aquatic animal models indicating that it is a potent neurotoxicant (Sturm *et al.,* 2007; Rao *et al.,* 2005). There is a wide range of half-lives in soil reported in the literature for Chlorpyrifos, ranging from a few days to 4 years, depending on application rate, ecosystem type and various environmental factors (Gebremariam *et al.,* 2012).

Pyrethroids are synthetic analogs, structural derivatives of the naturally occurring pyrethrins, which are known for their high bioactivity against a wide range of insect pests and their non-persistency in the environment. Several of the earlier SPs were successfully commercialized mainly for the control of household insects, and newer pyrethroids have been introduced as agricultural insecticides. Pyrethroids are widely used on paddy in many countries (Lawler *et al.,* 2003; Dennett *et al.,* 2003; Biever *et al.,* 2003; Das *et al.,* 1995; Haddad *et al.,* 1989), but their application in paddy agroecosystem has been prohibited in some countries such as China because of its high toxicity to aquatic organisms (Koprucu and Aydin, 2004; Weston *et al.,* 2004). No such regulations are adapted in India. Both pyrethroids and pyrethrins are extremely toxic to aquatic organisms, including fish such as the Blue Gill and Lake Trout, with $LC_{50}$ values less than 1.0 µg/L (Mueller-Beilschmidt, 1990). The $LC_{50}$ levels are similar to the application rates for mosquito, blackfly, and tsetse fly larvae, often the actual target of the pyrethroid application. Two pyrethroids with α-cyano substitution of the phenoxybenzyl alcohol moiety, Cypermethrin and Fenvalerate are more toxic to aquatic organisms, with their 96-h $LC_{50}$'s for salmon, lobsters and shrimp being 2.0, 0.04, and 0.01 µg/L, respectively (Mueller-Beilschmidt, 1990). However, the real environmental risk of any pesticide is not

only determined by the toxicological data but also by their application rate, environmental fate and metabolism in the ecosystem (Rand and Clark, 2000). The use of pyrethroid insecticides is increasing for agriculture, commercial pest control, and residential consumer use. In addition, there is a trend toward the use of newer and more potent compounds. Little is known about the toxicity of sediment associated pyrethroid residues to aquatic organisms, yet recent work has shown that they commonly are found in aquatic sediments (Amweg *et al.*, 2005).

The SPs provide highly efficacious control of paddy field arthropod pests at low dose and present low toxicity to birds and mammals and thus low risks to applicators. Pyrethroid's toxicity is highly depended on its stereochemistry, each isomer (molecules consisting of the same atoms, but with different stereochemistry) has its different toxicity (Liu *et al.*, 2005a). λ-Cyhalothrin is highly toxic to fish, and even more toxic to the arthropod and shrimp. Moreover, it had short persistence both in water and soil (Laskowski, 2002). Due to the lipophilicity, SPs have a high rate of gill absorption. So they have been reported to be extremely toxic to fish (Benli *et al.*, 2009; Saha and Kaviraj, 2008; Polat *et al.*, 2002).

According to Gu *et al.* (2007), although λ-Cyhalothrin exhibits high acute toxicity to aquatic organism such as crab and fish, but it degrades quickly in the aquatic environment. Hence, application of suspension concentrate (SC) of λ-Cyhalothrin is much safer than emulsion in water (EW), wettable powder (WP), emulsifiable concentrate (EC), and microemulsion (ME) formulations should be used to reduce the environmental risks (Gu *et al.*, 2007). In agricultural soils, Fenvalerate is tightly adsorbed to soil particles, does not easily move laterally or to lower soil layers with groundwater, and almost always remains in the application site because of its extremely low solubility in water (Miyamoto, 1988; Hill, 1981). Studies have indicated that SPs are highly toxic to a number of non-target organisms such as honeybees, aquatic arthropods, fishes and amphibians even at low concentrations (Palmquist *et al.*, 2011). The known effects of SPs on frogs at sublethal concentrations include incoordination, lack of limb control, hyperactivity, tremors and writhing. Tadpoles of leopard frog had exhibited convulsions and twitching following sublethal (1.3 μg/L) Fenvalerate exposure, with behavioral homeostasis re-established only one week after removal to clean systems (Materna *et al.*, 1995).

Carbamate insecticides have a half-life of 30-120 days in soil and its break down in sunlight and have a high potential for groundwater contamination (Howard, 1991). Commonly used as an insecticide, nematicide and acaricide for the control of all types of stem borers in rice, sugarcane, fruits and vegetables. Granular formulations of carbamates have been reported to be highly toxic to wildlife and particularly to birds (Otieno *et al.,* 2009; Mineau, 1993; Flickinger *et al.* 1980). While one of the carbamates, Carbofuran ingestion in small doses show symptoms such as nausea, headache, eye irritation, muscle weakness, vomiting, excess salivation and ingestion may lead to death in birds (Elliott *et al.,* 2008). In 2006, US EPA identified significant dietary, ecological and worker risks from the use of Carbofuran in granular form. EPA estimated that prior to cancellation of the granular formulation, up to two million birds were killed each year by Carbofuran. In general, Cb insecticides rapidly inhibit cholinesterase via carbamylation of the enzyme, killing the exposed animal as a result of neuromuscular and central nervous system (CNS) blockage (Hernandez and Margalida, 2009).

### 2.1 Status of Organochlorine Pesticides in India

Although India is in the process of phasing out persistent OC pesticides, a few are still being used in substantial quantities either for agriculture or civic health. For example, DDT which has been banned for agriculture in India in 1989, but still it is being used, in public health programmes, especially for malaria eradication (Agarwal, 2001). Although it has been limited to an annual ceiling of 10,000 MT till an acceptable substitute is identified, DDT could continue to pose problems to the environment. Although technical HCH is banned in many countries, such as Canada, the USA, and Mexico, use of HCH continued unabated until it was banned in 1997 in India, but restricted use of lindane (99% $\gamma$-HCH) was allowed till 2011. However, now in India, HCH is totally banned for manufacture, import or formulate and use (http://cibrc.nic.in/retrieved on 17$^{th}$ April 2013). Endosulfan is an off-patent OC insecticide and acaricide that is being phased out globally. Endosulfan became a controversial agrochemical due to its acute toxicity, potential for bioaccumulation, and role as an endocrine disruptor (Malik, 2013). Because of its threats to human health and the environment, a global ban on the manufacture and use of Endosulfan was negotiated under the Stockholm Convention in April 2011. It

may be noted that Kerala government banned Endosulfan way back in 2002 particularly in Kasargod district. More than 80 countries, including the European Commission, Australia, New Zealand, several West African nations, the US, Brazil and Canada had already banned it or announced phase outs by the time the Stockholm Convention's ban was agreed upon. The Supreme Court of India has banned Endosulfan in the country with effect from May, 2011, with certain exemptions for five additional years (Malik, 2013).

Despite the proliferation of different types of new generation pesticides, OCs are sure to be of concern for many more years to come. Based on the information that we have gathered over the last two decades, impacts of pesticides on birds, including many threatened species, are abundantly clear. We have proof beyond doubt for a few species of birds, while there are indications of toxic effects in other cases. Long-term effects of secondary exposure to many new generation pesticides need to be evaluated as these chemicals present great risks to the wildlife. If the present trend of pesticide usage continues unchecked, birds will certainly be one of the worst affected non-target organisms. Since agriculture has a place of prime importance in India's economy, large scale and unscrupulous use of pesticides in this sector is likely to continue, which might lead to many adverse effects not only to birds, but also man (Muralidharan *et al.*, 2014).

**2.2 Surface and groundwater contamination**

The industrialization, agricultural activities and urbanization have led to deterioration of water quality due to various contaminants. Pesticides can reach surface water through runoff from treated plants and soil. Presence of OC and OP residues in surface and groundwater of Kanpur (Sankararamakrishnan *et al.*, 2005), OCs in River Gomti (Mudiam *et al.*, 2012; Singh *et al.*, 2005a) and groundwater aquifers of Gangetic plains (Singh *et al.*, 2005b), Uttar Pradesh were reported. Around 58% of drinking water samples collected from hand pumps and wells in Bhopal were contaminated with OCs above the EPA standards (Kole and Bagchi, 1995).

The threat of pesticides in groundwater is that any toxic chemical present will reach the drinking water system, which may prove to be harmful for human consumption (Pankaj

*et al.*, 2012). Rao *et al.* (1994) reported residues of DDT, HCH and Carbofuran associated with high organic contents in the waters of Ooty Lake. Vijayan and Muralidharan in 1999 conducted a study on pesticide contamination in sediment, water, fishes and birds collected from major water bodies of Nilgiri district in Tamil Nadu, India. Mahapatra *et al.* (1995) found that the groundwater from Farrukhabad in the vicinity of River Ganges was contaminated with OCs, namely ∑-DDT (BDL-1.657 ng/mL), Dieldrin (BDL-0.908 ng/mL) and Heptachlor (BDL-0.129 ng/mL) were higher than their respective MRL values in some samples of drinking water. Bakre *et al.* (1990) monitored the OCPs in water in Mahala reservoir, Jaipur. The OC insecticides in water samples ranged between 1.07 and 81.23 ng/mL. γ-HCH was the most frequently detected pesticide (91%, 0.10 to 44.72 ng/mL) followed by α-HCH (88%, 0.11 to 20.12 ng/mL).

The Centre for Science and Environment (CSE, 2006) reported residues of 32 most commonly used pesticides in India (16 OCs, 12 OPs and 4 SPs) in carbonated waters of Pepsi and Coca-Cola. Recent study by Pankaj *et al.* (2012) reported the HCH, DDT, Dicofol, Endosulfan and Chlorpyrifos residues in cold drinks and groundwater collected from Delhi. Once groundwater is polluted with toxic chemicals, it may take many years for the contamination to dissipate or be cleaned up. Cleanup may also be very costly and complex, if not impossible (USEPA, 2002). The present study did not measure residues in water due to constraints; it was decided to study sediments.

### 2.3 Soil/ sediment contamination

Practically sediments act as a sink to the contaminants present in the over lying water. Quality of aquatic sediment is critical to assess the health of aquatic ecosystems. The study of sediments can provide historical records of both the natural background and man-induced accumulation of elements and compounds over extended period of time (Malik *et al.*, 2009; Li *et al.*, 1999). A large number of transformation products (TPs) from a wide range of pesticides have been documented (Gomez *et al.*, 2011; Roberts and Hutson, 1999). Persistency and movement of these pesticides and their TPs are determined by some parameters, such as water solubility, soil-sorption constant ($K_{oc}$), octanol/ water partition coefficient ($K_{ow}$), and half-life in soil ($DT_{50}$). Pesticides and TPs

could be grouped into (a) Hydrophobic, persistent and bioaccumulable pesticides those are strongly bound to soil. Pesticides that exhibit such behavior include the organochlorines such as DDT, Endosulfan, Endrin, Heptachlor, Lindane and their TPs. Most of them are now banned for agriculture but their residues are still present. (b) Polar pesticides are represented mainly by herbicides but they also include carbamates, synthetic pyrethroids, fungicides and some organophosphorous insecticides and their TPs. They can be moved from soil by runoff and leaching, thereby causing problem for supply of safe drinking water to the population. The most researched pesticide TPs in soil are undoubtedly those from herbicides. Pesticides and their TPs are retained by soil to different degrees, depending on the interactions between soil and pesticide properties. The most influential soil characteristic is the organic matter, the higher the organic content, the greater the adsorption of pesticides (Andreu and Pico, 2004).

Ramesh *et al.* (1991) studied the HCH and DDT residue levels in the paddy and sediment samples collected from the Vellar River water-shed in Tamil Nadu, India from 1987 to 1989. HCH showed higher levels in soil during wet season, while DDT residues were low and did not show a significant seasonal trend in soil or sediment. Sarkar *et al.* (1997) reported the OC levels in marine and estuarine sediments along the west coast of India. The concentration of various pesticides in sediments from the mouth of different estuaries along the coast were in the range of 0.85-7.87 ng/g (∑-HCH), 0.10-0.26 ng/g (Aldrin), 0.70-3.33 ng/g (Dieldrin), and 47-25.17 ng/g (∑-DDT). Among the OCPs, ∑-HCH and ∑-DDT were the most dominant in both the estuarine and offshore sediments. Rajendran *et al.* (2005) revealed HCH and DDT in sediment samples collected from six locations along the east coast of India. Sediment from Chennai harbour contained higher concentration of all the OCs. Based on sediment quality guidelines, DDTs and γ-HCH levels were above the threshold levels in some sediments of Bay of Bengal.

Nhan *et al.* (2001) determined the levels of OCs in sediments and freshwater mollusc *Angulyagra* sp. from canals in the region of Hanoi city, Vietnam. Levels of ∑-DDT in sediment ranged from 7 to 80 ng/g and from 6 to 864 ng/g in the soft tissues of mollusc. The concentrations of ∑-DDTs were higher in populated sites and much lower in rural sites, indicating that the DDT has been used for mosquito control and not only as a crop

protection chemical. Taking into consideration the high DDT levels measured in these molluscs, their consumption by man is worrisome and may expose the population to high levels of endocrine disrupting substances.

Babu *et al.* (2003) reported the OCs in samples of soil and rice plants collected from ten different villages of a well-known Basmati rice growing area in Dehradun, India. Residues of both DDT and HCH residues were found in all samples of soil and different parts of rice plants except for a few grain samples. The levels of DDT and HCH in grains were well below the maximum residue limit of 0.1 mg/kg for DDT and 0.05 mg/kg for HCH prescribed by the Government of India and WHO/FAO. They concluded that the pesticide residue levels in this export commodity are not of hazardous nature.

Barlas *et al.* (2002) found levels of OCs found to be more in sediments than water samples from Uluabat Lake, Turkey. Amweg *et al.* (2005) found acute toxicity and growth impairment on amphipod *Hyalella azteca* for six pyrethroids in sediments. In the order of decreasing toxicity of sediment-associated residues, the compounds tested were Bifenthrin (average 10-d $LC_{50}$: 50.18 mg/g), λ-Cyhalothrin (0.45 mg/g), Deltamethrin (0.79 mg/g), Esfenvalerate (0.89 mg/g), Cyfluthrin (1.08 mg/g), and Permethrin (4.87 mg/g).

Arora *et al.* (2008) studied soil, water and rice grains from field trials conducted under the Integrated Pest Management (IPM) and non-IPM modules in Kaithal, for Pendimethalin, Atrazine, Lindane and Chlorpyrifos residues, and in Dehradun, Uttarakhand, samples were analyzed for Carbendazim residues respectively. The pesticide residues were found below the detection limit in the soil and water samples of the Kaithal region. From Dehradun region the residues of Carbendazim in rice grains were detected at 0.001 mg/kg level, and in soil they were in the range of 0.03 - 0.001 mg/kg.

Rama Krishna and Philip (2008) studied the adsorption and desorption characteristics of three insecticides on four types of Indian soils. The order of adsorption of pesticides on soils was: Lindane > Methyl Parathion > Carbofuran. Compost soil had shown the maximum adsorption capacity. The order of adsorption capacity of various soils was:

Herbicides can also be toxic to birds. Although trifluralin was considered "practically nontoxic to birds" in studies of acute toxicity, the exposure resulted in diminished reproductive success due to cracked eggs (USEPA, 1996). Exposure of eggs to 2,4-D, reduced successful hatching of chicken eggs (Duffard *et al.,* 1981) and caused feminization or sterility in pheasant chicks (Lutz *et al.,* 1972). Herbicides can also adversely affect birds by destroying their habitat. Effects of some OCs on many species of fish-eating birds and marine mammals have been extensively documented in North America and Europe (Barron *et al.,* 1995; Kubiak *et al.,* 1989). Despite the continuing usage, little is known about the impacts of OCs in bird populations in developing countries. Among the countries that continue to use OCs, India has been one of the major producers and consumers in recent years. As a consequence, wild birds in India are exposed to great amounts of OC pesticides (Tanabe *et al.,* 1998). Use of OCs in tropical countries may not only result in exposure of resident birds but also migratory birds when they visit tropical regions in winter. The Indian sub-continent is a host to a multitude of birds from western Asia, Europe and Arctic Russia in winter (Woodcock, 1980). Hundreds of species of waterfowl, including wading birds such as plovers, terns and sandpipers, migrate each winter to India covering long distances (Grewal, 1990).

Senthilkumar *et al.* (2001) measured levels of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in sediments, aquatic organisms, birds, bird eggs and bat collected from coastal areas of South India. Misra and Bakre (1994) studied the levels of organochlorine in a few biological and non-biological components in Rajasthan. Reports on the impact of agricultural chemicals on agroecosystem components and avifauna are also available (Muralidharan *et al.,* 2004; Rajendran *et al.,* 2003). Muralidharan *et al.,* (2011, 2008) documented the status on the use of pesticides and their impact on birds. Kannan *et al.* (1992) reported that the persistent organochlorine residues in agricultural food stuffs from India and their implications on human dietary exposure. The presence of organochlorines in tissues of Ganga River dolphin was also reported (Senthilkumar *et al.,* 1999). Singh *et al.* (2005a,b) and Singh, (2001) documented organochlorine residues in sediments and groundwater of Gomati River, Agra and Gangetic plains of Uttar Pradesh, India. Ramesh *et al.* (1991) reported the HCH, DDT and other OC residues in sediments of River Cauvery, Tamil Nadu. Pillai, (1986) reported that

the pesticide residues in soil, water and air in Delhi area, India. Guzzella *et al.* (2005) reported total HCH, DDT, HCB, PCB and PAH in the sediments of Hugli estuary, West Bengal, Northeast India. Hans *et al.* (1999) reported the OC pesticide residues in agricultural produce (paddy and wheat straw) from dry bed of the River Ganges, Kanpur. Sankar *et al.* (2006) revealed the organochlorine residues and heavy metals in fishes and shellfish from Calicut region, Kerala, India. Kumari *et al.* (2001) and Kannan *et al.* (1992) reported high concentration of HCH in fish and food products and higher dietary intake through fishes to man.

Amaraneni, (2002) determined select OC and OP pesticides in water, sediment and fish from 12 aquaculture farms in Kolleru Lake, Andhra Pradesh, India. Results indicate that the pesticide levels in fish decreased in the order of sediment > fish > water. The pesticide levels in fish on a wet weight basis were 0.5 mg/kg for α- and γ-HCH, 1 mg/kg for Malathion, Chlorpyrifos and Dieldrin, 2 mg/kg for Endosulfan and 5 mg/kg for *p,p'*-DDT. These levels were higher than the standards for human consumption set by various statutory agencies.

An extensive study on inland wetlands of India by Vijayan *et al.* (2004) documented threats and conservation issues in many wetlands across the country. The study pointed out two overwhelming issues of immediate concerns: huge loss of wetlands and contamination of the remaining ones. Compounding to the loss of wetlands is that almost all the wetlands studied from 14 states in the country were contaminated at varying levels. About 80% of fishes from the wetlands in the country, even from relatively remote regions had residues of HCH, while Heptachlor Epoxide was detected in about 78% and Endosulfan in 66% of the wetlands studied. The most persistent DDT and its homologs (*p,p'*-constituents) were detected in 76% of fishes. In many cases, the fishes were not fit for human consumption, because of the high levels of heavy metals or pesticides. It is a cause for great concern that not even one of the several hundred fishes studied from 115 wetlands was free from pesticides or heavy metals.

Kaur *et al.* (2008) reported the OCs in five freshwater fishes from Punjab, India. DDTs were the predominant OC contaminants in all species with *p,p'*-DDT and *p,p'*-DDE as the

main pollutants. HCH isomers and Dieldrin were also found at lower levels in fish species. The α-HCH was the dominant isomer of HCH in all fish species followed by γ-, β- and δ-HCH. The residues of Aldrin, Chlordane, Heptachlor and Endosulfan were BDL in many fish samples. Levels of OCs detected in present study were below the tolerance limits recommended by national and International agencies.

Peter, (2008) studied the concentration of OC residues in the muscle tissues of 164 fishes comprising five species collected from Sewri, Mahul and Nhava, Mumbai. Total HCH residues were the highest (673 ng/g) in *Otolithes rubber*. *Coilia dussimierie* had the maximum concentration of ∑-DDT (61.7 ng/g).

Dhananjayan and Muralidharan, (2010) reported OCP residues in fishes collected from different inland wetlands in Karnataka, India and their suitability for human consumption. Isomers of HCH were most frequently detected with β- and γ-HCH as the main pollutants. Among various fish species tested, the highest pesticide burden was recorded in *Anguilla bicolor bicolour* (77.9 µg/kg) and the lowest in *Heteropneustes fossilis* (2.1 µg/kg). OCPs detected in the referred study were well below the tolerance limits recommended for fishes. The calculated daily dietary intake of OCPs in all the species examined was lower than the maximum acceptable dietary intake (ADI) limits prescribed for human consumption. However, continuous monitoring is recommended to facilitate the early identification of risks.

Jayanthi, (2012) reported OC pesticide residues in select species of commercially important marine fishes, received from Cochin and Rameshwaram, and sold at Coimbatore market. Among the species of fishes analysed, concentration of γ-HCH and *p,p'*-DDT were higher in *Scoberomorus commersonn* (4.8 ± 1.0 ng/g) and *Sardinella longiceps* (13.2 ng/g), respectively. However, Endosulfan residues were detected at lower concentrations (< 2.2 ng/g). Based on the human consumption survey, the daily per capita consumption of marine fishes in Coimbatore city was 47 g/ day. Based on this, the average dietary intake concentration of organochlorines irrespective of the species preference was estimated to be 4.31 µg/person/day. Higher dietary intake of OC residues mainly through *Scoberomorus commersonn* (5.65 µg/person/day) followed by *Sardinella*

*longiceps* (4.73 µg/person/day) was reported. These are the species which are the most preferred and highly consumed by the local people. These fishes are rich in fat content and thereby accumulate higher quantum of residues. Calculated dietary intake values were below the ADI limits prescribed by existing statutory guidelines (European Commission, 2000; FDA, 2001, 2000; Health Canada, 1997).

The impact of endocrine-disrupting chemicals in the environment is also of special concern in amphibians, which are declining globally (Adams, 1999; Bishop *et al.,* 1999). Synthetic pesticides and agricultural chemicals are known to cause malformation or death of frogs (Kiesecker, 2002; Sparling *et al.,* 2001), altered sexual development, deformed limb development (Kayes, 2013) when present in sufficient concentrations. In an experimental study, mortality of frogs, fishes and other aquatic lifes was reported to have been caused by combination of pesticides, namely Chlorpyrifos, Atrazine and Metolachlor (USDA-ARS, 2003). Thus, agrochemical contamination of surface water is considered to be one of the causative agents for population decline and deformities in amphibians. Although information is available on acute toxicity of many pesticides to amphibians, their effects on long-term basis are not adequately studied.

Sparling *et al.* (2001) reported drastic population declines of amphibians in the Sierra Mountains, east of the Central Valley and downwind of San Joaquin Valley California, United States using *Hyla regilla* as a sentinel species, we found that cholinesterase (ChE) activity in tadpoles was depressed in mountainous areas, east of the Central Valley compared with sites along the coast or north of the Valley. Cholinesterase was also lower in areas where ranid population status was poor or moderate compared with areas with good rain fall. Up to 50% of the sampled population in areas with reduced ChE had detectable OP residues, with concentrations as high as 190 ng/g wet weight. In addition, up to 86% of some populations had measurable Endosulfan concentrations and 40% had detectable *p,p'*-dichlorodiphenyldichloroethylene, *p,p'*-DDT, and *o,p'*-DDT residues.

Sundar *et al.* (2010) reported the presence of OCs in Green Mussel *Perna viridis* and water samples collected from Ennore creek, Chennai, India. Mussel samples showed very low concentrations of OCPs in the order of DDT (5.83 ng/g) > Endosulfan (2.84 ng/g)

> HCH (2.34 ng/g). Concentrations of OCPs in water samples were in the order of Endosulfan (29.21 ng/L) > HCH (17.14 ng/L) > DDT (14.63 ng/L). Study recommended that continuous monitoring in creek is necessary to assess possible impact on human health.

Measurement of chemicals in human diet provides the best estimates of the exposure and potential risk. The risk of consumers may then be evaluated by comparison with toxicologically acceptable intake levels (Kashyap *et al.,* 1994). Fatty food was the main source of these contaminants. Chlorpyrifos, a common contaminant of urban streams (USGS, 1999), is highly toxic to fish, and has caused fish kills in waterways near treated fields or buildings (USEPA, 2000a).

Herbicides can also be toxic to fish. Weed-killers such as Ronstar and Roundup are also toxic to fish (Shafiei and Costa, 1990). In addition to direct acute toxicity, some herbicides may produce sublethal effects on fish that lessen their chances for survival and threaten the population as a whole. Glyphosate or glyphosate-containing products can cause sublethal effects such as erratic swimming and laboured breathing, which increase the chance of fishes being eaten by predators (Liong *et al.,* 1988). 2,4-D an herbicide is reported to have caused physiological stress responses in Sockeye Salmon (McBride *et al.,* 1981) and reduced food-gathering abilities in Rainbow Trout (Little *et al.,* 1990).

Pazou, (2014) reported the OC residues in sediment, fish, shrimp and oyster species collected from Lake Nokoue and Cotonou Lagoon in the Republic of Benin. The main pesticides identified in sediment were *p,p'*-DDT and its metabolites *p,p'*-DDE and *p,p'*-DDD, with residue levels ranging up to 24.4 ng/g. Fish species commonly consumed such as *Elops lacerta*, *Podamasys jubelini*, *Gobbienellus occidentalis*, *Ethmalosa fimbriata*, *Mugil cephalus* and *Hemichromis fasciatus* were contaminated with residues of nine pesticides, namely *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDT, *p,p'*-DDT, α-Endosulfan, Aldrin, Dieldrin and γ-HCH. The levels ranged up to 289 ng/g. The same pesticides were also detected in other aquatic species, such as shrimp and oysters. A summed risk assessment, comparing pesticide intake levels through fish consumption with tolerable daily intake levels proposed by the World Health Organization, showed in all cases a low risk for human health.

Gebremichael *et al.* (2013) studied OCP residues in human and cow milk in the towns of Asendabo, Serbo and Jimma in South-Western Ethiopia. They found that mean levels of ∑-DDT in the human and cow-milk samples in the three areas were 12.68, 4.38 and 0.389 µg/g respectively. The proportion of *p,p'*-DDT was the highest in all the three cases, comprising 55-71% of ∑-DDT, followed by *p,p'*-DDE, 26-39%, and the least, *p,p'*-DDD of 2-5%. The mean ratio of DDT/ DDE concentration for the three areas was calculated to be 2.01%. This value was much higher than the values reported from other countries in earlier studies and indicates the existence of a higher quantity of DDT from a fresh input in the three study areas. The mean estimated daily intake of DDT by infants from mother's milk in the three locations was found to be 62.17 µg/g body weight, which is about three times higher than the acceptable daily intake set by WHO/FAO for ∑-DDT, 20 µg/g of body weight. This alarmingly high daily intake value is a cause for concern, since children are highly susceptible to effects from such environmental contaminants. The study has revealed that people in the study areas are facing exposure to DDT from recent use. The observed contamination of mother's milk and the possible transfer of the contaminant from mother to child is an obvious risk associated with breast-feeding in the study areas and possibly in other parts of the country too.

The present study aims to monitor the residues of pesticides in organic and conventional farms in Padayetti village, Palakkad district, India. Samples of components, namely sediment, fish, crab, frog, snail, paddy, fodder and cow-milk were collected from organic and conventional paddy agroecosystem on seasonal basis and processed for multiresidue estimation.

# MATERIALS AND METHODS

# 3. MATERIALS AND METHODS

## 3.1. Study area

Kerala, the southernmost state of India, lies between 8° 18′ and 12° 48′ N latitude and 74° 52′ and 77° 24′ E longitude. The total geographical area of the state is 38,863 $km^2$ ($17^{th}$ in area among the states of India). Palakkad district is one of the 14 districts of Kerala with the area of 4,480 $km^2$. About 1,360 $km^2$ of land is covered by forests which accounts for 11.5% of the state's area (http://en.wikipedia.org /wiki/Palakkad_district). Palakkad is the gateway to Kerala due to the presence of the Palakkad Gap, in the Western Ghats. This district is bordered on the North-West by Malappuram district, on the South-West by Thrissur district and on the East by Coimbatore district of Tamil Nadu.

The net cultivable area in Palakkad district which is referred as "Granary of Kerala" is 2,18,336 ha i.e. around 49% of the total geographical area. Major portion of the cultivable area is used for raising food crops. Paddy is cultivated in about 1,07,467 ha (Dept. of Agriculture, 2011 http://palakkad.nic.in/agri.htm). About 80% of the rural population of this district are agriculturists or agricultural labourers. Annual production of rice in the district is 2,37,800 tons out of the total production of 7,26,800 tons in the state, i.e. Palakkad produces about $1/3^{rd}$ of the total production of rice in the state.

The study was conducted in Padayetti village, Palakkad district of Kerala. The area selected for the present study is around 100 acres of paddy field adopting both organic and conventional farming (50 acres each) situated in a valley sandwiched between hills. The organic farming area lies within the longitude of 10° 41′106″ N and latitude of 76° 32′ 900″ E and conventional farming lies within the longitude of 10° 40′ 615″ N and latitude of 76° 32′ 289″ E (fig. 3.1). This hamlet was particularly chosen because the organic farming area is considerably elevated than the conventional farming area which prevents spray drifts (subject to wind pattern) and surface runoffs, but contiguous (fig. 3.1). Human settlements are about 300-500 m away from the farms on a higher elevation. There are also plantations of rubber, coconut and orchards of mango, jack fruit, sapota

and guava located nearby. Crops such as ginger, red gram, tapioca, banana and vegetables such as brinjal, chilli, okra, ash gourd, bottle gourd, snake gourd and little gourd are also cultivated in the village without any chemical input.

Padayetti village is predominantly rural with an agriculture-based economy. The climate of the village is classified into three seasons. Under rain fed condition, farmers cultivate two crops. Period from March to end of May is the summer season. This is followed by South-West monsoon season, which continues till middle of October (*Kharif*, first cropping season: locally known as *Viruppu*). From the middle of October begins the North-East monsoon season, which lasts up to the end of February (*Rabi*, second cropping season: locally known as *Mundagan*). The climate is pleasant from September to February. Summer months (March to May) are uncomfortable due to high temperature and humidity. The main water source is atmospheric precipitation during South-West and North-East monsoons. The greater portion of the rain is from South-West monsoon which starts between mid-June and lasts up to September. Both the crops are cultivated under rain fed conditions. *Viruppu* crop generally starting as a dry broadcast crop (direct sowing) ends up in wet conditions. The varieties grown have such plasticity that they are conditioned to grow under a wide spectrum of moisture regimes from moist seedbed to standing water. *Mundakan* (winter) crop is generally a transplanted crop in which rice plant grows under continuous wet conditions. The fag end of the crop might be subjected to moisture stress if the North-East monsoon tapers off suddenly (Devi, 2007).

Average annual rainfall is around 1200 mm, and comes largely from the South-West monsoon. *Thula varsham*, the North-East monsoon, unlike other parts of Kerala, is negligible here. Temperature is moderate (21 - 36°C) and humidity is very high. Mean monthly maximum temperature varies from 29°C (July) to 38°C (April) and mean monthly minimum temperature varies from 21°C (July) to 24°C (December). The mean monthly maximum humidity is 95% (July - August) and the mean monthly minimum is 84% during April and May.

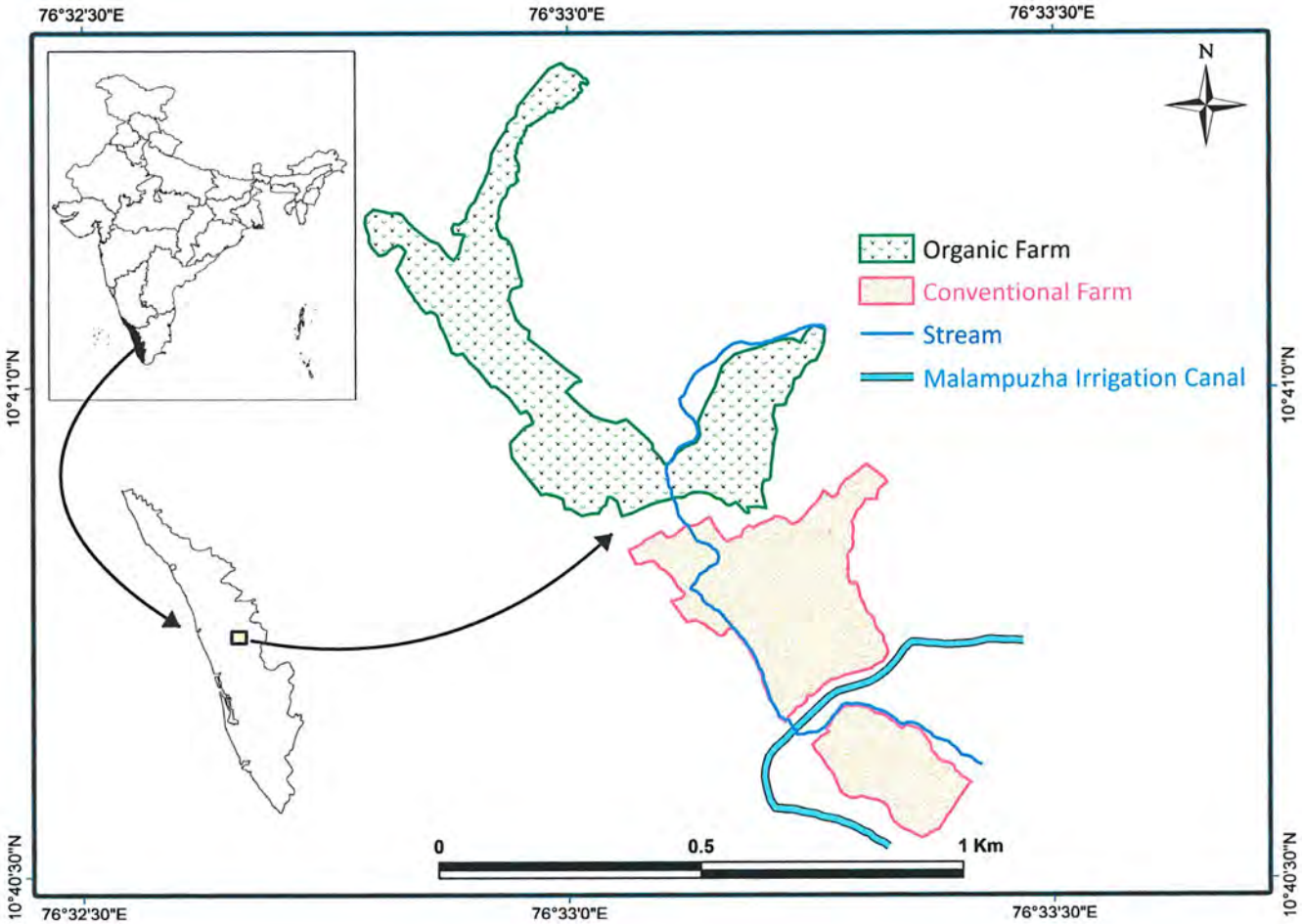



Fig. 3.1. Digital map of the study area: Padayetti village, Kerala, India

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## 3.2. Methodology

### 3.2.1. Field survey

Questionnaire survey was conducted among the farmers to know the farming practice and chemicals used. During the survey, information on pesticide application, source, frequency of application, chemical group, quantity, mode of application, cropping pattern and common crop-pests were collected, and complied. In addition to documenting the pesticides used presently, information on the past-use was also gathered. Information collected were recorded on pre-formatted data sheet (table 3.1).

### 3.2.2. Sample collection

Samples of sediment, crab, snail, fishes, frogs, paddy, fodder and cow-milk (fig. 3.2a-n) were collected seasonally (Season I: *Kharif* (*Viruppu*): mid May to mid October; Season II: *Rabi* (*Mundagan*): mid October to end February, and Season III: Summer: March - May) from both organic and conventional farms (table 3.2) between 2009 and 2012, to quantify pesticide residues. Choice of species was based on availability and abundance in the study area throughout the year.

Sediment/ soil samples were collected and transferred into pre-labelled zip-lock pouches. Samples were kept on ice until they were brought to the laboratory, where they were homogenized manually, large debris (e.g. gravel, sticks) removed, and frozen at 4°C until extraction was performed. Freshwater snail, *Pila globosa* and paddy field crab, *Geothelphusa dehaani* were collected by hand-picking in the paddy fields. Both snail and crab are found abundantly and are widely distributed in the study sites. They largely feed on detritus. Since the freshwater field crabs and snails are smaller, entire body tissues except shells were used for pesticide analysis. As far as fish samples are concerned, five commonly available species, namely Spotted Snakehead *Channa punctatus*, Climbing Perch *Anabas testudineus*, Spot-fin Barb *Puntius sophore*, Long-snouted Barb *Puntius dorsalis* and Mrigal *Cirrhinus mrigala* were collected using gill net. Muscle tissues were used for residue estimation. Sometimes when

we could not get larger fishes, small fishes were pooled and analysed for pesticide residues. List of components included in the study are shown in fig. 3.2a-n.



3.2a. Sediment



3.2b. Freshwater field crab *Geothelphusa dehaani*



3.2c. Freshwater snail *Pila globosa*



3.2d. Mrigal *Cirrhinus mrigala*



3.2e. Spotted Snakehead *Channa punctatus*



3.2f. Climbing Perch *Anabas testudineus*



3.2g. Spot-fin Barb *Puntius sophore*



3.2h. Long-snouted Barb *Puntius dorsalis*



3.2i. Mrigal *Cirrhinus mrigala*



3.2j. Jerdon's Bullfrog *Hoplobatrachus crassus*



3.2k. Indian Rice Frog *Fejervarya limnocharis*



3.2l. Paddy *Oryza sativa*



3.2m. Paddy straw (fodder)



3.2n. Cow-milk

**Fig. 3.2a-n: List of components included for the multiresidue analysis**

Frog samples were collected by nocturnal visual encounter survey (VES) which yielded greater number of individuals and species per unit area (Crump and Scott, 1994). Observations were made fortnightly between 18 00 and 20 30 hrs. Belt transects (50x4m) for VES were placed randomly and four observers walked inside the flooded paddy fields with light-emitting diode (LED) torch (Britelite) and each member visually searched the entire 4m width of the transect. During each survey, 150 min were spent in an area of 2.5 ha. Identity of the frogs was confirmed referring to Daniels (2005).

Samples were cleaned, morphometric measurements recorded, labelled and packed in clean polythene bags, transported over ice in thermal insulated box to the laboratory. On reaching laboratory, tissue samples were minced into small pieces and about 10 g of the homogenate was weighed separately using a top loading electronic balance (Mettler AE420) and transferred to clean specimen vials for pesticide analysis and stored in deep freezer at -20°C till the processing was carried out.

Three commonly cultivated paddy (*Oryza sativa* L.) varieties, namely *Aishwarya*, *Jothi matta* and *Thavalakannan* were chosen for the study. Paddy and fodder samples were collected soon after harvest from both organic and conventional fields. The samples were transported immediately to the laboratory over thermal insulation box, stored in deep freezer and processed subsequently.

The samples of liquid milk in prewashed-containers were collected during every season from cows fed with feeds grown organically and conventionally. It should also to be noted that we could not collect milk from the same cow for all the 3 years of study.

### 3.2.3. Pesticides chosen for this study

Pesticides considered in the study are compounds which were frequently used in the past in this region as well as being used nowadays, and are associated with health risks.

## Table 3.1. DATA SHEET FOR SURVEY ON PESTICIDE CONSUMPTION

| **Name of the person interviewed:**<br>**Mr. P.S. Sulaiman; Age: 58; (ORGANIC FARMER)**<br>**Education : 8th std; Occupation: Agriculturist** | | | **Date : 13/09/2009** | | **Data Sheet No. 1**<br>**Recorded by: Mr. K. GANESAN** | |
|---|---|---|---|---|---|---|
| **Crop under cultivation** | **Growth stage of** | **Area under cultivation** | **Name of the pest & nature of** | **Whether the crop attracts birds? If so** | **Migrant/local others (specify)** | **Feeding habit of the bird (s)** |
| 1. Paddy : *Jothi matta* | Vegetative | 1.81 acres | Rice leaf-folder, Stem feeder and White leafhopper | Cattle Egret, Indian Pond Heron, Black Drango, Green bee-eater and Indian Peafowl | Local | Insectivores/ Omnivore |
| 2. Rubber | Fully grown | 2 acres | - | - | - | - |
| 3. Coconut, banana, mango, ginger & turmeric | Fully grown | 0.6 acres | - | - | - | - |
| **Pesticide name** | **Source** | **Group** | **Forms** | **Total No. of application during the life span of crop** | **Quantity of pesticide applied per spray (kg/lit/ acre)** | **Total quantity of applied during the life span of the crop (kg/lit/ acre)** |
| Vermicompost , Neem oil, Castor oil, Groundnut oil cakes,<br>Liquid manure, '*Panjakavya*'<br>N: *Azospirillum*<br>P: *Bacillus megatherium*<br>K: *Frateuria aurantia* | Govt: KSBB through Krishi bhavan<br>Pvt: -<br>Others: - | OC: -<br>OP : -<br>SP : -<br>Cb : -<br>Others | E. liquids:<br>Dust:<br>Granules: | -<br>-<br>- | -<br>-<br>- | -<br>-<br>- |

**Table 3.2. Details of samples analysed by GC-MS from organic and conventional farms between 2009 and 2012**

| S.No. | Component | Common name | Scientific Name | No. of samples collected | | | | | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Organic farm | | | | | | | | | Conventional farm | | | | | | | | |
| | | | | 2009-10 | | | 2010-11 | | | 2011-12 | | | 2009-10 | | | 2010-11 | | | 2011-12 | | |
| | | | | K | R | S | K | R | S | K | R | S | K | R | S | K | R | S | K | R | S | |
| 1. | Sediment | - | - | 3 | 4 | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 4 | 3 | 3 | 4 | 3 | 4 | 60 |
| 2. | Crab | Fresh water paddy field crab | *Geothelphusa dehaani* | 5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 74 |
| 3. | Snail | Fresh water snail | *Pila globosa* | 4 | 3 | 4 | 3 | 4 | 4 | 4 | 3 | 3 | 4 | 3 | 4 | 3 | 4 | 4 | 4 | 3 | 3 | 64 |
| 4. | Fish | Spotted Snakehead | *Channa punctatus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 18 |
| | | Climbing Perch | *Anabas testudineus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 18 |
| | | Spot-fin Barb | *Puntius sophore* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 18 |
| | | Long-snouted Barb | *Puntius dorsalis* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 18 |
| | | Mrigal | *Cirrhinus mrigala* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 18 |
| 5. | Frog | Indian Rice Frog | *Fejervarya limnocharis* | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 36 |
| | | Jerdon's Bull Frog | *Hoplobatrachus crassus* | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 36 |
| 6. | Paddy | *Aishwarya* | *Oryza sativa* | 2 | 2 | - | 2 | 1 | - | 2 | 1 | - | 2 | 1 | - | 2 | 1 | - | 2 | 1 | - | 19 |
| | | *Jothi matta* | *Oryza sativa* | 2 | 2 | - | 2 | 2 | - | 2 | 2 | - | 2 | 2 | - | 2 | 2 | - | 2 | 2 | - | 24 |
| | | *Thavalakannan* | *Oryza sativa* | 1 | 1 | - | 1 | 2 | - | 1 | 2 | - | 1 | 2 | - | 1 | 2 | - | 1 | 2 | - | 17 |
| 7. | Fodder | *Aishwarya* | *Oryza sativa* | 2 | 2 | - | 2 | 1 | - | 2 | 1 | - | 2 | 1 | - | 2 | 1 | - | 2 | 1 | - | 19 |
| | | *Jothi matta* | *Oryza sativa* | 2 | 2 | - | 2 | 2 | - | 2 | 2 | - | 2 | 2 | - | 2 | 2 | - | 2 | 2 | - | 24 |
| | | *Thavalakannan* | *Oryza sativa* | 1 | 1 | - | 1 | 2 | - | 1 | 2 | - | 1 | 2 | - | 1 | 2 | - | 1 | 2 | - | 17 |
| 8. | Cow-milk | - | - | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 70 |

Note: K- *Kharif*; R- *Rabi*; S- Summer

### 3.2.4. Sample processing

#### 3.2.4a. Sediments

Samples were taken out of deep freezer at the time of analysis. About 1 kg of wet sediment from every sample was allowed to dry under room temperature for 48-96 hrs, ground with pestle and mortar following the method described by You *et al.* (2004). Dried samples were sieved through 300 µ mesh size and only 25 grams of dry sediment was taken for analysis. Samples were extracted with 200 mL mixture of acetone and dichloromethane (1:1 ratio) sonicated for 30 mins using ultrasonic processor (Crest-275 2.7 lts). The solvents were decanted through a Whatman No.1 filter paper containing 4 gm of activated anhydrous $Na_2SO_4$. This procedure was repeated twice with a sonication time of 15 minutes. Extracts were combined and concentrated to approximately 2 mL using rotary evaporator (Buchi R-215/V) under vacuum at 40°C. The co-extractives were removed from the concentrated extract by passing it through a column (25 cm x 1 cm) packed with 10 g of activated Florisil (60-80 µ mesh size) column, and a 1 cm layer of $Na_2SO_4$ was used to cap the Florisil. The concentrated extract was placed over the deactivated Florisil in the column. The tube that previously contained the extract was rinsed twice with 2 mL hexane, and these rinses were also introduced into the column. Further, the column was eluted with 50 mL of 30% diethyl ether in hexane (vol/ vol) and the eluents were concentrated by rotary evaporator and reconstituted to 1 mL with hexane for analysis in GC-MS.

#### 3.2.4b. Animal tissues

As far as tissue samples (fish, crab, mollusc and frog) are concerned, multiresidue extraction procedure was followed for extracting pesticide residues (Anastassiades *et al.*, 2003). Ten grams of tissue was taken and ground with pestle and mortar to get a homogenous mixture. Then the homogenate was transferred into a 50 mL centrifuge tube (Oakridge, Tarsons) and 20 mL of Acetonitrile (Merck) was added to it and shaken vigorously for a minute. To the centrifuge tube, 4 g of anhydrous Magnesium Sulphate ($MgSO_4$) (Himedia), 1 g of Sodium Chloride (Merck) were added and centrifuged at 10,000 rpm for 10 min. Then the organic

layer of the sample (4 mL) was transferred into a 15 mL centrifuge tube which contained 100 mg of primary secondary amine and 600 mg of $MgSO_4$ and further centrifuged at 5,000 rpm for 5 minutes. From the centrifuged extract, 2 mL was transferred into test tube, evaporated to near dryness and reconstituted with 1 mL of HPLC grade n-Hexane and stored in deep freezer at -20°C until final qualitative and quantitative analyses were carried out with GC-MS.

### 3.2.4c. Paddy and straw

Paddy and paddy straw samples were ground to fine powder and processed following multiresidue extraction methods adopted by Nguyen *et al.* (2008). Ten gram of paddy/ straw was transferred into a 50 mL Teflon centrifuge tube, 5 mL of HPLC-grade water was added to the centrifuge tube and mixed with 10 mL of acetonitrile in acetic acid (0.5%), vigorously vortexed for 3 min and further twice for 1 min at 15 min intervals. The centrifuged sample was then put into a refrigerator for 30 min (till sample mixture reached 4°C); Magnesium Sulfate (4 g) and Sodium Chloride (1 g) were added to the well-mixed paddy or fodder/ Acetonitrile/ water mixture, and the mixture was vortexed immediately for 1 min. The sample was centrifuged for 5 min at 4000 rpm. The acetonitrile extract (2 mL) was transferred to a 5 mL microcentrifuge tube containing 50 mg PSA, 300 mg $MgSO_4$ and 20 mg Graphitized Carbon Black (GCB), and vortexed for 1 min. Subsequently, the 5 mL microcentrifuge tube was centrifuged at 4000 rpm for 5 min at 4°C. The extract (1.2 mL) was transferred to a 1.8 mL Eppendorf vial and was reduced to near dryness using a vacuum concentrator. Acetonitrile (0.4 mL) containing 0.1 mg/L of TPP was added to the Eppendorf vial. The vial was vortexed and centrifuged at 1000 rpm for 5 min. The extract was then transferred to the GC auto-sampler vial for GC-MS analysis.

### 3.2.4d. Cow-milk

About 5 mL of fluid milk was mixed thoroughly with 20 g of freshly activated silica gel (60-100 µ mesh size, Qualigens fine chemicals) and 20 g of anhydrous $Na_2SO_4$ to form a free-flowing powder using pestle and mortar following the method described by Battu *et al.*

(2004). The powdered sample was packed into an extraction glass column (Borosil; 30 cm length x 1 cm i.d.) containing about 40 mL dichloromethane (DCM) over a plug of pre-washed cotton. Care was taken to prevent air bubbles from being trapped inside the column. DCM was eluted drop-wise from the column. When DCM was about to reach the level of the adsorbent, the column was re-eluted with 150-mL mixture of DCM: acetone (1:2, v/v). To remove the moisture, anhydrous $Na_2SO_4$ was added to the eluate. The volume was concentrated to about 2-3 mL under vacuum at 35°C. Hexane (10-15 mL) was added and again the mixture was concentrated (rotary flask evaporator; Model: Buchi) to about 5 mL. This was repeated to completely remove DCM and reconstituted with 1 mL of HPLC grade n-Hexane. Final analyses were carried out with GC-MS.

### 3.2.5. Chemical Analysis

Qualitative and quantitative analyses of OCs, OPs, SPs and Cbs were made with GC (Agilent Model 7890A Series) equipped with MS (5975 Quadruple) using HP-5ms fused silica capillary column (15m x 0.25 mm I.D x 0.25 µm film thickness) coated with 5% phenyl and 95% dimethyl polysiloxane. While Helium (IOLAR) was the carrier gas (flow rate: 1.0 mL/ min), chromatographic operating conditions were as follow: detector temperature was set at $325^0$C; the injector temperature was programmed as 85°C for 12 sec, increased at 600°C/ min to 325°C and held for 5 min, then again increased at 10°C/ min to 200°C (0 min); similarly oven temperature programmed as 70°C (1min), increased at 25°C/ min to 150°C (0 min), again increased at 3°C/ min to 200°C (0 min) and then 8°C/ min to 280°C (10 min). MS source temperature: 230°C; MS quadruple: 150°C; Technique: Electron ionization (EI); total run time: 40.867 min. The calibration standards with a minimum of five calibration points (1.56, 3.26; 6.25, 12.5, 25, 50 and 100 ng/g) spanning the expected concentrations in the samples were used for each analyte. All the samples were analyzed for organochlorine pesticides, namely alpha-Hexachlorocyclohexane (α-HCH), beta-Hexachlorocyclohexane (β-HCH), gamma-Hexachlorocyclohexane or lindane (γ-HCH), delta Hexachlorocyclohexane (δ-HCH), *o,p'*-Dichlorodiphenyltrichloroethane (DDT), *p,p'*-Dichlorodiphenyltrichloroethane (DDT), *o,p'*-Dichlorodiphenyldichloroethylene (DDE), *p,p'*-Dichlorodiphenyldichloroethylene (DDE),

*o,p'*-Dichlorodiphenyldichloroethane (DDD), *p,p'*-Dichlorodiphenyldichloroethane (DDD), α-Endosulfan, β-Endosulfan, Endosulfan Sulphate, Heptachlor, Heptachlor Epoxide, Aldrin, Dieldrin, Endrin, Chlordane and Mirex, organophosphate pesticides, namely Phorate, Methyl Parathion, Malathion, Chlorpyrifos, Chlorpyrifos Methyl, Parathion Ethyl, Pirimiphos Ethyl, Quinalphos, Phosphamidon, Phenthoate, Ethion and Triazophos, Synthetic Pyrethroids, namely λ-Cyhalothrin-I, λ-Cyhalothrin-II, Permethrin-I, Permethrin-II, Fenvalerate-I and Fenvalerate-II, and Carbamate pesticides, namely Carbaryl and Carbofuran (Fig. 3.3a-d).

An equivalent mixture manufactured by Sigma-Aldrich was used as standard. Concentrations of individual compounds were quantified from the peak area of the sample to that of the corresponding external standard (Fig. 3.3a-d). Recoveries of the compounds from fortified samples (50 µg/kg) ranged from 91 to 102%. Results were not corrected for per cent recovery and expressed in wet weight basis. The limits of detection (LOD) for the method were in the range of 0.25-0.75; 0.29-0.62; 0.09-0.40 and 0.25-0.80 ng/g for the OC, OP, SP and Cb respectively. Quantification limits were set at three times the instrumental detection limit for each chemical, and they ranged from 0.25 ng/g to 0.80 ng/g. Recoveries of compounds from fortified samples (50 ng/g) ranged from 91 to 102% and the results were not corrected for percent recovery and the results were expressed in wet weight basis. Analyses were run in batches of 10 samples plus four quality controls (QCs) including one reagent blank, one matrix blank, one QC check sample and one random sample in duplicate.

### 3.2.6. Data Analysis

Statistical tests were performed with SPSS (Ver 16) and a preset p value of 0.05 was used to evaluate statistical significance. For statistical comparisons, contaminants below detection limit were treated as zeros. Contaminants detected, but were below the limit of quantification (LOQ), were also not included in the analyses. Analysis of variance (ANOVA) was used to test the differences in pesticides residues among the components and also among the sampling seasons, years, group of pesticides of both organic and conventional farms. Values below detection limits are reported as below detection limit (BDL).

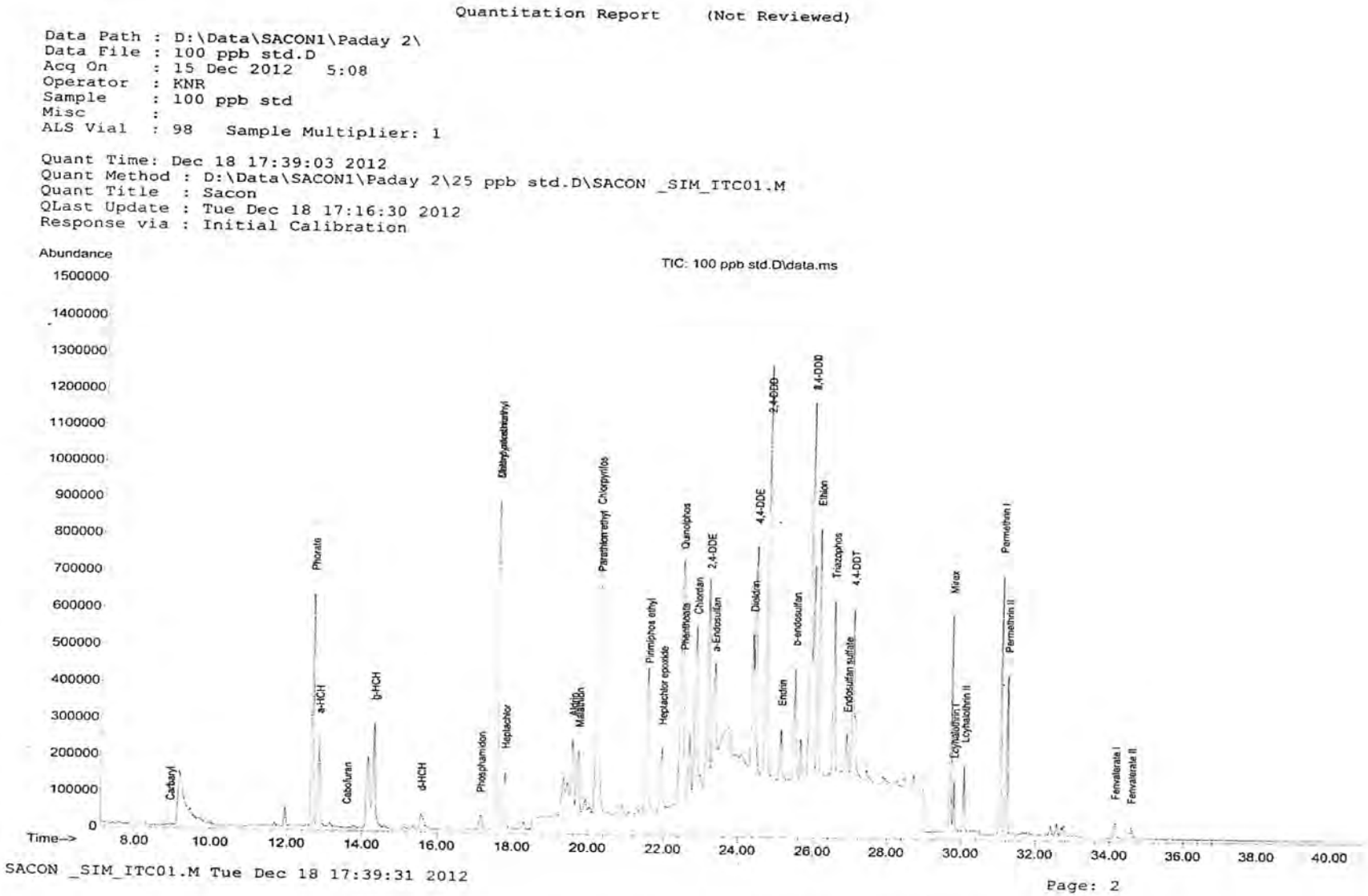



**Fig. 3.3a. Standard chromatogram of 40 pesticides calibrated in GC-MS**

Quantitation Report (Not Reviewed)

Data Path : D:\Data\SACON1\Paday 2\
Data File : 100 ppb std.D
Acq On : 15 Dec 2012 5:08
Operator : KNR
Sample : 100 ppb std
Misc :
ALS Vial : 98 Sample Multiplier: 1

Quant Time: Dec 18 17:39:03 2012
Quant Method : D:\Data\SACON1\Paday 2\25 ppb std.D\SACON _SIM_ITC01.M
Quant Title : Sacon
QLast Update : Tue Dec 18 17:16:30 2012
Response via : Initial Calibration

| Compound | R.T. | QIon | Response | Conc | Units | | Dev(Min) |
|---|---|---|---|---|---|---|---|
| Target Compounds | | | | | | | Qvalue |
| 1) Carbaryl | 8.932 | 144 | 7013 | 114.13 | ppb | | 100 |
| 2) Phorate | 12.643 | 75 | 11557524 | 101.04 | ppb | | 100 |
| 3) a-HCH | 12.827 | 183 | 3431719 | 98.23 | ppb | | 100 |
| 4) Cabofuran | 13.586 | 164 | 2126 | 117.47 | ppb | | 100 |
| 5) b-HCH | 14.309 | 181 | 2421569 | 98.12 | ppb | | 100 |
| 6) g-HCH | 14.309 | 181 | 2421569 | 98.12 | ppb | | 100 |
| 7) d-HCH | 15.588 | 181 | 673753 | 93.58 | ppb | | 100 |
| 8) Phosphamidon | 17.154 | 127 | 797864 | 92.76 | ppb | | 100 |
| 9) Methyl parathion | 17.551 | 263 | 1012063 | 102.35 | ppb | | 100 |
| 10) Chlorpyrifos methyl | 17.553 | 286 | 5802893 | 100.39 | ppb | # | 100 |
| 11) Heptachlor | 17.797 | 100 | 3072900 | 101.07 | ppb | | 100 |
| 12) Aldrin | 19.575 | 66 | 3518848 | 99.63 | ppb | | 100 |
| 13) Malathion | 19.729 | 125 | 2187011 | 94.64 | ppb | | 100 |
| 14) Chlorpyrifos | 20.216 | 197 | 5716109 | 101.00 | ppb | | 99 |
| 15) Parathion ethyl | 20.262 | 291 | 2634272 | 102.67 | ppb | | 100 |
| 16) Pirimiphos ethyl | 21.561 | 318 | 3606050 | 99.57 | ppb | | 100 |
| 17) Heptachlor epoxide | 21.949 | 81 | 1215119 | 99.90 | ppb | | 100 |
| 18) Quinolphos | 22.451 | 146 | 7202692 | 99.98 | ppb | | 100 |
| 19) Phenthoate | 22.501 | 274 | 4159337 | 99.11 | ppb | | 100 |
| 20) Chlordan | 22.850 | 373 | 4783233 | 99.29 | ppb | | 100 |
| 21) 2,4-DDE | 23.166 | 246 | 12975583 | 99.17 | ppb | | 100 |
| 22) a-Endosulfan | 23.329 | 195 | 872804 | 94.87 | ppb | | 100 |
| 23) Dieldrin | 24.350 | 79 | 5420122 | 98.61 | ppb | | 100 |
| 24) 4,4-DDE | 24.425 | 246 | 10465098 | 99.65 | ppb | | 100 |
| 25) 2,4-DDD | 24.722 | 235 | 17673097 | 98.91 | ppb | | 100 |
| 26) Endrin | 25.126 | 263 | 1061412 | 99.42 | ppb | | 100 |
| 27) b-endosulfan | 25.476 | 195 | 1758204 | 92.77 | ppb | | 100 |
| 28) 2,4-DDT | 25.877 | 235 | 15773438 | 98.66 | ppb | | 100 |
| 29) 4,4-DDD | 25.877 | 235 | 15773438 | 98.66 | ppb | | 100 |
| 30) Ethion | 26.100 | 231 | 10266637 | 99.87 | ppb | | 100 |
| 31) Triazophos | 26.512 | 161 | 5885815 | 99.23 | ppb | | 100 |
| 32) Endosulfan sulfate | 26.880 | 272 | 863063 | 96.81 | ppb | | 100 |
| 33) 4,4-DDT | 27.034 | 235 | 5768207 | 99.45 | ppb | | 100 |
| 34) Mirex | 29.703 | 272 | 6345206 | 99.37 | ppb | | 100 |
| 35) Lcyhalothrin I | 29.799 | 181 | 1172048 | 91.81 | ppb | | 100 |
| 36) Lcyhalothrin II | 30.068 | 181 | 1557620 | 95.83 | ppb | | 100 |
| 37) Permethrin I | 31.031 | 183 | 12041586 | 99.57 | ppb | | 100 |
| 38) Permethrin II | 31.209 | 183 | 6883496 | 99.65 | ppb | | 100 |
| 39) Fenvalerate I | 34.139 | 167 | 598767 | 99.97 | ppb | | 100 |
| 40) Fenvalerate II | 34.576 | 167 | 415080 | 97.79 | ppb | | 100 |

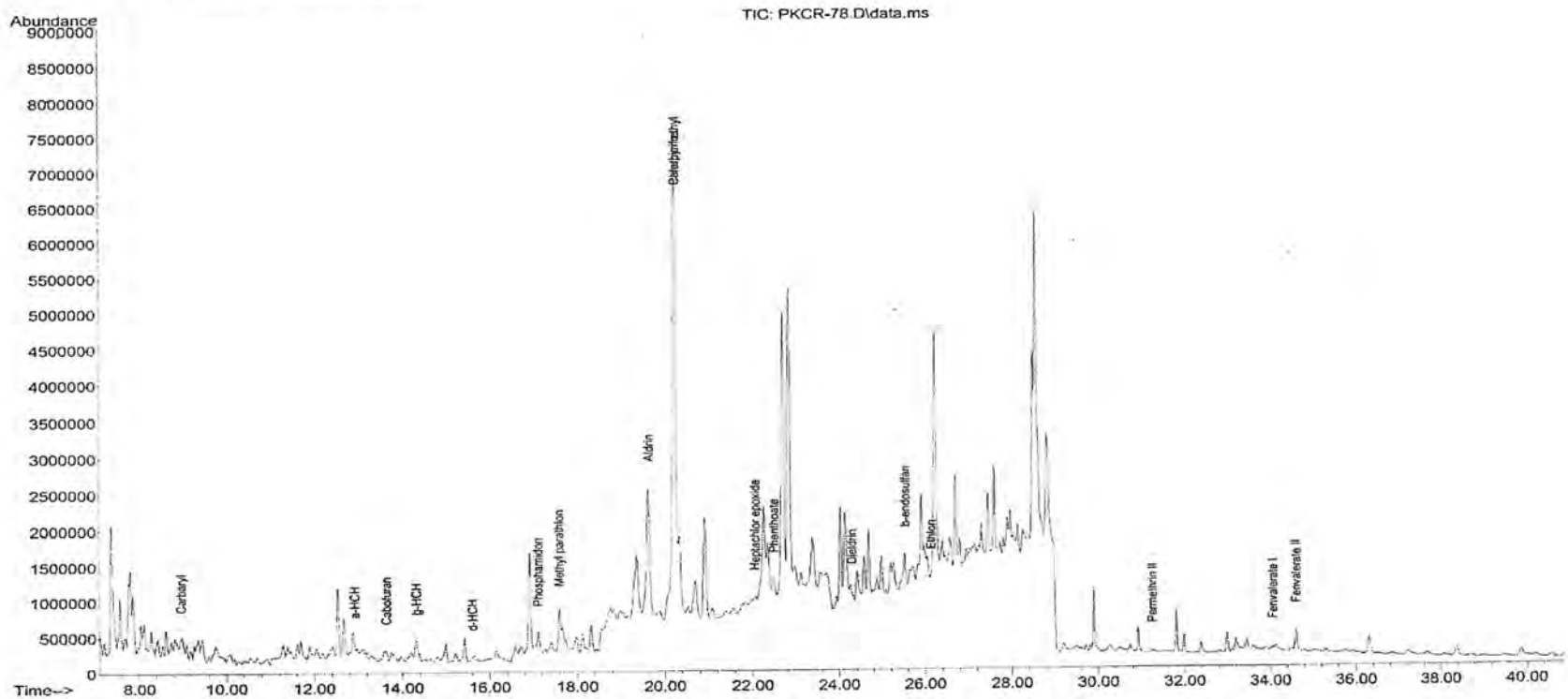
(#) = qualifier out of range (m) = manual integration (+) = signals summed

**Fig. 3.3b. Quantification report of 40 calibrated pesticides quantified and qualified by GC-MS**

Quantitation Report (Not Reviewed)

Data Path : D:\Data\SACON1\Paday 2\
Data File : PKCR-78.D
Acq On : 17 Dec 2012 14:42
Operator : KNR
Sample : PKCR-78
Misc :
ALS Vial : 148 Sample Multiplier: 1

Quant Time: Dec 18 18:44:57 2012
Quant Method : D:\Data\SACON1\Paday 2\25 ppb std.D\SACON _SIM_ITC01.M
Quant Title : Sacon
QLast Update : Tue Dec 18 17:16:30 2012
Response via : Initial Calibration




SACON _SIM_ITC01.M Tue Dec 18 18:45:02 2012 Page: 2

**Fig. 3.3c. Conventional farm crab, *Geothelphusa dehaani* chromatogram of individual pesticides quantified and qualified by GC-MS**

Quantitation Report (Not Reviewed)

Data Path : D:\Data\SACON1\Paday 2\
Data File : PKCR-78.D
Acq On : 17 Dec 2012 14:42
Operator : KNR
Sample : PKCR-78
Misc :
ALS Vial : 148 Sample Multiplier: 1

Quant Time: Dec 18 18:44:57 2012
Quant Method : D:\Data\SACON1\Paday 2\25 ppb std.D\SACON _SIM_ITC01.M
Quant Title : Sacon
QLast Update : Tue Dec 18 17:16:30 2012
Response via : Initial Calibration

| Compound | R.T. | QIon | Response | Conc | Units | | Dev(Min) |
|---|---|---|---|---|---|---|---|
| Target Compounds | | | | | | | Qvalue |
| 1) Carbaryl | 8.967 | 144 | 254789 | 3769.92 | ppb | # | 1 |
| 2) Phorate | 12.648 | 75 | 853 | N.D. | | | |
| 3) a-HCH | 12.922 | 183 | 625010 | 15.30 | ppb | # | 7 |
| 4) Cabofuran | 13.637 | 164 | 367630 | 25280.83 | ppb | # | 1 |
| 5) b-HCH | 14.319 | 181 | 4441233 | 182.57 | ppb | | 81 |
| 6) g-HCH | 14.319 | 181 | 4441233 | 182.57 | ppb | # | 77 |
| 7) d-HCH | 15.606 | 181 | 664632 | 91.98 | ppb | # | 8 |
| 8) Phosphamidon | 17.100 | 127 | 121818 | 11.70 | ppb | # | 79 |
| 9) Methyl parathion | 17.585 | 263 | 2916 | 3.70 | ppb | # | 1 |
| 10) Chlorpyrifos methyl | 17.564 | 286 | 859 | N.D. | | | |
| 11) Heptachlor | 17.845 | 100 | 980 | N.D. | | | |
| 12) Aldrin | 19.634 | 66 | 918339 | 25.85 | ppb | # | 14 |
| 13) Malathion | 19.790 | 125 | 58226 | Below Cal | | # | 7 |
| 14) Chlorpyrifos | 20.237 | 197 | 45993740 | 811.40 | ppb | # | 78 |
| 15) Parathion ethyl | 20.240 | 291 | 354476 | 19.07 | ppb | | 83 |
| 16) Pirimiphos ethyl | 0.000 | | 0 | N.D. | | | |
| 17) Heptachlor epoxide | 22.058 | 81 | 384198 | 32.07 | ppb | # | 36 |
| 18) Quinolphos | 22.521 | 146 | 49796 | N.D. | | | |
| 19) Phenthoate | 22.499 | 274 | 137641 | 4.29 | ppb | # | 58 |
| 20) Chlordan | 22.858 | 373 | 1960 | Below Cal | | # | 70 |
| 21) 2,4-DDE | 23.202 | 246 | 46901 | Below Cal | | # | 7 |
| 22) a-Endosulfan | 23.426 | 195 | 5763 | Below Cal | | # | 1 |
| 23) Dieldrin | 24.291 | 79 | 661561 | 10.28 | ppb | # | 54 |
| 24) 4,4-DDE | 24.469 | 246 | 44595 | N.D. | | | |
| 25) 2,4-DDD | 24.680 | 235 | 49570 | Below Cal | | # | 1 |
| 26) Endrin | 25.031 | 263 | 8500 | Below Cal | | # | 1 |
| 27) b-endosulfan | 25.534 | 195 | 272926 | 5.53 | ppb | # | 69 |
| 28) 2,4-DDT | 25.922 | 235 | 304439 | Below Cal | | # | 1 |
| 29) 4,4-DDD | 25.922 | 235 | 304439 | Below Cal | | # | 1 |
| 30) Ethion | 26.127 | 231 | 201466 | 3.33 | ppb | # | 1 |
| 31) Triazophos | 26.467 | 161 | 7547 | N.D. | | | |
| 32) Endosulfan sulfate | 26.811 | 272 | 27295 | Below Cal | | | 91 |
| 33) 4,4-DDT | 27.099 | 235 | 104165 | N.D. | | | |
| 34) Mirex | 29.634 | 272 | 2943 | Below Cal | | # | 77 |
| 35) Lcyhalothrin I | 29.791 | 181 | 1590 | Below Cal | | # | 1 |
| 36) Lcyhalothrin II | 30.091 | 181 | 12867 | Below Cal | | | 81 |
| 37) Permethrin I | 31.041 | 183 | 10021 | Below Cal | | # | 1 |
| 38) Permethrin II | 31.240 | 183 | 332167 | 4.43 | ppb | # | 52 |
| 39) Fenvalerate I | 34.049 | 167 | 595159 | 99.28 | ppb | | 89 |
| 40) Fenvalerate II | 34.599 | 167 | 831725 | 209.89 | ppb | # | 1 |

(#) = qualifier out of range (m) = manual integration (+) = signals summed

**Fig. 3.3d. Quantification report of conventional farm crab sample quantified and qualified by GC-MS**

# RESULTS AND DISCUSSION

# 4. RESULTS AND DISCUSSION

Organic farming in the study area (Padayetti village) was started in 2009 with the support of Kerala State Biodiversity Board (KSBB). Forty nine out of 69 households in Padayetti village are engaged in paddy cultivation. Around 30 farmers practice organic farming while the rest of them use chemical fertilizers and pesticides. People who do not own any land, work as agricultural labourers on daily wages in nearby farms. A few farmers adopted convenient farming. Most of them have kitchen gardens and cultivate vegetables without any chemical input. Information on educational status of the farmers was also gathered. About 69% of farmers have studied up to $10^{th}$ standard. Their experience in farming ranges between 20 and 40 years. Paddy is the major crop followed by rubber, banana, ginger and vegetables such as brinjal, chilli, okra, ash gourd, bottle gourd, snake gourd and little gourd. Pesticides used by farmers in conventional farms for pest and weed control, and their relevant details are furnished in table 4.1.

Instead of chemical pesticides and fertilizers, organic farmers used farm yard manure, vermicompost, biogas slurry, neem oil cake, castor oil cake, groundnut oil cake, biopesticides (Liquid manure *'Panchagavya'*), plant extracts such as garlic, ginger, chilli and N, P and K bionutrients (Table 4.2 and Fig. 4.1). It is to be noted that Kerala State Biodiversity Board as part of Agricultural Biodiversity Restoration Programme through Thiruvananthapuram based non-governmental organisation, *'Thanal'* provided cows to every farmer and constructed tanks for preparing vermicompost. Besides, all the farmers and their school going children were trained in every weekend for a period of two years. As part of the farmers field school programme, all the farmers were trained for system of rice intensification (SRI) technique, various biological pest control measures, organic manure and vegetable bed preparations in their fields with the help of experts from the nearby states. They also conducted awareness programmes and field camps on the impacts of chemical fertilizers and pesticides with case studies, biodiversity conservation and sustainable usage of natural resources.



**Plate 1: Farmer takes stock of his pesticides**



**Plate 2: Factamfos being applied before transplantation in conventional farms**



**Plate 3: Organic farming - Seed bed preparation**



**Plate 4: Farmer applying bionutrient in organic farm nursery**



**Plate 5: Transplantation of paddy in progress**



**Plate 6: Farm yard manure application**



**Plate 7: System of Rice Intensification**



**Plate 8: Plant extracts (biopesticide) application**



**Plate 9: Congregation of birds during vegetative stage**



**Plate 10: Egrets flock in organic field after harvesting**

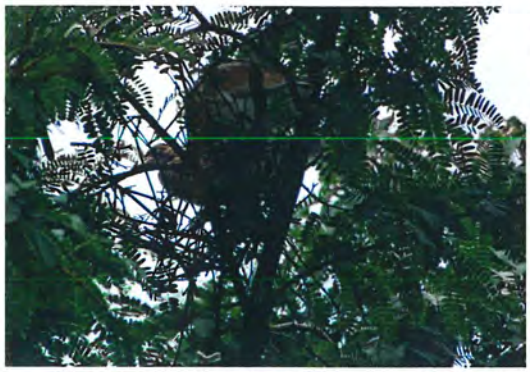


Plate 11: Indian Pond Heron on its nest



Plate 12: Bayaweaver weaving the nest



Plate 13: A view of organic turmeric farm



Plate 14: View of an organic vegetable garden




**Fig. 4.1. Bionutrients supplied by Kerala State Biodiversity Board to farmers**

**Table 4.1. List of pesticides currently-used in conventional paddy cultivation in Padayetti village, and their relevant details**

| S.No | Common Name | Group | Trade name | Formulation | Dose/ ha |
|---|---|---|---|---|---|
| 1. | Phorate | OP | Thimate | 10% G | 750-1250 g |
| 2. | Methyl Parathion | OP | Folidol | 2% DP | 250-750 g |
| 3. | Triazophos | OP | Hostathion | 40% EC | 1000-1250 mL |
| 4. | Phosphamidon | OP | Kindon plus | 40% SL | 500-750 mL |
| 5. | Chlorpyrifos | OP | Classic | 1.5% DP | 100-250 g |
| 6. | Phenthoate | OP | Cidial | 2% DP | 250-500 g |
| 7. | Malathion | OP | Devimalt | 50% EC | 500g |
| 8. | Quinalphos | OP | Ekalux | 25% EC | 325-375 g |
| 9. | Lambda (λ) Cyhalothrin | SP | Karate | 5% EC | 400 mL |
| 10. | Fenvalerate | SP | Fenvol | 20% EC | 500-1500 mL |
| 11. | Carbofuran | Cb | Furadan 3G | 3% G | 750 g |
| 12. | Carbaryl | Cb | Sevin | 5% DP | 500-1500 g |
| 13. | 2,4-Dichlorophenoxyacetic acid | Herbicide | 2,4-D | 80% w/w | 850 g |

Note: EC - Emulsifying Concentration; G - Granule; DP - Dustable Powder; w/w - weight by weight; SL- Soluble Liquid

**Table 4.2. List of biopesticides and bionutrients used in organic paddy cultivation in Padayetti village, and their relevant details**

| S.No. | Biopesticides/ Bionutrients | Dose/ Acre |
|---|---|---|
| 1. | Farm yard manure | 2000-3000 kg |
| 2. | Vermicompost | 250-400 kg |
| 3. | Biogas slurry | 500-1000 kg |
| 4. | Neem oil cake | 100-150 kg |
| 5. | Castor oil cake | 75-100 kg |
| 6. | Groundnut oil cake | 75-100 kg |
| 7. | Liquid manure, *'Panchagavya'* | 20-30 litre |
| 8. | Plant extracts: garlic, ginger and chilli | 1-2.5 kg |
| 9. | N: *Azospirillum* inoculant | 2 kg |
| 10. | P: *Bacillus megatherium* inoculant | 2 kg |
| 11. | K: *Frateuria aurantia* inoculant | 2 kg |
| Note: N - Nitrogen; P - Phosphorus and K - Potassium | | |

Data on pesticide residues (group wise) have been compiled to check the overall load of pesticides in both organic and conventional farms during different seasons (*Kharif*, *Rabi* and summer), years and trophic levels. Further, within a group, data on the levels of individual pesticides and their isomers or metabolites have also been compiled to understand the implications with reference to usage pattern or policy of the government in existence.

### 4.1 Pesticide residues in sediments in the agroecosystem in Padayetti village, Palakkad district

Pesticides, particularly OCs and their transformation products (TPs) are mainly hydrophobic, persistent and bioaccumulable. Pesticides those exhibit such behaviors include the OCs such as HCH, DDT, Endosulfan, Dieldrin, Endrin, Heptachlor and their breakdown products. Most of them are now banned for agriculture (Clarke *et al.*, 2013; Pazou *et al.*, 2013; Vijayan *et al.*, 2008). Contemporary pesticides such as OPs, SPs, Cbs, herbicides, weedicides are retained by soil to different degrees, depending on the interactions between soil and pesticides (Andreu and Pico, 2004).

A total of 90 sediment samples (each 45 from organic and conventional farms) were collected seasonally (*Kharif*, *Rabi* and summer) between May 2009 and April 2012, and analysed for residues of 20 OCs, 12 OPs, six SPs and two Cbs in 60 samples (29 from organic and 31 from conventional farms). Five out of the 31 sediments samples (16.13%) from conventional farm contained persistent OC contaminants, namely HCH isomers. The highest level of OC (i.e. HCH: 30.90 ± 9.83 ng/g) residue was in the sediments of conventional farms collected during *Kharif* season (2009-10) and lowest (2.34 ng/g) during summer season (2010-11). None of the sediment samples collected from organic farm had detectable levels of any OC residues.

Among the twelve currently-used OPs for paddy cultivation in Padayetti agroecosystem, 18.33% of sediments collected from conventional farms had Chlorpyrifos or Triazophos residues with maximum (22.07 ± 4.80 ng/g) during *Rabi* season (2009-10), followed by 17.35 ± 3.19 ng/g in the same farm sediments collected during summer in the same year i.e. 2009-10. Levels of these compounds were recorded in trace amounts (3.26 ng/g) in one of the organic farm sediment collected during *Rabi* season (2011-12). This could be because a few farmers practicing organic farming resorted to using OP pesticide, namely Chlorpyrifos to contain pests on paddy, namely stem borer, leaf roller, gall midge, planthoppers and rice hispa when the biological methods adopted for pest control were not effective in their farm. It was observed that the farmers of organic farming did not apply any other pesticide.

Levels of SP such as ∑-Fenvalerate (Fenvalerate-I and -II) were fairly high in only one sediment sample collected from conventional farms (62.80 ng/g) during *Rabi* season (2011-12), whereas levels of these compounds were at BDL in other samples from conventional and organic farms in all the seasons during the study period (2009-12). The levels of Carbaryl and Carbofuran (carbamates) were found at BDL in all sediments collected from both the farms. The mean variations in the OCs, OPs and SPs residue levels in sediments are presented in fig. 4.1a. Levels of currently-used pesticide residues (i.e OPs) varied significantly between organic and conventional farm sediments ($p < 0.05$; $F = 0.79$). Since samples with detectable levels of OCs and SPs were not many, no statistical test was performed.

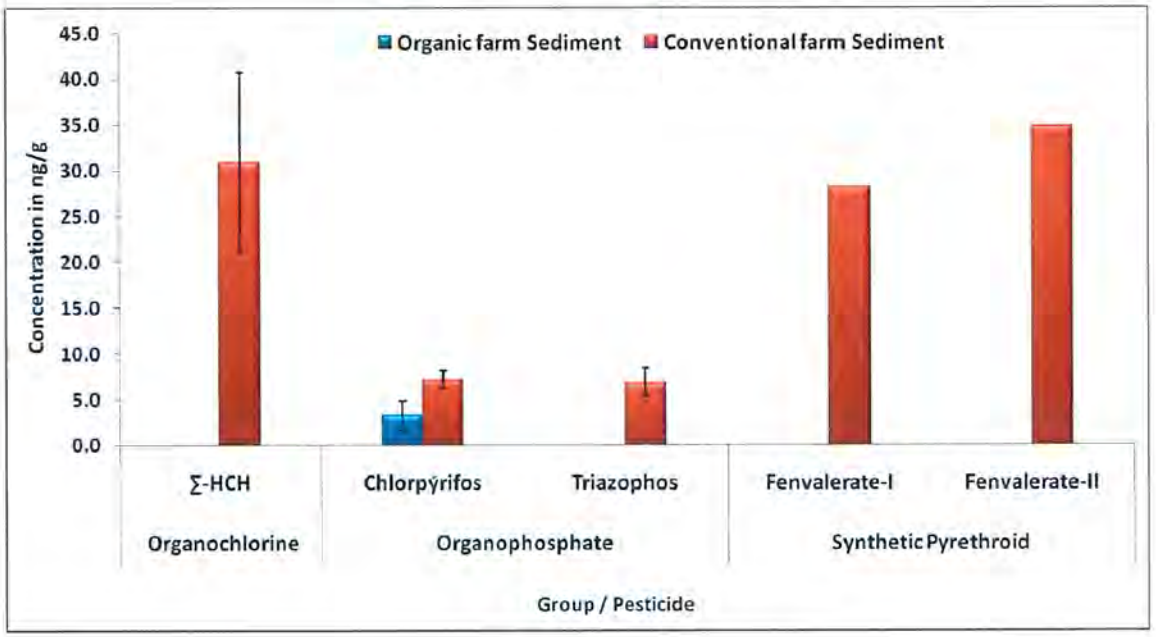



**Fig. 4.1a Mean ( ± SE) levels of OC, OP and SP residues in sediments (n=60; ng/g wet wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12)**

### 4.1.1 Residues of major isomers/ metabolites of organochlorine pesticides in sediments in Padayetti village, Palakkad district

#### 4.1.1a) HCH and its isomers

Among the four isomers of HCH, namely alpha (α), beta (β), gamma (γ) and delta (δ), only residues of β- and γ-HCH were detected in four and three samples of sediments respectively collected from five out of 31 conventional farm sediments in Padayetti village. While levels of α- and δ-HCH were below detection limit, both γ-HCH (24.42 ± 5.74 ng/g) and β-HCH (6.48 ± 2.02 ng/g) were high in sediments collected from conventional farms during *Kharif* (2009-10). Further γ-HCH was in traces (2.34 ng/g) during summer (2010-11). However, levels of these compounds were at BDL in sediments collected from organic farms during the entire study period. Absence of α- and δ-HCH in sediments might indicate that there has been no recent use of technical mixtures of HCH (Doong *et al.,* 2002) in this area.

Residues of Σ-HCH were detected in 16.13% of sediment samples from conventional farm and it ranged between BDL and 30.90 ng/g. Similar level of HCH contamination of soil/ sediment in elsewhere in India and other parts of the world have been reported earlier (Garcia-de la Parra *et al.*, 2012; Hu *et al.*, 2010; Carvalho *et al.*, 2009; Wang *et al.*, 2008; Malik *et al.*, 2009; Stringer *et al.*, 2003; Zhang *et al.*, 2003; Sahu *et al.*, 1990). Levels of HCH isomers, namely γ- and β-HCH observed in the conventional farm sediments are higher than the levels reported (Vijayan and Muralidharan, 1999) in sediments of Pykara in Nilgiris, Tamil Nadu, India. Further, levels of Σ-HCH in conventional farm are higher than the levels reported by them from sediments of various reservoirs, namely Avalanche, Upper Bhavani, Emerald, Kamaraj Sagar, Pykara and Maravakandy, Ooty lake and rivers, namely Coonoor and Moyar in Nilgiris in Tamil Nadu, India. Σ-HCH recorded in the present study are lower than the levels recorded in the coastal sediments of Chennai, Cochin and Visakhapatnam (Senthilkumar *et al.*, 2001) and the river sediments of Gomati, Lucknow, India (Malik *et al.*, 2009). Kumari *et al.* (2008) reported HCH residues (β-HCH: 15.0 ng/g and γ-HCH: 41.0 ng/g) in soil/ sediment collected from different crop lands (paddy, cotton, wheat and sugarcane) in Hisar, Haryana, India, which are comparatively higher than the levels recorded in the present study.

Mean concentrations of γ-HCH (24.42 ng/g) and β-HCH (6.48 ng/g) observed in conventional farm sediment in the present study are lower than the levels reported by Barlas *et al.* (2006) in Uluabat Lake (γ-HCH: 266.6 ng/g and β-HCH: 377.6 ng/g) from the Bursa Province, Turkey. Contamination of soil with ∑-HCH up to 32 ng/g had been reported from the states of Northern India, i.e. Uttar Pradesh, Punjab and Delhi during 1965-1978 (Anonymous, 1988a) which are comparable with the levels recorded in the present study. Levels of β-HCH was comparable (8.32 ng/g) and γ-HCH was relatively higher than those detected (4.30 ng/g) in the sediments of agricultural drains in the Culiacan Valley, Sinaloa, Mexico (Garcia-de la Parra *et al.*, 2012) and sediments collected from various sites in the Neemsar, Bhatpur, Gaughat, mid-Lucknow, Pipraghat, Barabanki, downstream of Sultanpur and Jaunpur of river Gomati (β-HCH: 1.73 ng/g and γ-HCH: 2.52 ng/g), Uttar Pradesh, India (Malik *et al.*, 2009).

The levels of β- and γ-HCH are higher than the levels reported in sediment (β-HCH: 1.63 ng/g and γ-HCH: 5.96 ng/g) samples collected from six locations (Chennai harbour, Cuddalore fishing harbour and Mandapam of Gulf of Mannar Biosphere Reserve) along the east coast of India (Rajendran *et al.,* 2005); marine and estuarine sediments (0.85-7.87 ng/g) along the west coast (Saraswati, Purna, Ambika, Damanganga, Savitri, Zuari, Kali, Netravati, Beypore and Ponnani) of India (Sarkar *et al.*, 1997) and sediment collected from Najafgarh, Shahadra, Barapulla, Sen Nursing Home, Power House, Gunchi, Civil mill and Nizamuddin (BDL-17.29 ng/g) drains in Delhi (Kumar *et al.,* 2011b) and lower than the levels reported in sediments collected from paddy fields (3.7-1100 ng/g) of Parangipetti and Pichavaram mangroves of Southeast coast of India (Ramesh *et al.,* 1991); sediments of agriculture, nursery, gardening and landfill sites (500 ng/g) in Agra city, India (Singh, 2001); surface and subsurface soils from agricultural sites of Delhi, Haryana, Haridwar, Uttar Pradesh (β-HCH: 2.5 - 4630 ng/g; γ-HCH: 0.08 - 43 ng/g) and around the HCH manufacturing plant of IPL (Indian Pesticide Limited), Delhi (Prakash *et al.,* 2004) and downstream creeks of Hindustan Lever Limited (Kuzhikandam Thodu) plant, Eloor, Kerala (Stringer *et al.,* 2003).

Most of the field studies referred in India called the levels as baseline information and did not report any adverse effects on soil dwelling organisms. Moreover levels of β- and γ-HCH isomers did not exceed the sediment quality guidelines (SQG: 50 ng/g), threshold effect level (TEL: 100 ng/g), probable effect level (PEL: 100 ng/g) (Buchman, 2008) and maximum permissible concentration (MPC: 100 ng/g) set by various International statutory agencies (Crommentuijn *et al.,* 2000). Hence, the present study indicates that these contaminants at present levels may not produce any adverse effects to soil dwelling organisms.

Of all the HCH isomers, only γ-HCH has significant insecticidal activity, although all of them exhibit both acute and chronic toxicity. The β-isomer particularly has lower vapor pressure, more resistance to hydrolysis and environmental degradation which also acts as an environmental estrogen (Kumari *et al.,* 2008; Li, 1999; Walker *et al.,* 1999). α-HCH and γ-HCH are more likely to be transported by air for a long distance (Brubaker and Hites, 1998).

Moreover, γ-HCH can be converted to α-HCH by sunlight (Malaiyandi and Shach, 1984), and biological degradation in the environment (Huehnerfuss *et al.*, 1992).

It is to be noted that technical grade HCH consists mainly 5 isomers, namely α-HCH (60-70%), β-HCH (5-12%), γ-HCH (10-15%), δ-HCH (6-10%) and Ɛ-HCH (3-4%) (Walker *et al.*, 1999), while lindane consists of mainly γ-HCH (> 99%). Therefore, the profiles of HCH isomers can reflect the sources and the fate of HCHs in the environment (Hu *et al.*, 2010). The presence of the β-HCH (the most persistent isomer) in sediments, (Phillips *et al.*, 2005), can be attributed to the degradation of α-HCH. Wu *et al.* (1997) demonstrated that the transformation of α-HCH occurs under both oxic and anoxic conditions, while β-HCH increased resistance to microbial degradation and lower volatility (Phillips *et al.*, 2005; Rajendran *et al.*, 2005). Considering that the half-life of lindane (γ-HCH) is 30-300 days in water and 2 years or more in soils (INE, 2004), its detection in trace amounts in the present study suggests its past usage for agricultural and public health activities in Padayetti hamlet.

Lindane can persist for a long time in the treated soils. Certain sources report half-life of lindane in soils to range 13 to 240 days (Howard *et al.*, 1991) depending on the soil type. Another study found that its half-life may be in the order of 3 to 4 years (Martijn and Schreuder, 1993). In the soil, lindane can undergo bioisomerization forming α-, β- and δ-HCH, but not ε-HCH (Waliszewski, 1993). Lindane is also used to a lesser degree in pharmaceutical preparations to treat lice and scabies in humans and ectoparasites in cattle in developing countries (Li, 1999). It is known that lindane causes endocrine disorders (CEC, 2003). Walker *et al.* (1999) assessed the factors responsible for the distribution of lindane and other HCHs and noted that HCH isomers have a relatively higher volatility and solubility in water compared to other OCs. HCH residues, therefore, tend to volatilise into the atmosphere, dissolve into precipitation, and end up in surface waters, especially in high altitudes where it is cold. As a result, HCH has become the most abundant OC insecticide in the Arctic, an area where it has never been used. Therefore, based on the isomer composition of HCH, the sources and the fate of HCHs in the environment can be reflected.

Moreover, Isomers of HCH can be degraded in the soil by various bacteria (Jacobsen *et al.*, 1991; Sahu *et al.*, 1990; Imai *et al.*, 1989), but the present study did not address the same. Although technical HCH was restricted since 80s in many countries, such as Canada, the USA, and Mexico, use of HCH continued unabated until 1997 in India, and restricted use of lindane (99% γ-HCH) was allowed till 2011. At present HCH is totally banned vide Gazette Notification No S.O. 637(E) for manufacture, import or formulate and use with effective from 25$^{th}$ March, 2011 and banned for use with effective from 25$^{th}$ March, 2013 (CIBRC, 2014). Residues of HCH in ecosystem components may come down in coming years in India as we have witnessed in the present study.

#### 4.1.1b) DDT and its metabolites

Sediment from both organic and conventional farms did not have any of the isomers (*o,p'*- DDT and *p,p'*-DDT) or metabolites (*o,p*-DDE, *p,p'*-DDE, *o,p'*-DDD and *p,p'*-DDD) of DDT. The restriction and banning of most of the OCs including DDT for agriculture in 1989 have resulted in reduction of their residues in the environmental components (table 4.1). In the present context, decreasing trend in DDT residues is a good sign.

#### 4.1.1c) Cyclodiene insecticides

None of the ten cyclodiene pesticides (α- and β-Endosulfan, Endosulfan Sulphate, Heptachlor, Heptachlor Epoxide, Chlordane, Aldrin, Dieldrin, Endrin and Mirex) tested in the sediment from both the farms had detectable levels of residues. When we compare the levels of cyclodienes in sediments reported from India and elsewhere, concentrations are coming down due to restricted usage as evidenced in the present study as well.

### 4.1.2 Residues of organophosphate pesticides in sediments in Padayetti village, Palakkad district

Among the 12 currently-used OPs analysed in sediments, residues of only two OPs, namely Chlorpyrifos (14.72 ng/g) and Triazophos (10.47 ng/g) were found to be high in conventional

farms during *Rabi* and summer seasons (2009-10), while trace amount of Chlorpyrifos (3.26 ng/g) was detected in one of the sediment samples from organic farm during *Rabi* (2011-12). This could be because a few farmers practicing organic farming resorted to using OP pesticide, namely Chlorpyrifos to contain stem borer, leaf roller, gall midge and rice hispa on paddy. Unfortunately biological control methods to contain the above-referred pest turned out to be futile. It was observed that the farmers of organic farming did not apply any other pesticide.

Triazophos residues were found to be high (10.47 ± 7.24 ng/g) in the conventional farm sediments collected during summer 2009-10. Sediment collected from organic farms did not have this residue. Residues of other OPs, namely Phorate, Phosphamidon, Methyl Parathion, Parathion Ethyl, Pirimiphos Ethyl, Quinalphos, Phenthoate and Ethion were BDL in sediments from both organic and conventional farms during the study period. Variation in residue levels of OP pesticides among years, seasons and between farms are given in fig. 4.1b. Among the individual OP pesticides analysed in sediments, Chlorpyrifos was detected in 18.33% and <1% of conventional and organic farm samples respectively. While residue of Triazophos was detected in 15% of conventional farm sediment, in organic farms it was BDL. Levels of Triazophos (trade name: Hostathion 40% EC) and Chlorpyrifos (trade names: Classic, Tarkash and Chlorguard 20% EC) residues in conventional farm sediments of Padayetti village reflect the current use of these compounds to control pests such as rice hispa, stem borer and stem fly on paddy.

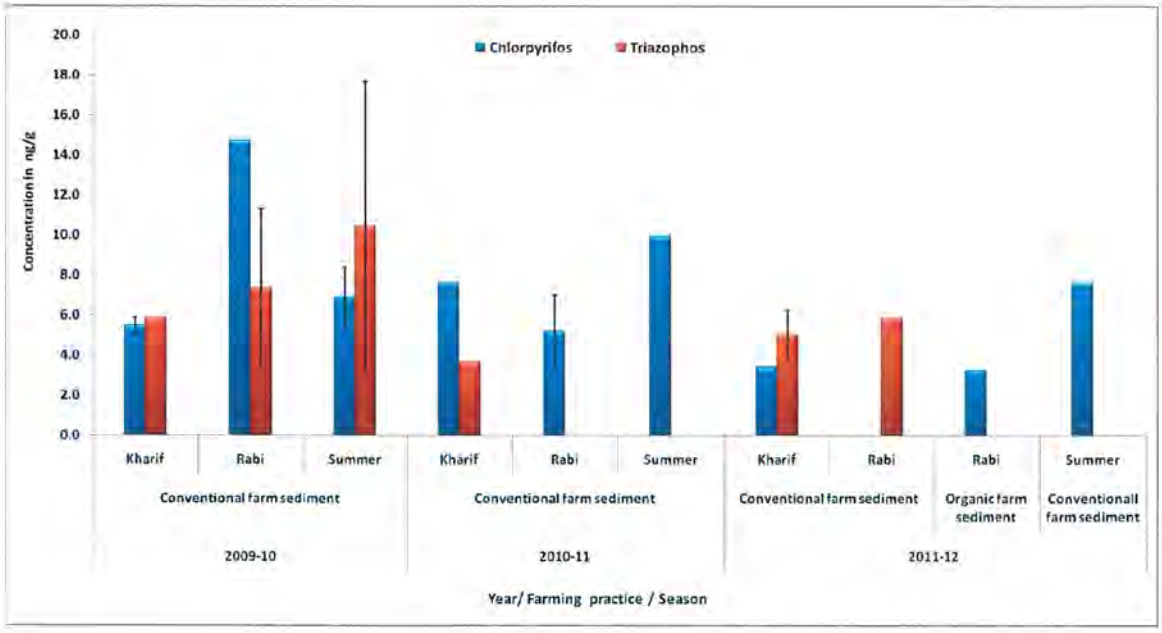



**Fig. 4.1b. Mean ( ± SE) levels of organophosphate residues in sediments in the agroecosystem (n=60; ng/g dry wt) in Padayetti village, Palakkad district (2009-12)**

Mean concentration of Chlorpyrifos (7.20 ± 0.96 ng/g) measured in the conventional farm sediments are higher than the levels reported in Basmati rice fields under Integrated Pest Management (1-3 ng/g) and non-IPM (1-4 ng/g) modules in Kaithal (Haryana) and Dehradun (Uttarakhand), India (Arora *et al.,* 2014). But Chlorpyrifos was lower than the levels reported in sediments collected from Najafgarh, Shahadra, Barapulla, Sen Nursing Home, Power House, Gunchi, Civil mill and Nizamuddin (17.29 ng/g) drains in Delhi, India (Kumar *et al.,* 2011b) and agricultural soils in Benguet (10 ng/g), Philippines (Lu, 2010). Chlorpyrifos is one of the most widely used insecticides in India. Due to its harmful POP properties and risks related to its widespread production and use, International action is warranted to control Chlorpyrifos (Watts, 2012). Triazophos is also one of the broad-spectrum, moderately toxic, nonsystemic contact pesticides. It is mainly used on paddy crop to control stem borer, leaf folder, hispa, green leafhopper, brown planthopper and white-back planthopper (Arora *et al.,* 2011). It is an acetylcholinesterase (AChE) inhibitor and neurotoxicant (Xiao *et al.,* 2010). Due to its stability and long half-life (Kunde *et al.,* 2004), Triazophos may harm non-target organisms and potentially threaten human health through food chain (Xiao *et al.,* 2010). It

also damages immune-related genes and membrane proteins of non-target organisms (Ma *et al.*, 2007; Zhong *et al.*, 2005). Indiscriminate use of Triazophos severely polluting surface water, soil, food and biota was reported in Southeastern China (Zhong *et al.*, 2006; Shen *et al.*, 2005). Microorganisms play a key role in the degradation of OP compounds and in eventually reducing their toxicity. Many bacteria have been reported to be capable of degrading these insecticides i.e. *Pseudomonas* (Liu *et al.*, 2005b; Horne *et al.*, 2002), *Flavobacter* (Sethunathan and Yoshida, 1973), *Arthrobacter* (Ohshiro *et al.*, 1999), and *Agrobacterium* (Harcourt *et al.*, 2002), *Diaphorobacter* sp. (Guo *et al.*, 2009), *Klebsiella* sp. (Wang *et al.*, 2005), and *Ochrobactrum* sp. (Dai *et al.*, 2005).

Although considerable quantities of OP pesticides are used in conventional farms in Padayetti agroecosystem, as they degrade comparatively faster in the environment, only trace levels of Chlorpyrifos and Triazophos residues were detected in sediments of conventional farms. It does not necessarily indicate that lack of impact of OP compounds on the environment. They may still cause harm before they break down to non-toxic products. According to USEPA (2000a) OPs are capable of creating deleterious effects such as reproductive failure, neurotoxicity, kidney/ liver damage and birth defects in addition to being a possible carcinogen. While the fate of OP compounds in the aquatic system is not clear, the most serious concern is that they are still suspected to create serious toxicological impacts upon terrestrial wildlife and man (Barlas, 2002).

### 4.1.3 Residues of synthetic pyrethroid pesticides in sediments in Padayetti village, Palakkad district

All the sediment samples collected from Padayetti village were screened for residues of SPs, namely Lambda (λ)-Cyhalothrin (I and II), Permethrin (I and II) and Fenvalerate (I and II). Among these, residues of ∑-Fenvalerate (62.80 ng/g) were detected only in one of the sediment samples collected from conventional farm during *Rabi* season (2011-12). Levels of other SPs were at BDL in all the other sediments analysed from both organic and conventional farms.

Residues of Fenvalerate (62.80 ng/g) recorded in the sediments of conventional farm are higher than the levels reported in sediments (1-22 ng/g) from paddy, cotton, wheat and sugarcane in Hisar, Haryana, India (Kumari *et al.,* 2008). It should be noted that Fenvalerate is one of the most persistent synthetic pyrethroids in soils (Cunninghame and Grant, 1987). It gets tightly adsorbed to soil particles, does not easily move laterally or to lower soil layers with groundwater, and almost always remains in the application site because of its extremely low solubility in water (Oros and Werner, 2005). Fenvalerate degradation rates from mineral soil surfaces are dependent on soil type, moisture, temperature and microbial activity. Although Fenvalerate is susceptible to chemical degradation by hydrolysis and oxidation, most authors agree that degradation in soils is primarily due to microbial activity. Microbial degradation is most rapid under aerobic conditions, and the transformed products do not persist longer than the parent compound (Eisler, 1992; Bennett *et al.,* 1983).

Pyrethroid insecticides are strongly hydrophobic (Oros and Werner, 2005); with octanol-water partition coefficients ($K_{OW}$) values of $10^5$-$10^7$. They tend to get adsorbed to soil and sediment rather than remaining in dissolved phase (Laskowski, 2002). High sediment concentrations of pyrethroids were reported to induce acute toxicity to sensitive aquatic species in the US (Luo and Zhang, 2011). But such kind of information on the SP residues in sediment is not available in Indian context for comparison and understanding adverse effects of these chemicals.

### 4.1.4 Residues of carbamate pesticides in sediments in Padayetti village, Palakkad district

All the samples collected from both conventional and organic farms were screened for residues of Carbaryl and Carbofuran. But the levels were BDL in both the farms, probably due to their comparatively short half-lives and higher polarity, which means that they would be absorbed and accumulated less as well as metabolized more rapidly than persistent organic contaminants.

Although we have no data on the total application of Carbofuran and Carbaryl in the study sites, farmers of the area spray up to 750 and 250 g/ha respectively. It may be worth mentioning that around 500 and 225 metric tons of Carbofuran and Carbaryl are being used annually in India for various crops (CIBRC, 2014). Below detectable levels of these residues in sediment from both organic and conventional farm is a good sign.

In India, till date there are two hundred and forty six molecules of pesticides registered under section 9(3) of the Indian Insecticides Act, 1968 (CIBRC, 2014). Based on the research carried out so far it appears that sediment could be used as one of the best contaminant indicators for assessing the quality of an agroecosystem.

### 4.2. Pesticide residues in crabs in the agroecosystem in Padayetti village, Palakkad district

Crabs are active predators and detritus feeders, occupying an intermediate position in both aquatic and terrestrial ecosystems. They are also an important food resource for fish, reptiles, birds and mammals (Collins *et al.*, 2007). Because of their capacity for bioaccumulation and their relatively elevated resistance to pesticides, crabs could transfer the pesticides to organisms in a higher trophic level, and eventually to humans (Negro *et al.*, 2012).

According to Sures (2004), there are many requirements that a species must meet to be a good biological indicator of pollution. The species must play a key role in the community, transporting the pollutants from the lower to the upper links of the food webs, or participating in the food chain of a species that is economically important to human. The use of native species as sentinel organisms is proposed as a more appropriate way to obtain information about a specific site (organic and conventional farms). Hence, *Geothelphusa dehaani,* a locally abundant crab that is widely distributed in the Padayetti agroecosystem and easy to collect was chosen to study the load of chemical contaminants.

Between May 2009 and April 2012, totally 197 freshwater paddy field crab *Geothelphusa dehaani* were collected from the organic (96 individuals) and conventional (101 individuals) farms in Padayetti village. Analyses for residues of OCs, OPs, SPs and Cbs were carried out. Among the 20 individual OCs analysed in 74 crabs from organic and conventional farms (each 37), nine samples i.e. 12.16% of crabs had detectable levels of residues with the maximum (72.30 ng/g) in crabs collected from conventional farms during summer (2011-12) and minimum (1.55 ng/g) during the same season in 2009-10. It should be noted that we did not study the biology and lifecycle of crabs in the paddy field. We collected fully grown crabs (50-100 g) by using garden hoe during summer for the study. During summer months, due to less availability of food and water the crabs residing in the mud move towards corners of paddy field bunds, dig and form a small hole like structure (20-20 cm depth). Crabs residing in the hole might have got exposed to the sinking pesticides up to 20-30 cm depth in to the sediments which were applied in the previous season i.e., *Rabi*. Moreover, shortage of water favors bioconcentration of these pollutants in the tissues. This might be applicable in the present study as well. In organic farm less than 3% of crabs collected contained persistent OC contaminants with the highest (21.32 ng/g) during *Rabi* (2011-12) and lowest (1.61 ng/g) during *Kharif* (2011-12) season. Even though these legendary chemicals have not been used for agriculture in both the farms in the recent past, physico-chemical characteristics of sediments and topography of conventional farms might have contributed to their persistence.

Among the twelve organophosphate compounds analysed, 14.86% of crabs collected from conventionally grown paddy farms had one or other residues with the highest concentrations (172.40 ng/g) during *Kharif* (2011-12), followed by 166.77 ng/g and 155.01 ng/g in crabs from the same farm and season. But the levels of all the OPs were at BDL in crabs from organic farms.

Synthetic pyrethroid residues were detected in 6 out of 74 samples (8.11%) analysed, with fairly high concentration of residues in only one of the crabs collected from conventional

farm (197.40 ng/g) during *Kharif* season (2011-12), whereas levels of these compounds were not detected in organic crabs in all the seasons during the study period.

Among the commonly used Carbamates, residues of Carbaryl and Carbofuran were analysed. One of the dead crabs collected from the conventional farm recorded 3501.91 ng/g of Carbaryl residues (*Rabi* 2011-12) very much suggestive of poisoning. Levels of Carbaryl residues were at BDL in other crabs collected from both the farms. Because of comparatively short half-lives in the biota and higher polarity, Carbaryl would be absorbed and accumulated less readily in tissues. It will be metabolized and excreted more rapidly than persistent organic contaminants. The mean concentrations of OC, OP, SP and Cb residues in *Geothelphusa dehaani* during 2009-12 are presented in fig. 4.2a.

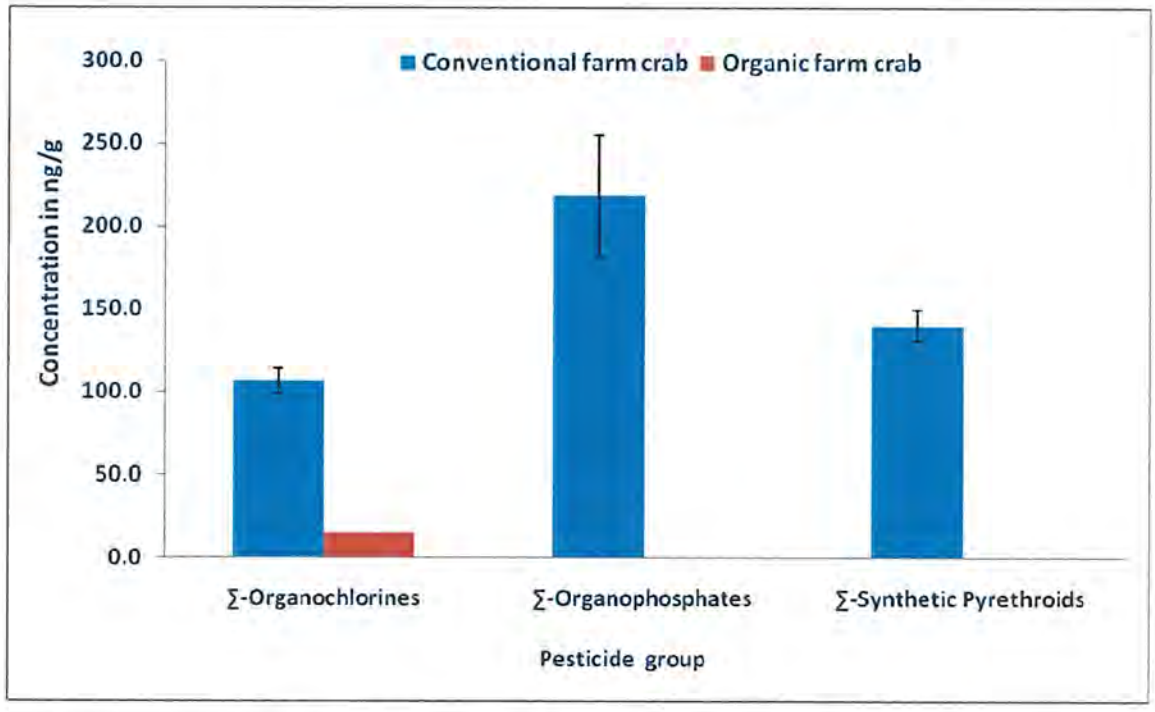



**Fig. 4.2a. Mean (± SE) levels of OC, OP and SP pesticide residues in *Geothelphusa dehaani* (n=74; ng/g wet wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12)**

Levels of currently-used pesticides (i.e OPs and SPs) varied significantly between organic and conventional farm crabs ($p < 0.05$; $F = 9.5$) but the levels of historical chemicals (i.e. OCs) did

farm (197.40 ng/g) during *Kharif* season (2011-12), whereas levels of these compounds were not detected in organic crabs in all the seasons during the study period.

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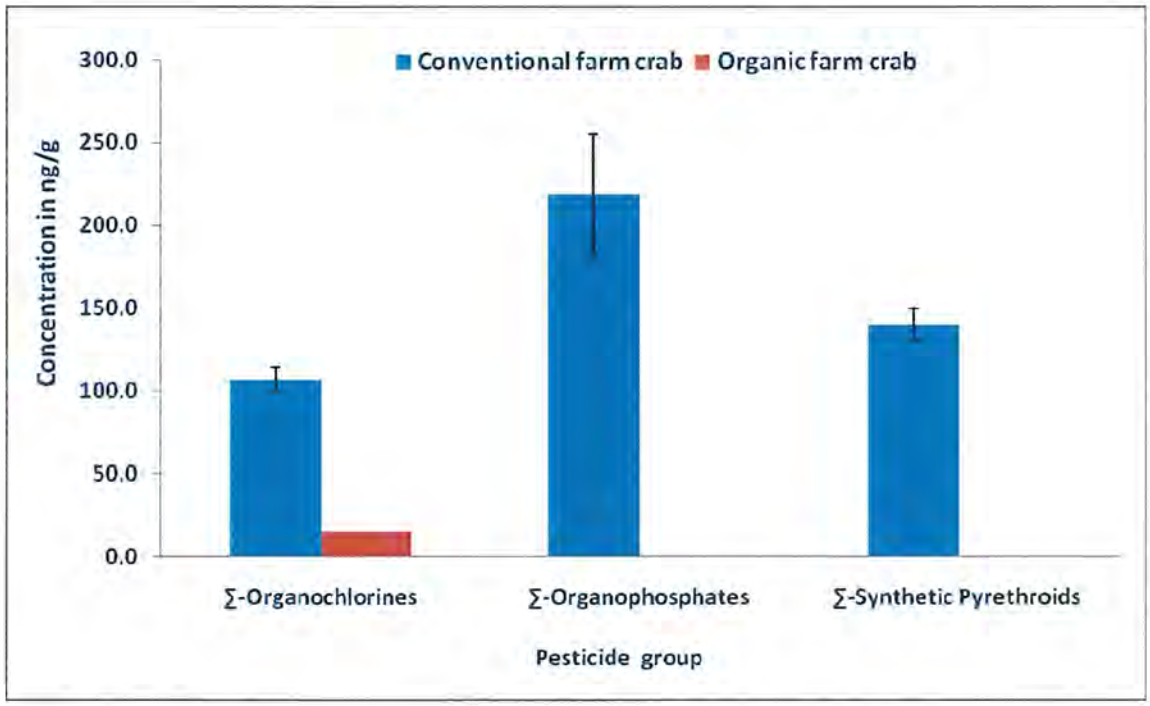



**Fig. 4.2a. Mean (± SE) levels of OC, OP and SP pesticide residues in *Geothelphusa dehaani* (n=74; ng/g wet wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12)**

Levels of currently-used pesticides (i.e OPs and SPs) varied significantly between organic and conventional farm crabs ($p < 0.05$; $F = 9.5$) but the levels of historical chemicals (i.e. OCs) did

not vary between farms ($p>0.05$; $F = 0.34$). Since carbamate residue was detected in only one of the sample, no statistical test was warranted.

### 4.2.1 Residues of major isomers/ metabolites of organochlorine pesticides in crabs in Padayetti village, Palakkad district

Among the four persistent OC contaminants measured, concentrations of ∑-HCH ranked first followed by DDT, Endosulfan and Dieldrin in the crabs collected from conventional farms. In the crabs collected from organic farms, trace amounts of ∑-HCH, ∑-Endosulfan and Dieldrin were detected and this could be due to past usage in this region. However, DDT was not detected in any of the crabs collected from organic farm.

#### 4.2.1a) HCH and its isomers

Among the four isomers of HCH analysed, namely alpha (α), beta (β), gamma (γ) and delta (δ), β- and γ-HCH were observed to be 36.15 and 25.39 ± 4.93 ng/g respectively in crabs collected from conventional farms during summer (2011-12), and traces of γ-HCH (13.54 ± 0.47 ng/g) were found in organic farm crabs collected during *Rabi* in the same year.

Among the seasons, levels of ∑-OCs were found to be high during summer (61.54 ng/g), followed by *Kharif* (25.22 ng/g) and low during *Rabi* (20.02 ng/g) season in conventional farm crabs, while in organic crabs levels were high during *Rabi* (13.54 ng/g) and low during *Kharif* (1.61 ng/g) (Fig. 4.2b). However, levels of α- and δ-HCH concentrations were at BDL in crabs from both the farms during the study period. This perhaps confirms that technical HCH is not in use presently in Padayetti village. The detection frequency of γ-HCH was 2.70% in organic farm crab *Geothelphusa dehaani*, whereas the same for β- and γ-HCH in conventional farm crabs were about 5.40 and 1.35% respectively. Moreover, in biotic components β- and γ-HCH are more stable than α- and δ-HCHs to enzymatic degradation (Ramesh *et al.*, 1992). Residues of HCH in crabs collected from both farms could only reflect its historical use.

Field studies on assessing adverse effects of HCH on wildlife, especially freshwater crabs are not available in India or elsewhere. Majority of the studies which reported residues of persistent contaminants were silent on adverse effects. In order to further elucidate, the specific residue pattern of toxic contaminants in different tropic levels, concentrations of HCHs in crabs recorded in the current study were compared with the data reported from other places in the world. It is understood that the mean concentrations of HCHs in paddy field crab *Geothelphusa dehaani* from both conventional (34.43 ± 13.07 ng/g) and organic (13.54 ng/g) farm were less than those reported in freshwater field crab *Paratelphusa gurini* (62 ng/g) and mangrove crab *Scylla serrata* (45 ng/g) collected from aquatic habitats in and around the Vellar river watersheds of Parangipettai, Southeast coast of India (Ramesh *et al.*, 1992) and marginally higher than in Burrowing crab *Chamagnathus granulata* (172 ng/g) collected from intertidal mudflats in Coastal Lagoon, Argentina (Menone *et al.*, 2000).

∑-HCH concentrations recorded in the present study are lower than the levels in crab *Oxypoda africanus* collected from Agboyi creek (155.4 ng/g) and comparable with the same species of crab (35.6 ng/g) from Lagos Lagoon in the western Nigeria (Williams *et al.*, 2013). Levels of γ-HCH (36.45 ng/g) and β-HCH (36.15 ng/g) recorded in crabs collected from conventional farms and γ-HCH (13.07 ng/g) and β-HCH (BDL) in organic farms were lower than that of the levels (γ-HCH: 2600 ng/g and β-HCH: 580 ng/g) reported by Caliskan and Yerli (2000) in blue crab *Callinectes sapidus* in Koycegiz Lagoon System in the South-western Turkey; mangrove mud/ red crab *Chasmagnathus granulate* (BDL-5.78 ng/g) collected near Rio de Janeiro, Brazil (Souza *et al.*, 2008); Mitten-handed crab *Eriocheir sinensis* (31.26 ng/g) in the paddy field ecosystem of Liaohe River plain, China (Teng *et al.*, 2013), and blue crab *Callinectus pallidus* and freshwater prawn *Macrobrachium rosenbergii* from Ologe lagoon, Lagos state, Nigeria (Clarke *et al.*, 2013).

With reference to the concentration of HCH isomers in crabs, β- and γ-HCH were slightly high. In biotic bodies β-HCH is more stable than α- and δ-HCH (Ramesh *et al.*, 1992). Hence the residues of HCH in crabs of both the farms in the present study reflect only historical use.

**4.2.1b) DDT and its metabolites**

In all the crab samples analysed, the DDT isomer *p,p'*-DDT and one of its breakdown product *p,p'*-DDE were detected only in two and one individual crabs collected from conventional farm during summer (2009-10) and *Rabi* (2011-12) respectively. To the best of our knowledge, most of the studies on pesticides in wildlife are just reports. They could be treated either as baseline information or as status report on the magnitude of contamination. None of them looked at adverse effects or impacts on any agroecosystem components and their associated biodiversity. ∑-DDT was the highest (7.46 ng/g) in crab collected from conventional farm during summer (2009-10) and lowest (2.65 ng/g) during *Rabi* season (2011-12). Moreover, incidences of DDT detection in conventional farm crabs were very low. None of the crabs collected from the organic field had residues of DDT. However, residues recorded in the present study was compared with the threshold levels prescribed by various International and national agencies wherever applicable. Since DDT was detected in few crabs, no seasonal variation comparison warranted (fig. 4.2b).

Mean concentrations of ∑-DDT in crabs collected from conventional farms was 3.89 ng/g. When this value was compared with published data, the ∑-DDT levels in freshwater paddy field crabs from the present study were slightly lower than the concentrations reported in freshwater crab *Paratelphusa gurini* (6.9 ng/g) and mangrove crab *Scylla serrata* (20.0 ng/g) collected from aquatic habitats in and around the Vellar river watershed of Parangipettai, Southeast coast of India (Ramesh *et al.*, 1992) and marine crabs *Scylla serrata* and *Ovalipes punctatus* (12.8 ng/g) reported from South China (Guo *et al.*, 2010). Further, the level is much less than those in burrowing crab *Chamagnathus granulata* (605 ng/g) collected from Coastal Lagoon in Argentina (Menone *et al.*, 2000); intertidal crab *Australoplax tridentata* from Behms creek, Burrum, Fitzroy, Logan, Maroochy, Mulgrave, Ningi, Noosa and Pimpama

Rivers (50 ng/g), Brisbane River (83 ng/g) and mud crab *Scylla serrata* (29-3200 ng/g) from Pine River and Oxley creek in Brisbane and Gladstone areas of Australia (Mortimer, 2000); River crab (526-821 ng/g) collected from Qiagtang River of China (Zhou *et al.,* 2008); sand crab (43-13000 ng/g) in California coasts of United States; Mangrove mud/ red crab *Chasmagnathus granulata* (6.53-98.22 ng/g) from Rio de Janeiro of Brazil (Souza *et al.* 2008) and Mitten-handed crab *Eriocheir sinensis* (48.26 ng/g) in the paddy agroecosystem of Liaohe River plain in China (Teng *et al.,* 2013). ∑-DDT levels reported in the conventional farm crabs in the present study were comparable with the levels in *Oxypoda africanus* (3.6-4.8 ng/g) from Lagos Lagoon of Nigeria (Williams *et al.,* 2013). Among chlorinated pesticides, DDT is the most important one, being decisive in both agricultural pest and vector control until the appearance of resistant lineages. DDT adversely affects reproduction due to its estrogen agonistic and neurotoxic properties (WHO, 1979).

**4.2.1c) Cyclodiene insecticides**

Although residues of cyclodienes, namely ∑-Heptachlor, Chlordane, Aldrin, Endrin and Mirex were BDL, trace levels of Endosulfan (2.79 ± 0.51 ng/g) and Dieldrin (4.0 ± 0.81 ng/g) were detected in crabs collected from conventional and organic farms respectively. Among the individual cyclodienes, residues of β-Endosulfan were detected at 3.80 and 2.32 ng/g in *Rabi* (2010-11) and *Kharif* (2011-12) seasons respectively in conventional farms. Dieldrin was the maximum (5.52 ng/g) during *Kharif* (2011-12), followed by 5.27 ng/g during *Rabi* (2010-11) and negligible (2.42 ng/g) during the same season in 2011-12 in conventional farms. Endosulfan sulphate (7.31 ng/g) was detected in only one crab collected from organic farm during *Rabi* (2011-12).

Levels of ∑-Endosulfan and Dieldrin reported in organic and conventional farm crabs of Padayetti village were lower than that of the levels reported by Souza *et al.* (2008) in mangrove mud/ red crab *Chasmagnathus granulate* (Endosulfan: 14.90 ng/g and Dieldrin: 7.07 ng/g) collected from Rio de Janeiro, Brazil; *Chamagnathus granulata* (Endosulfan: 605 ng/g) collected from Coastal Lagoon in Argentina (Menone *et al.,* 2000); *Oxypoda africanus*

collected from Agboyi creek (Endosulfan: 332.9 ng/g and Dieldrin: 92.8 ng/g) and comparable with the same species of crab collected from Lagos Lagoon (Endosulfan: 4.6 ng/g and Dieldrin: 7.1 ng/g) in the western part of Nigeria (Williams *et al.,* 2013). However, the trace amounts of Endosulfan and Dieldrin observed in the present study does not seem to cause any adverse effects in crabs in the study site.

Dieldrin was largely used as agriculture insecticides and also for the control of tsetse fly causing human and animal trypanosomiasis in India (Mukherjee *et al.,* 2011). Although the manufacture, use and import of Dieldrin have been banned in India since 2003, these pesticides are still persistent in the environment and may be associated with adverse neurological and reproductive effects (Mustafa *et al.,* 2010). Endosulfan continues to act long after its application due to its persistence, and it affects many nontarget organisms (Jergentz *et al.,* 2005; Ernst *et al.,* 1991). But it has a very short persistence in Crustacea (Negri *et al.,* 2009), because crab muscle tissue is not an accumulation site and possibly because of the less amount of lipids.

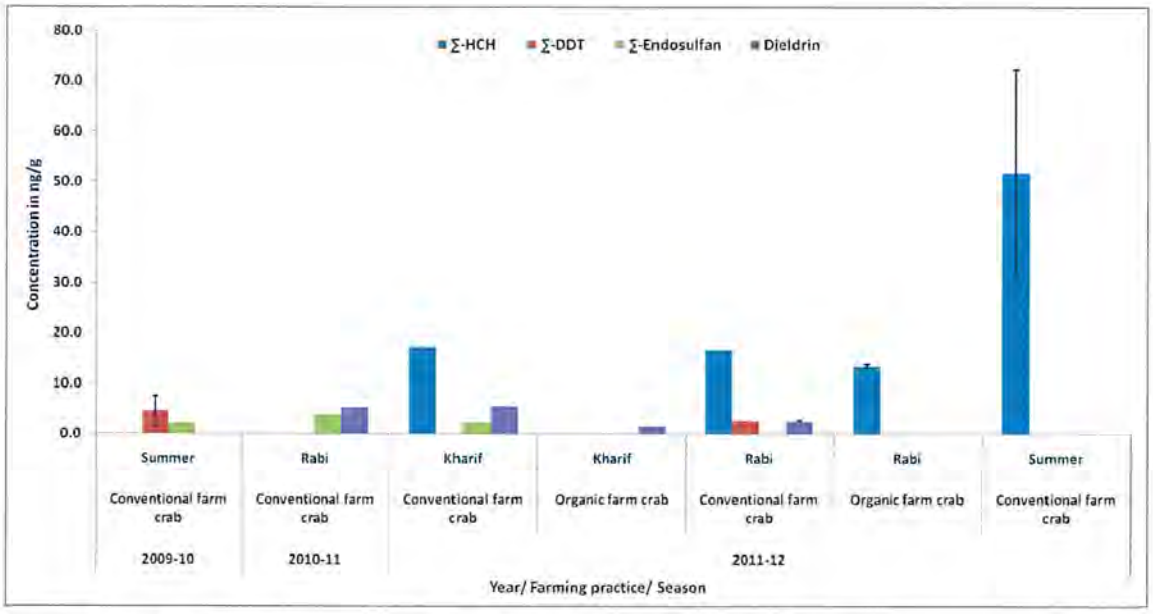



**Fig. 4.2b. Variation (season, year and farming practice wise) in organochlorine pesticide residues in crabs in the agroecosystem in Padayetti village, Palakkad district (Mean ± SE; n=74 ng/g wet wt; 2009-12)**

The field studies on Endosulfan carried out by Mortimer (2000) in intertidal burrowing crabs *Australoplax tridentata* and a Portunid *Scylla serrata* sampled from estuaries on the east coast of Australia between Cairns and Brisbane and sublethal studies done by Negro *et al.* (2012) in *Zilchiopsis collastinensis* in Parana River floodplain, Argentina and Penaeid shrimp, *Metapenaeus monoceros* collected from aquaculture pond located in Chorao Island, Goa by Suryavanshi *et al.* (2009) found that bioaccumulation of Endosulfan was found to be higher in the hepatopancreas and gonads than in the muscle tissues. But experimental exposures of Crayfish *Procambarus clarkii* to Endosulfan revealed that about 200 ng/g of Endosulfan accumulated after 8 weeks, and around 2% remained in the Crayfish after 4 weeks of depuration (Naqvi and Newton, 1991). Despite its low potential for bioaccumulation among most of the contemporary insecticides and herbicides, Endosulfan has been detected, although at extremely low concentrations, in the crabs in Padayetti agroecosystem.

In some studies Endosulfan was found mainly as Endosulfan Sulfate, with minimal accumulation as Endosulfan the parent compound, indicating that the Endosulfan may have been metabolized (Cobb *et al.,* 2003). Endosulfan is an off-patent OC insecticide and acaricide that is being phased out globally. Endosulfan became a highly controversial agrochemical in India due to its acute toxicity, potential for bioaccumulation, and its role as an endocrine disruptor (Malik, 2013). Because of its threats to human health and the environment, a global ban on the manufacture and use of Endosulfan was negotiated under the Stockholm Convention in April 2011. It may be noted that Kerala government banned Endosulfan way back in 2002 particularly in Kasargod district, Kerala. More than 80 countries, including the European Commission, Australia and New Zealand, several West African nations, the United States, Brazil and Canada had already banned it or announced phase outs by the time the Stockholm Convention's ban was agreed upon. The Supreme Court of India banned the toxic Endosulfan in the country with effect from May, 2011, with certain exemptions for five additional years (Malik, 2013).

In the present study, the concentration of OCs was low in crabs collected from both organic and conventional farms, often just above the limits of reporting and it is likely that many of the apparently uncontaminated crabs did contain OC concentrations below the level of detection. By and large low concentrations contributed to patchy detection in some samples and made a definitive comparison difficult among seasons and years (fig. 4.2b). Nevertheless in medium to long term, the residues of OCs such as HCH, DDT and Dieldrin are likely to drop in the body burdens of crabs and may be difficult to be detected in 10 years from now.

### 4.2.2 Residues of OP pesticides in crabs in Padayetti village, Palakkad district

Residues of organophosphate pesticides such as Chlorpyrifos (8 samples), Phenthoate (3 samples), Phosphamidon (2 samples), Methyl Parathion (1 sample) and Parathion Ethyl (1 sample) were detected in crabs from conventional farms. Levels of other OPs (Phorate, Malathion, Pirimiphos Ethyl, Quinalphos, Ethion and Triazophos) were at BDL. However, levels of all the OPs were BDL in all the crabs studied from organic farms.

$\Sigma$-OP in conventional farm crab was found to be the highest (164.59 ± 5.39 ng/g) during *Kharif* season in 2011-12 and lowest (1.54 ± 0.12 ng/g) during summer (2009-10). The season, year and farming practice wise variation in OPs are presented in fig. 4.2c. Among the individual OP pesticides analysed in crabs, Chlorpyrifos was detected in 10.81% of samples collected from conventional farms, while Methyl Parathion, Parathion Ethyl, Phosphamidon and Phenthoate were detected in less than 4%. Levels of all these OPs analysed were at BDL in crabs collected from organic farms. High concentration of Chlorpyrifos (158.0 ± 5.34 ng/g) in the conventional farm crabs during *Kharif* 2011-12 and trace amounts of Methyl Parathion (1.06 ng/g) during the same season and at the same site were recorded. As mentioned elsewhere in the report, high levels of Chlorpyrifos residues in conventional farm crabs indicate the excessive use of this pesticide for the control of various insect pests on paddy.

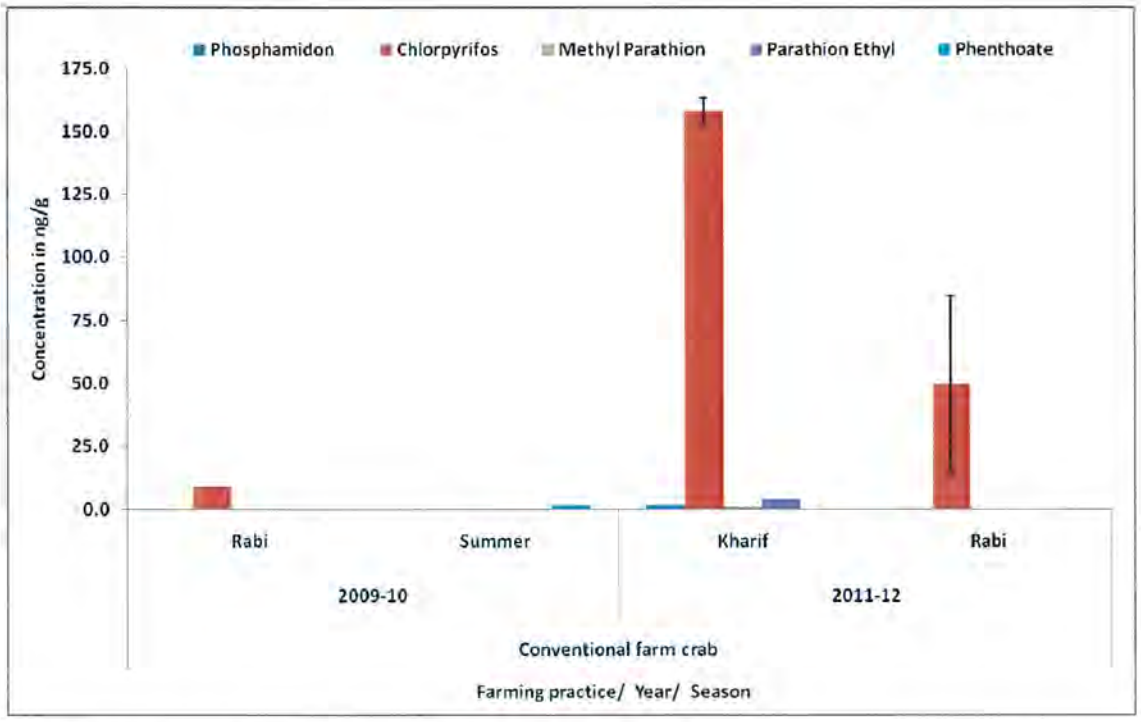



**Fig. 4.2c. Variation (season, year and farming practice wise) in organophosphate pesticide residues in crabs in the agroecosystem in Padayetti village, Palakkad district**

Studies documenting the residue levels of currently-used pesticides on wildlife are scattered all over the world. Mean concentration of Chlorpyrifos (98.62 ± 25.78 ng/g) recorded in the conventional farm crabs are higher than the levels reported in mud crab *Scylla serrata* (7 ng/g) collected in Oxley creek in Gladstone area of Australia (Mortimer, 2000) and comparable with shore crabs *Hemigrapsus oregonensis* and *Pachygrapsus crassipes* collected from Stege Marsh in Northern California state of the US (Smalling *et al.,* 2010). But there are hardly any field based assessment studies on OP residues available in Indian context.

The OP pesticides are known to affect the crustaceans (Srivastava *et al.,* 2013). There are not many studies available on sub lethal toxicity of OP pesticides in crabs. However, toxicity of Chlorpyrifos to freshwater field crab *Oziotelphusa sensex sensex* (Radhakrishnaiah and Renukadevi, 1990), acute toxicity in edible crab *Paratelphusa hydrodromus* (Chalasani and Sandeep, 2004), acute toxicity of Chlorpyrifos (460.9 µg) to the Chinese mitten-handed crab, *Eriocheir sinensis* (Li *et al.,* 2006), effects on survival and growth in *Palaemonetes*

*argentines* (Montagna and Collins, 2007) and also increased oxygen consumption and ammonia excretion in freshwater crab *Trichodactylus borellianus* (Montagna and Collins 2008) are available. Moreover, Monocrotophos disturbance of neuroendocrine regulation in freshwater crab, *Barytelphusa guerini* was also reported (Patil and Paul, 2008).

Senthilkumar *et al.* (2007) reported $LC_{50}$ of Chlorpyrifos to freshwater crab *Siralothelphusa hydrodroma* and inferred that crabs showed high tolerance to Chlorpyrifos than the fishes (De Mel and Parthiratne, 2005; Rao *et al.*, 2005) possibly due to the hard chitinous exoskeleton in crabs. A very recent study on acute toxicity and behavioural responses of freshwater crab *Barytelphusa guerini* from River Gomti near Sultanpur, India to Chlorpyrifos revealed that the exposed crab exhibited restlessness, escape behavior, increased scaphognathite activity, topsy-turving response and increased $O_2$ consumption with increasing toxicant concentration and exposure time (Srivastava *et al.*, 2013). But information on the OP residues in tissues of crabs is not available.

Chlorpyrifos is used extensively for the management of domestic and agricultural pests and is effective in controlling Coleoptera, Diptera, Homoptera and Lepidoptera in soil or on foliage in over 100 crops (Tomlin, 2001). Chlorpyrifos is available in India in different trade names, namely Classic, Tebafam, Radar, Fentum, Tarkash, Nuclor, Kohiban, Suban, Strike, Trishul, Chlorguard and Command (CIBRC, 2014). It is available in two forms (10% G and 20% EC) and also used for controlling paddy stem borer, leaf roller, gall midge and rice hispa (Arora *et al.*, 2011). It is the second largest OP insecticide used in India (Srivastava *et al.*, 2013). The OP pesticides also inhibit AChE activity (Ghedira *et al.*, 2009) and affect neuronal control of heart in crab (Lundebye *et al.*, 1997). Narra *et al.* (2012) reported alterations in enzyme activity (increased LDH; decreased SDH and ACP) in nervous tissues of crab. However, levels reported in the present study may not cause any adverse effects.

Trace amounts of Parathion Ethyl (3.78 ng/g), Phosphamidon (1.75 ± 0.56 ng/g), Phenthoate (1.54 ± 0.12 ng/g) and Methyl Parathion (1.06 ng/g) were observed in the conventional farm

crabs. Although they do not stay in the system for long time, they are also capable of creating ecological imbalance at various levels and cause serious physiological effects.

Information on the toxicity of Phosphamidon to common South Indian Earthworm *Lampeto mauritii* (Bharathi and Subba Rao, 1984), and American lobsters *Homarus americanus* in Canada (Mcleese, 1974) is available. Histopathological alterations have previously been reported in the giant estuarine crab *Chasmagnathus granulatus* exposed to Methyl Parathion (Bianchini and Monserrat, 2007) at 0.05 mg/kg/day (72 hr-$LD_{50}$).

Phosphamidon is a widely used pesticide in India on paddy against stem and leaf borers, green leaf hoppers and brown plant hoppers. Sublethal concentrations of Phosphamidon which could inhibit glutamate oxidation, aspartate, alanine and aminotransferases in Penaeid Prawn, *Metapenaus monoceros* in Buckingham canal near the Kavali coast, India are available (Reddy and Rao, 1990). Nevertheless, the amount of Phosphamidon observed in the present study does not seem to cause any adverse effects in crabs in the study site.
Organophosphorus compounds are one of the most widely used pesticides in the world replacing the organochlorines. OP pesticides have a common mechanism of action even though each of their chemical structure varies in nature. They inhibit of the acetylcholinesterase (AChE) activity (Chandrasekara and Pathiratne, 2005). Responses to OP insecticides by aquatic organisms are broad ranged depending on the compound, exposure time, water quality, organism, sex, age and the species (Narra *et al.*, 2011). However, there is not much of research on crabs that live in the freshwater agroecosystem. The long-term exposure to these new generation pesticides need to be evaluated as these chemicals present great risks to wildlife, environment and man. Regulatory agencies in India should consider additional restrictions on the use of these problem pesticides. The OP insecticides such as Chlorpyrifos, Methyl Parathion, Parathion Ethyl and Phosphamidon are widely used to control various insect pests on paddy crops in India, but their uses have been restricted due to their ecotoxicity (CIBRC, 2014). These pesticides are likely to be completely prohibited

in India in the near future. Hence, pesticides posing less environmental risk should be introduced to substitute OP insecticides to control the referred pests on rice.

### 4.2.3 Residues of synthetic pyrethroid pesticides in crabs in Padayetti village, Palakkad district

Of the six SPs analysed, residues of λ-Cyhalothrin, Fenvalerate-I and Fenvalerate-II were detected in one, two and three samples of crabs from conventional farms respectively. None of the crabs from organic farm had any detectable residues of any of the SPs. ∑-SP residues were found to be the highest (59.18 ± 27.94 ng/g) in conventional farm crabs collected during *Kharif* season (2011-12) and lowest (15.97 ng/g) during the same year in *Rabi* season.

Among the individual SPs measured, residues of Fenvalerate-I (40.8 ng/g) were detected the maximum in conventional farm crabs collected during *Kharif* (2009-10), followed by both Fenvalerate-II (39.59 ng/g) and Fenvalerate-I (39.39 ± 13.95 ng/g) during the same season in 2011-12 and at the same site (fig. 4.2d). Whereas levels of other SP (Permethrin-I and II) were not detected in any other crabs analysed from both organic and conventional farms.
The SPs effectively control paddy field arthropod pests at low doses. However, pyrethroids' toxicity highly depend on their stereochemistry and moreover each isomer has its own toxicity (Liu *et al.*, 2005a). Among pyrethroids, Fenvalerate highly toxic to aquatic organisms, with their acute toxicity levels for lobsters and shrimp as low as 0.04 and 0.01 µg/L respectively (Mueller-Beilschmidt, 1990). λ-Cyhalothrin similar to OCs is lipophilic, which favors adsorption to biota and highly toxic to aquatic invertebrates (Hand *et al.*, 2001). Moreover, it is less persistence both in water and soil. Photo- and hydrolysis are the main degradation processes (Laskowski, 2002). Although faster dissipation of λ-Cyhalothrin will mean low ecotoxic effects (Benli *et al.*, 2009), however, the real environmental risk will not only be determined by the toxicological data but also by their application rate, environmental fate and metabolism (Rand and Clark, 2000). Although λ-Cyhalothrin exhibits toxicity to crab, it degrades quickly in the aquatic environment. Hence, application of suspension concentrate (SC) of λ-Cyhalothrin is much safer than emulsion in water (EW),

wettable powder (DP), emulsifiable concentrate (EC) and microemulsion (ME) formulations. (www.fao.org/ag /againfo/programmes/manuals/vol5_2.html retrieved on 21 Oct. 2014).

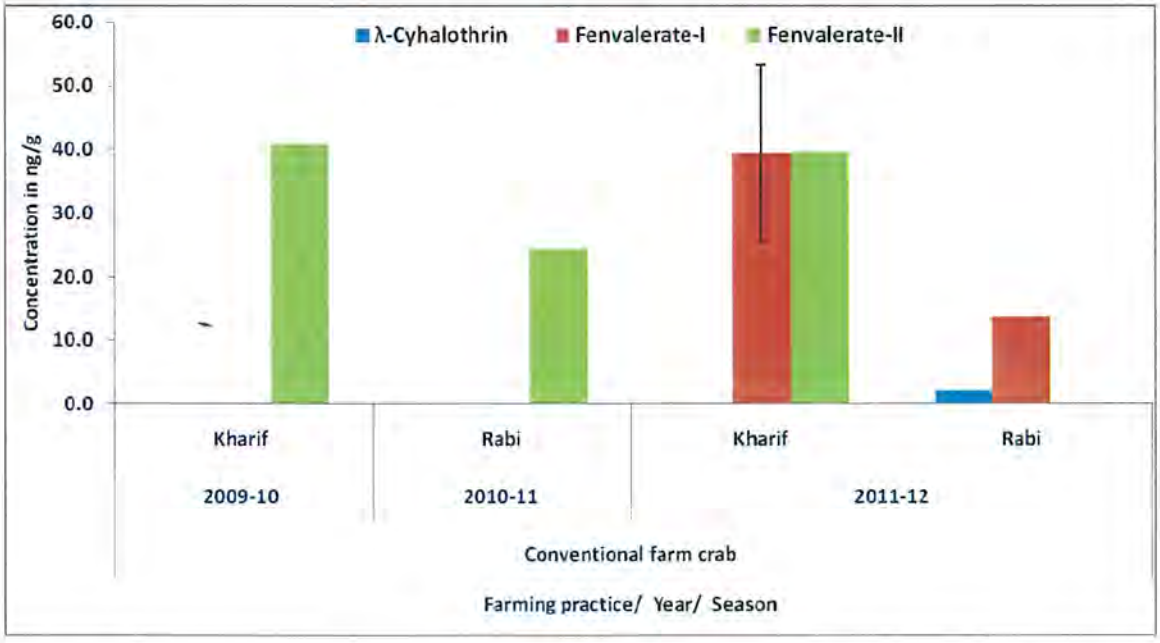



**Fig. 4.2d. Variation (season, year and farming practice wise) in synthetic pyrethroid pesticide residues in crabs in the agroecosystem in Padayetti village, Palakkad district**

### 4.2.4 Residues of carbamate pesticides in crabs in Padayetti village, Palakkad district

Commonly used carbamates such as Carbaryl and Carbofuran were analysed in all the samples of crabs collected. One of the dead crabs *Geothelphusa dehaani* (fig. 4.2e) collected from the conventional farm during *Rabi* season (2011-12) recorded very high concentration of Carbaryl (3501.91 ng/g). The observation at the site was that the crab was found dead in a place where Carbaryl had been applied the previous day. It should be noted that one of the farmers in conventional farm applied Carbaryl 5% DP (trade name: Sevin) for controlling leaf roller/ folder and brown planthopper and disposed off the empty plastic bag adjacent to the paddy field. The crab residing nearby was exposed to high concentration of Carbaryl. Levels of Carbofuran were at BDL in other crabs from both organic and conventional farms.



**Fig. 4.2e. Dead crab, *Geothelphusa dehaani* in the conventional farm, Padayetti village (Inset: Empty pesticide sachet disposed off in the paddy field)**

Carbaryl is used to control brown planthopper and other sap sucking insects (leaf roller/ folder) on paddy. It is sold under the trade names of Sevin, Dhanuvin, Anuvin, Devicarb, Carvit, Corovin and Cabin in India. Products containing Carbaryl is available in seven different forms (5 & 10% DP, 50% & 85% WP, 4% G, 40% LV and 42% flow) in India (Arora *et al.,* 2011).

Similar to other members of the carbamate family, Carbaryl works by attacking the nervous system. Specifically, it inhibits the essential action of the enzyme, acetylcholinesterase (AChE). AChE works to break down another chemical, acetylcholine, which is essential in transmitting impulses between nerves. Therefore, when Carbaryl is used, AChE becomes incapable of breaking down acetylcholine, which consequently accumulates in nerve cells. This abnormal acetylcholine build-up causes incoordination, rapid twitching, paralysis and eventually death (Gallo and Lawryk, 1991).

Carbaryl has deleterious effects on a variety of wildlife including beneficial arthropods, birds, fish, earthworms, plants and bacteria as well as crustaceans. Since it is a broad spectrum insecticide with a mode of action common to the majority of living organisms, its target, the mites are not the only organism killed by its use, but numerous beneficial insects. This has

been found in both agricultural and non-agricultural systems (Price and Schuster, 1991). One of the chemicals used in the manufacture of Carbaryl is methyl isocyanate (MIC). On December 3, 1984, a gas leak occurred at the Union Carbide factory in Bhopal where both Carbaryl and Aldicarb were produced (Everest, 1986). MIC is slightly lighter than water but heavier than air, so it escapes into the atmosphere and stays low to the ground. Approximately 40 tons of MIC poured out of the leaky tank for 2 hours, escaping into the air and spreading as far as eight kilometers downwind over a city of nearly 9,00,000 people. Although estimates ranged as high as 5,000, at least 3,000 people died in the immediate aftermath, and over half a million seriously injured within the first three days. Since then at least 20,000 other deaths have occurred. Despite the damages that MIC exposure caused among people such as eye and lung damage, emphysema, gastrointestinal, muscoskeletal gynecological and vision problems and neurological disorders. Union Carbicide, now Dow chemical, claimed that MIC is only a "mild throat and ear irritant" (http://www.sierraclub.ca/ retrieved on 22$^{nd}$ Feb. 2014).

Carbaryl is toxic to crustaceans, with a sublethal dose of 320 ng/g for blue crab (Mayer, 1987). It does not readily volatize into the atmosphere and unlikely to volatize from water to air. The inability of crabs to accumulate measurable concentrations of contemporary pesticides is due to their chemical nature such as relatively higher polarity and lower environmental persistence. Herbicides, namely 2,4-D and 2,4,5 T were not analysed in the present study since they rapidly degrade (Spain and van veld, 1983) and get eliminated soon from the body (Wang *et al.*, 1994a,b). Although these herbicides are non-persistent in biota, this does not mean that they do not have significant impacts. The capacity of *Geothelphusa dehaani* to accumulate OC, OP, SP and Cb pesticides makes it suitable to be considered as bioindicator.

### 4.3 Pesticide residues in snails in the agroecosystem in Padayetti village, Palakkad district

Freshwater snail *Pila globosa* has been considered as one of the bioindicator organisms for monitoring pesticides in paddy agroecosystem because of its general abundance, sedentary

and filter feeding habit. Moreover, these factors make the exposure to agricultural chemicals quite high (Moulton *et al.*, 1996). Snails are easily available for collection (Wang *et al.*, 2007), highly tolerant to many pollutants and exhibit high accumulation property (Kim *et al.*, 2002). Hence, the analyses of OC, OP, SP and Cbs in snail could help monitor the quality of the agroecosystem.

A total of 476 individuals of *Pila globosa* (234 from organic and 242 from conventional farms) were collected seasonally (*Kharif, Rabi* and summer) between May 2009 and April 2012. The present study reports the levels of 40 pesticide residues (20 OCs, 12 OPs, six SPs and two Cbs) in 64 snail samples (each 32 from organic and conventional farms).

Among the OCs and their metabolites or isomers analysed in snails, the residues of HCH (α-, β-, γ-, δ-HCH), DDT (*o,p*-DDT, *p,p'*-DDD, *p,p'*-DDT) and Dieldrin were detected in 6 out of 32 samples (18.75%) collected from conventional farms with the highest (83.91 ± 4.17 ng/g) during *Kharif* season (2009-10) and lowest (3.33 ± 0.17 ng/g) during the same season in 2010-11. The levels of these residues were also detected fairly high (80.87 ± 0.03 ng/g) in snails collected from organic farm during *Kharif* (2009-10) (fig. 4.3a). It should be noted that most of the persistent OCs are banned for agricultural use in India except DDT which is being used for vector control. Further, due to the past usage of these pesticides in the region snails from both the farms had slightly high levels of them. The statistical analyses revealed that the ∑-OCs especially γ-HCH varied significantly among the years ($p < 0.05$; $F = 8.03$) during the study period.

Among a set of currently-used OP compounds analysed, trace amounts of Triazophos (9.96 ng/g) and Phorate (5.18 ng/g) were detected in each one of the snail samples collected from conventional farms during 2009-10 *Kharif* and *Rabi* seasons respectively. However, levels of all the OPs were at BDL in all the snails studied from organic farms (fig. 4.3a).

∑-SP residues were detected in two and one of the conventional and organic farm snails respectively. Maximum of 20.33 ng/g was measured in conventional farm snails collected during *Kharif* 2009-10 and minimum of 6.76 ng/g in organic snails collected during the same season in 2011-12 (fig. 4.3a). Presence of SP residues in snails from organic farm could be because a few farmers practicing organic farming resorted to using Fenvalerate to contain sucking insect such as rice bugs *Leptocorisa* sp (Hemiptera: Alydidae) and stink bugs *Pygomenida* sp (Hemiptera: Pentatomidae) on paddy. Unfortunately biological control methods were not adopted perhaps in time to contain the above-referred pest. It was further observed that the farmers of organic farming did not apply any other pesticide.

Residue levels of two Carbamate pesticides, namely Carbaryl and Carbofuran were at BDL in all the snails collected from both the farms during the study period. Mean concentrations of the OCs, OPs and SPs residue levels in snails are presented in fig. 4.3a.

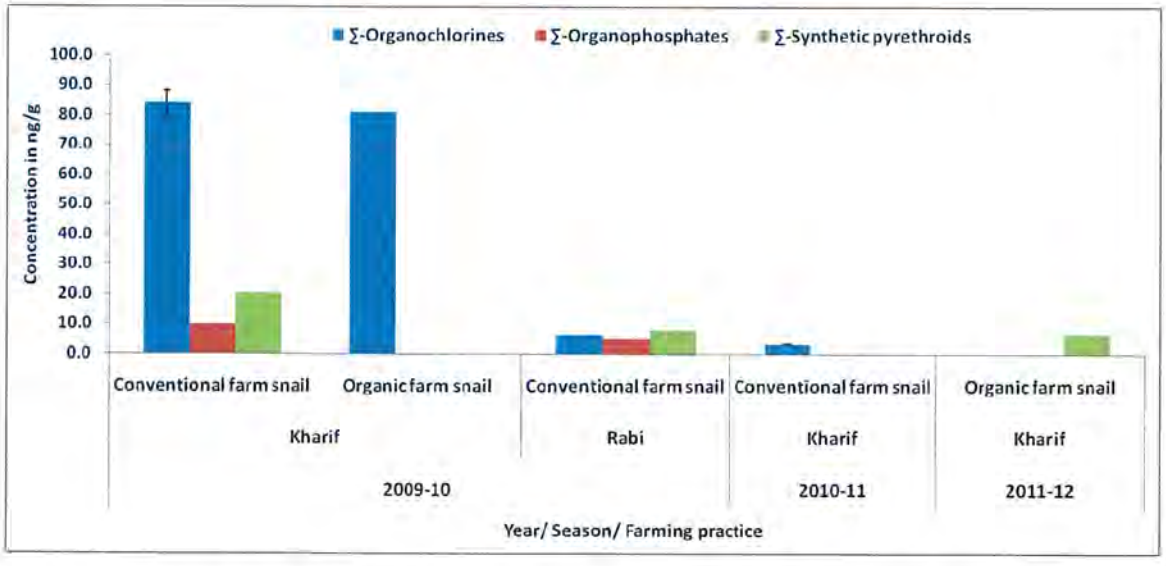



**Fig. 4.3a. Mean ( ± SE) levels of OC, OP and SP residues in snails (n=64; ng/g wet wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12)**

### 4.3.1 Residues of major isomers/ metabolites of organochlorine pesticides in snails in Padayetti village, Palakkad district

Although snails had majority of the OCs studied including HCH isomers (α-, β-, γ- and δ-HCH), DDT metabolites (*o,p*-DDT, *p,p'*-DDD, *p,p'*-DDT) and Dieldrin, the levels and detection frequencies were low. Levels of OCs in snail tissues were in the order of HCHs > cyclodienes > DDTs. Between the farms, concentrations of HCHs were higher in snails from conventional farm than Dieldrin and DDTs, whereas in snails from organic farm, only detectable amounts of HCHs and Dieldrin were detected. This may be due to their past usage in this region. Further these compounds were detected only during the first year of the study i.e. 2009-10. However levels of DDT and Dieldrin were not detected in snails of both the farms collected during the subsequent years, except the trace levels of ∑-HCH (3.33 ± 0.17 ng/g) measured in conventional farm snail during *Kharif* 2011-12. Levels of γ-HCH in snails did not vary significantly between the farms. Further, due to small detection frequency of residues, we did not attempt any statistical tests for other HCH isomers (α-, β-, δ-HCH), DDT or Dieldrin.

#### 4.3.1 a) HCH and its isomers

Mean concentrations of Σ-HCH were found to be 75.66 ± 5.24 ng/g and 29.12 ± 13.61 ng/g in organic and conventional farm snails respectively during *Kharif* 2009-10. However, the variations of HCH concentration between organic and conventional farms were not significant ($p > 0.05$; $F = 2.62$). γ-HCH was the dominant contaminant among the HCH isomers (α, β, γ and δ) analysed, varying from BDL to 63.52 ng/g in snails collected from conventional farm and BDL to 80.90 ng/g in snails from organic farm (fig. 4.3b). Although α-, β- and δ-HCH were detected in only one of the conventional farm snails, γ-HCH was detected in 5 out of 32 samples from conventional farm, but in snails from organic farm, the residues of γ-HCH were detected only in two samples. Among the other isomers of HCH, the levels of α-, β- and δ-HCH were found to be 23.63, 14.90 and 3.39 ng/g respectively in snail collected from conventional farm during *Kharif* 2009-10. The seasonal variation in γ-HCH residues was found to be significant ($p < 0.05$; $F = 8.03$).

Since, there is little information available on organic pollutants in freshwater snail from Padayetti village, Kerala or any part of India, levels documented in the present study were compared with information available in similar life forms elsewhere in the world. Concentrations of ∑-HCH observed in snails from the conventional (BDL - 63.52 ng/g) and organic (BDL - 80.90 ng/g) farms were lesser than those in mussel *Perna viridis* (16.2 - 183.4 ng/g) collected from coastal waters of Xiamen, China (Khaled *et al.*, 2004); Gulf of Thailand (1.0 - 23800 ng/g: Siriwong *et al.*, 1991).

Reported HCH levels were reasonably higher than in fresh water snail *Angulyagra* sp from Hanoi, Vietnam (0.07-3.12 ng/g: Nhan *et al.*, 2001); green mussel *Perna viridis*, blue mussel *Mytilus gallorovincialis*, and ezo mussel *Crenomytilus grayamus* collected from various locations in India (Mumbai : 210 ng/g; Goa : 67 ng/g; Cochin: 180 ng/g; Kannniyakumari : 20 ng/g; Akkaraipattinam: 87 ng/g; Nagapattinam: 110 ng/g; Portonovo: 430 ng/g; Cuddalore: 100 ng/g; Pondicherry: 58 ng/g; Kasimedu: 19 ng/g; Ennore: 20 ng/g; Machilipatinam: 130 ng/g; Kakinada: 120 ng/g; Visakapatnam: 100 ng/g; Gopalpular: 120 ng/g; Subaranarekha: 110 ng/g and Digha: 140 ng/g and other Asian countries (Cambodia: 0.40-1.1 ng/g; China: 11-110 ng/g; Hong Kong: 2.1-29 ng/g; Indonesia: 0.80-5.3 ng/g; Korea: 1.9-80 ng/g; Japan: 13-50 ng/g; Malaysia: 0.10-9.4 ng/g; Philippines: 0.60-4.0 ng/g; Far East Russia: 34-57 ng/g; Singapore: 12 ng/g and Vietnam: 2.9-12 ng/g) as a part of assessing the quality of coastal waters in Asia-Pacific region using mussels as sentinel organism (Monirith *et al.*, 2003); Bivalves *Arca senisis* and *Crassostera gasar* and gastropods (*Conus* sp. and *Pugilina morio*) from Senegalese stations (0.2-1.6 ng/g) (Bodin *et al.*, 2011).

The detection percentage of γ-HCH was 16.63 and 6.25% in snails from conventional and organic farm respectively. However, the variation in residues between the farms was not quite significant ($p > 0.05$; $F = 4.11$). The detection frequency of α-, β- and δ-HCH in snails from conventional farm was 1.56%. Absence of detectable levels of these compounds in snails collected from both the farms during the subsequent study period indicates that the pesticide residues perhaps are on the path of disappearance.

#### 4.3.1b) DDT and its metabolites

Measurable concentrations of metabolites of DDT, namely *p,p'*-DDT (12.13 ng/g), and *o,p*-DDT and *p,p'*-DDE (each 2.40 ng/g) were observed in snails collected from conventional farms during *Kharif* 2009-10. However, these residues were detected in < 0.5% of samples. Levels of DDT isomers and its breakdown products in all the snails collected from organic farm were at BDL (fig. 4.3b).

Concentrations of ∑-DDT reported in the present study were lower than the levels reported in mussels and oysters (424 ng/g) in USA (Wade *et al.*, 1998) and *Perna viridis* (124.9-772.3 ng/g) collected from aquaculture cages in coastal waters of Xiamen, China (Khaled *et al.*, 2004); green mussel *Perna viridis*, blue mussel *Mytilus gallorovincialis*, and ezo mussel *Crenomytilus grayamus* collected from various locations in India (Mumbai: 3000 ng/g; Goa: 230 ng/g; Cochin: 770 ng/g; Kannniyakumari: 160 ng/g; Akkaraipattinam: 130 ng/g; Nagapattinam: 120 ng/g; Portonovo: 71 ng/g; Cuddalore: 130 ng/g; Pondicherry: 450 ng/g; Kasimedu: 230 ng/g; Ennore: 78 ng/g; Machilipatinam: 58 ng/g; Kakinada: 50 ng/g; Visakhapatnam: 29 ng/g; Gopalpular: 50 ng/g; Subaranarekha: 520 ng/g and Digha: 280 ng/g) and other Asian countries (Cambodia: 16-48 ng/g; China: 830-54,000 ng/g; Hong Kong: 640-61,000 ng/g; Indonesia: 15-120 ng/g; Korea: 14-350 ng/g; Japan: BDL-790 ng/g; Malaysia: 16-270 ng/g; Philippines: 3.3-38 ng/g; Far East Russia: 520-730 ng/g; Singapore: 110 ng/g and Vietnam: 220-34,000 ng/g) as part of assessing the quality of coastal waters in Asia-Pacific region using mussels as sentinel organism (Monirith *et al.*, 2003); River shed *Hilsa ilisha* from south patches of Bay of Bengal (1771.67-2254.12 ng/g: Das and Das, 2004); and Bivalves *Arca senisis* and *Crassostera gasar* and gastropods (*Conus* sp. *Hexaplex duplex* and *Pugilina morio*) from Senegalese stations (1.1-14.8 ng/g) (Bodin *et al.*, 2011). Although studies in India or elsewhere reported high concentrations of residues in snails but they did not report any adverse effects. However, the levels observed in the present study indicate that DDT residues in environmental components are on the decline.

While manufactured DDT contains the byproducts of DDD and DDE, the technical DDT generally contains 75% *p,p'*-DDT, 15% *o,p'*-DDT, 5% *p,p'*-DDE (Kim *et al.,* 2002). The estimated environmental half-life for DDT from studies on mollusc is estimated at 10-20 years (Sericano *et al.,* 1995), and in this process, DDT is transformed to DDE and DDD (Klumpp *et al.,* 2002). Since DDT could undergo biological degradation to DDD under anaerobic conditions and DDE under aerobic conditions, the ratio of *p,p'*-DDT to the sum of two metabolites (*p,p'*-DDD and *p,p'*-DDE) could be used as an indicator to identify a recent input of technical DDT or retarded degradation according to environmental conditions (Zhang *et al.,* 2009). Trace amounts of ∑-DDT observed in the conventional farm snails in the present study confirms environmental stability of DDD and DDE in biological components.

The use of DDT in agriculture was banned in 1989 in India. It is one of the reasons for the decrease in concentration of OCP residues in lower level organisms in the agroecosystem of Kerala. The emission sources of a number of POPs (such as DDTs and HCHs) in the last 20 years have shifted from industrialized countries of northern hemisphere to developing countries in tropical and subtropical regions, owing to the delayed production ban or still being used both legally and illegally in agriculture and disease control such as malaria and typhus in India and other Asian countries (Subramanian and Tanabe, 2007; Senthilkumar *et al.,* 2001). Their long persistence is the reason that residues of DDT and its metabolites could still be detected in the environment. The degree of persistence of DDE varies considerably, as persistence depends on the medium and many environmental parameters. Due to their long environmental half-life, atmospheric depositions from remote sources, and sanitary use of DDT entering nearby agricultural field, concentrations of chlorinated hydrocarbons, although decreasing with time, will remain at measurable levels for years to come.

#### 4.3.1c) Cyclodiene insecticides

Among the cyclodienes, mean concentrations of Dieldrin were found to be 26.97 ± 3.32 ng/g in snails from conventional farm with highest (30.29 ng/g) during *Kharif* (2009-10) and lowest (6.35 ng/g) during *Rabi* season in the same year (fig. 4.3b). Levels of other cyclodienes

(α- Endosulfan, β- Endosulfan and Endosulfan Sulphate, Heptachlor and Heptachlor Epoxide, Chlordane, Aldrin, Endrin and Mirex) were below detectable levels or concentrations of residues lower than the detection limit in samples of snails collected from both the farms. Dieldrin was detected in only one snail (10.40 ng/g) collected from organic farm during *Kharif* (2009-10).

The residues of Dieldrin in the snails in the present study were relatively low in both conventional and organic farms, when compared with the residues reported in other areas; mussel *Perna viridis* (2.2-58.7 ng/g) collected from aquaculture cages in coastal waters of Xiamen, China (Khaled *et al.,* 2004); *Mytilus galloprovincialis* (1.8-36.0 ng/g) in France and Italy (Villeneuve *et al.,* 1999) and reasonably higher than freshwater snail *Angulyagra* sp from Hanoi, Vietnam (0.01-0.15 ng/g: Nhan *et al.,* 2001).

Dieldrin and Aldrin were used extensively in the past (Hanley *et al.,* 2002). While Aldrin was banned in 1996, Dieldrin was prohibited for use in agricultural activities in India since 2003 (Mustafa *et al.,* 2010). Hence, unless there is illegal use, environmental residue levels of Dieldrin should decline. Levels of HCH, DDT and Dieldrin in snails showed a clear decreasing trend when we compared with earlier studies reported in snail in India. In the case of DDT and HCH the studies show that the restrictions imposed on their usage by government of India are effective and India is becoming cleaner in terms of these classical OCs (Subramanian and Tanabe, 2007).

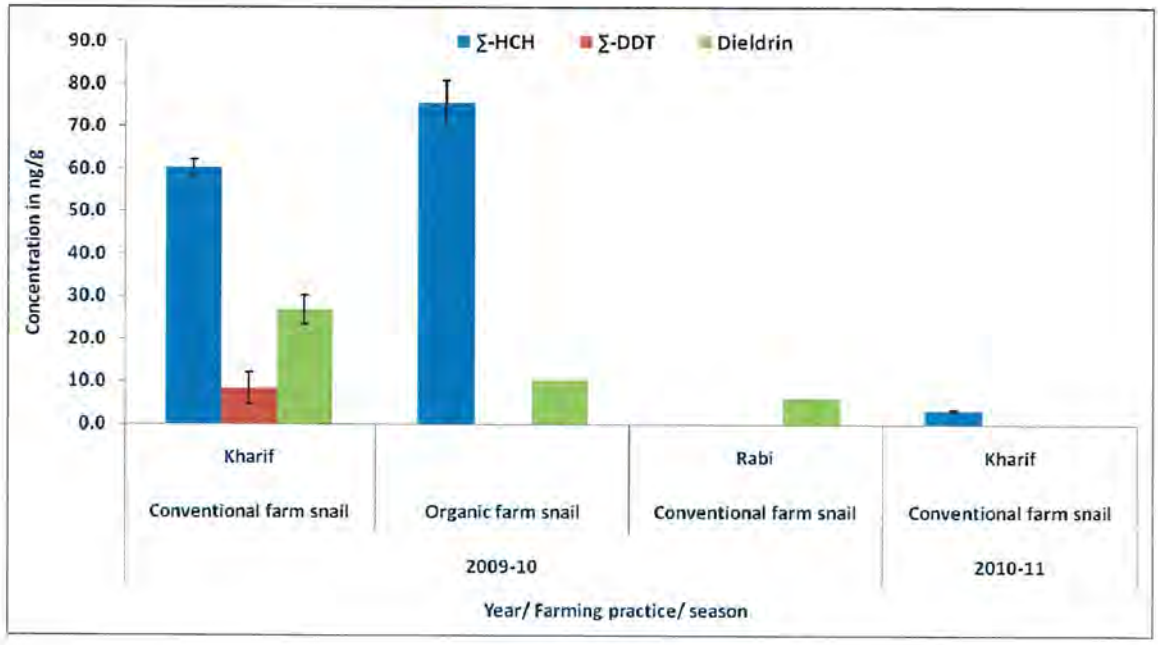



**Fig. 4.3b Variation (season, year and farming practice wise) in OC residues in snails in the agroecosystem in Padayetti village, Palakkad district (Mean ± SE; n=64; ng/g wet wt)**

### 4.3.2 Residues of organophosphate pesticides in snails in Padayetti village, Palakkad district

Among the OP pesticides, while residues of Phorate and Triazophos were detected at 5.18 and 9.96 ng/g respectively in snails collected from conventional farm during *Rabi* and *Kharif* seasons (2009-10), other OPs, namely Chlorpyrifos, Malathion, Methyl Parathion, Parathion Ethyl, Pirimiphos Ethyl, Phenthoate, Quinalphos and Ethion were at BDL in both the farms during the entire study period.

As we mentioned elsewhere, concentrations of pesticides being used these days in freshwater snails are scattered all over the world. Although information on 96 hr sublethal dose on Phorate is available for freshwater snail *Lymnaea acuminata* (15000-22,000 ng/g: Odenkirchen and Turner, 2003; 12,000 ng/g: Mahendru and Agarwal, 1983), there are no field based studies to assess the impact on snails or other life forms. Due to the biodegradability and short residence time in the environment, OPs are being increasingly used in recent years. However, Phorate is extremely toxic to mammals and other non-target

organisms including birds (Elliott *et al.*, 1997). It should be noted that during early December 2012, around 40 Demoiselle cranes *Anthropoides virgo* in Amreli and Surendranagar districts of Gujarat died. Mortality continued in Victor dam, Mansarovar Lake, Kodinar and Kutch for about two weeks. Field survey was carried out to ascertain the reason. Kathiyavadar, Victor, Thalavu were the dams near Rajula where large flocks of cranes with many sick birds were noticed congregating. Feral dogs and Marsh Harriers predated upon dying and moribund cranes. After post-mortem, tissues were screened for various chemical contaminants in the laboratory, and residues of Phorate in tissues of dead birds (14 ng/g in liver) confirmed poisoning (Muralidharan *et al.*, 2013). Granular formulations of Phorate (10%) are the most commonly used in the trade names of Jackpot, Thimate, Foril, Dahn 10G and Phoradox (CIBRC, 2014). It is moderately persistent in the soil with a half-life of up to 173 days depending on the environmental conditions. Most of the uses of Phorate have been cancelled in Thailand, Latin America and Kuwait and it is labelled as "HIGHLY TOXIC-POISON" and as a restricted use pesticide. But it is not listed as a UNEP-POP and registered for use only in around half of the world countries including the US, Canada, UK, Australia, New Zealand and India among others (PAN, 2007).

Triazophos is marketed in two different formulations (20 and 40% EC ) under trade names, namely Hostathion, Sutathion, Tarzon, Trizele, Tirkon and Triazocel to control paddy stem borer, leaf folder, hispa, green leafhopper, brown planthopper and white-back planthopper in India (Arora *et al.*, 2011). It has been extensively used in agricultural operations throughout the world (Naveed *et al.*, 2011). It also affects the non-target organisms such as fish, crab and snail (Reddy *et al.*, 1983). The FAO/WHO Joint Meetings on Pesticide Residues (2010) established an ADI of 0-0.001 mg/kg and acceptable reference dose (ARfD) of 0.001 mg/kg. Due to the persistent nature of Triazophos in the environment, it undergoes biomagnifications through food-chain, thereby causing serious concern even to the human health (Naveed *et al.*, 2011). However, field based studies on impacts of Triazophos on snail or other life forms are not available in India or elsewhere.

### 4.3.3 Residues of synthetic pyrethroid pesticides in snails in Padayetti village, Palakkad district

The levels of SP, namely ∑-Fenvalerate (sum of Fenvalerate I and II) were found to be 14.15 ± 6.19 ng/g and 6.76 ng/g in conventional and organic farm snails respectively, whereas levels of other SPs such as λ-Cyhalothrin (I and II) and Permethrin (I and II) were at BDL in all the snails in both the farms during the study period. Among the individual SPs, both Fenvalerate-I and II were detected in each one samples of conventional and organic farm snails (< 1%). The levels of Fenvalerate-I residues were found to be higher (20.33 ng/g) in conventional farm snails collected during *Kharif* season (2009-10) than (4.61 ng/g) during the same season in the following year (2011-12). While Fenvalerate-II were 7.96 and 2.14 ng/g in snails from conventional (*Rabi* 2009-10) and organic farm (*Kharif* 2011-12 ng/g) respectively, levels SP compounds were below detectable levels during other seasons and years.

It is to be noted that a few farmers adopting organic farming used Fenvalerate (trade name: Fenvol) as a chemical agent to control grain-sucking insect pest on rice during milk and dough stage as they found biopesticides to be ineffective. This might be one of the reasons for detecting trace levels of Fenvalerate residues in snails collected from organic farm during *Kharif* season (2011-12). According to US Fish and Wildlife Service report (Eisler, 1992), Fenvalerate is highly toxic to insects, mammals and aquatic organisms including snails.

As evidenced from the literature survey, there are a few laboratory and field based studies available on effects of Fenvalerate in environmental components. The lethal dose of commercially available Fenvalerate under field conditions to two aquatic snails, namely *Cipangopaludina japonica* (0.4-0.7 mg/L; Bennett *et al.*, 1986) and *Helisoma trivolvis* (0.021 µg/L; Anderson, 1989). In laboratory tests, Fenvalerate was highly toxic for aquatic organisms with $LC_{50}$ values ranging between 0.008 µg/L for newly hatched myriad shrimps and 2 µg/L for stoneflies. The no-observed-effect level in life-cycle tests using *Daphnia* was <0.005 µg/L. There are a few recent studies on biochemical aspects of Fenvalerate in snails available in the literature. The lethal dose (20 and 60% of 24h $LC_{50}$) of commercially available

Fenvalerate caused a significant reduction in the protein and amino acid levels in hepatopancreas of snail *Lymnaea acuminata* (Singh and Tiwari, 2013). Ray *et al.* (2013) observed induced stress by commercially available Fenvalerate (0.5, 1.0 and 3.0 mg/L) pesticide caused apoptosis, immunotoxicity, morphological damage in hemocytes of two Indian freshwater snails, namely *Bellamya bengalensis* and *Lamellidens marginalis*. Moreover, the levels reported in the present study could be considered as safe and do not produce any adverse effects on snails. However, effects of Fenvalerate on aquatic organisms need to be frequently monitored in the agroecosystem to understand the role of this pesticide in long-term basis.

### 4.3.4 Residues of carmabate pesticides in snails in Padayetti village, Palakkad district

Levels of two Carbamate pesticides, namely Carbaryl and Carbofuran analysed in snails from both organic and conventional farms were below detection limit. Although carbamate pesticides do not persist in the environment, there may still be short-term cumulative effects on the reproduction of aquatic organisms. For instance, Tripathi and Singh (2004) found that doses of 2, 5 and 8 mg/L Carbaryl altered the biochemical parameters in nervous, hepatopancreatic and ovotesticular tissues of the freshwater snail, *Lymnaea acuminate*. Specifically, glycogen, pyruvate, total protein and nucleic acid levels were reduced after 96 hours of exposure to Carbaryl while lactate and free amino acid levels increased (Tripathi and Singh, 2004). They also reported that the number of eggs for the freshwater snail, *Lymnaea acuminate* was reduced by 49% at 2 mg/L while no eggs were laid at 5 and 8 mg/L.

The toxicity of carbamates is similar to OPs. However, carbamates are higher in toxicity and exhibit a greater sensitivity to the inhibition of AChE activity than OPs. *In vivo*, the gastropod, *Biophalaria glabrata* was exposed to 1 mg/L of Carbaryl, which significantly inhibited enzyme activity (Kristoff *et al.*, 2010). Granular form of Carbofuran generally displays high avian toxicity. The formulated granules look very similar to seeds, certain birds incorporate into their diet mistakenly, they seize and die upon ingestion, leading to bird-kill incidents. Secondary poisoning of raptors such as vultures feeding on baited carcasses has also been

reported (Otieno, 2010). Since, both Carbaryl and Carbofuran residues were at BDL in snails from both the farms. As on date, both these pesticides are expected to cause no harm.

### 4.4. Pesticide residues in fishes in the agroecosystem in Padayetti village, Palakkad district

Fishes from organic and conventional farms were selected for monitoring because pesticides might bind with sediments and bioaccumulate/ bioconcentrate through food or directly from water (Pazou, 2013; Kaur *et al.*, 2008; Sarkar *et al.*, 2008; Amaraneni, 2002). Although numerous studies have investigated the occurrence of OCs in inland wetland and marine fishes (Williams *et al.*, 2013; Jayanthi, 2012; Dhanajayan and Muralidharan, 2010; Malik *et al.*, 2007; Muralidharan *et al.*, 2009; Kaur *et al.*, 2008; Sankar *et al.*, 2006; Vijayan *et al.*, 2004; Kumari *et al.*, 2001; Kannan *et al.*, 1992) in India, data on OPs, SPs and Cbs are very limited, especially for fishes in an agroecosystem. Fish being an indicator of pollution, their contamination is also a matter of concern for human health.

Pesticide residues in five species of fishes, namely Spotted Snakehead *Channa punctatus* (n=54), Climbing Perch *Anabas testudineus* (n=48), Spot-fin Barb *Puntius sophore* (n=76), Long-snouted Barb *Puntius dorsalis* (n=67) and Mrigal *Cirrhinus mrigala* (n=52) collected from organic and conventional farms were estimated and compiled. These species of fishes were selected based on their abundance and availability throughout the year. Samples were pooled species, season and farm wise depending on their number and size. Data have been compiled to check the overall load of pesticides, variation among the species, seasons and between farms with reference to groups, namely OCs, OPs, SPs and Cbs. Further, within a group, levels of individual pesticides have been compiled, wherever applicable, to understand the implications with reference to usage pattern or policy of the government in existence.

None of the fishes studied showed any accumulation of OCs from both the farms, and also had a very less level of OP and SP residues. Among the OPs analysed, trace levels of Phosphamidon (2.98 ng/g), Methyl Parathion (3.23 ng/g) and Phenthoate (2.78 ng/g) were

measured in three fishes collected from conventional farms. None of the fishes collected from organic farm had detectable levels of any of the OPs analysed. Similarly, λ-Cyhalothrin was the only SP pesticide detected at trace levels in two species of fishes collected from conventional farms, while other SPs were at BDL. Levels of Cbs were also BDL in fishes from both the farms during the study period (2009-12). Statistical tests were not performed as there was less number of detections.

### 4.4.1 Residues of major metabolites/ isomers of organochlorine pesticides in fishes in Padayetti village, Palakkad district

The concentrations of persistent OCs such as isomers of HCH, Endosulfan and Heptachlor, DDT and its breakdown products, Aldrin, Dieldrin, Endrin, Chlordane and Mirex were below detectable levels in all the species of fishes collected from both the farms. This may be because the size of the fishes collected from the respective farm wells and ponds (known as *Kokkarni*) were small (9.61 ± 4.36 g). We have also noticed that most of the fishes in ponds, paddy fields and a few small wells located in the study area either died or were predated by birds during summer months (February-April). Further these water bodies get filled during the subsequent monsoon (May-June) and the surviving fishes already available in the big wells of high land areas starts reproducing and it leads to spreading of fingerlings across the paddy fields and nearby ponds, when it rains heavily, due to overflow of water, it get mixed with the farming area. Moreover, since the study area is under rain fed condition, and each crop period is around three to four months, these fishes do not get opportunities to live more than 3-4 months. Hence they do not increase in size like the fishes in wetlands or any other water bodies such as rivers and dams.

Present study clearly indicates that the levels of chlorinated pesticides in fishes from both organic and conventional farms are not of great concern. Even the only historical OC compound, HCH isomers namely γ-HCH (24.42 ± 5.74 ng/g) and β-HCH (6.48 ± 2.02 ng/g) measured in the sediments of conventional farm did not bioaccumulate in the fish tissues. Normally bioaccumulation of persistent OCs in fishes can be viewed as a result of the interaction among numerous factors. Besides the level of contaminant (exposure and

dosage) input to the freshwater system, biological aspects (species, sex, age, size, trophic level, reproductive status and metabolism) and surrounding environmental parameters (temperature, depth and habitat) influence the accumulation (Kaur *et al.*, 2008). One or combination of factors might influence the accumulation of these compounds.

### 4.4.2 Residues of organophosphate pesticides in fishes in Padayetti village, Palakkad district

Among the various OPs analysed in muscle tissues, trace levels of three compounds were detected in three species collected from conventional farms. Methyl Parathion and Phenthoate were found at 3.23 and 2.78 ng/g in *Channa punctatus* and *Puntius sophore* collected during summer 2009-10, while Phosphamidon (2.98 ng/g) was detected in *Cirrhinus mrigala* collected during *Rabi* season of the same year. Levels of other OPs, namely Phorate, Malathion, Chlorpyrifos, Chlorpyrifos Methyl, Parathion Ethyl, Pirimiphos Ethyl, Quinalphos, Ethion and Triazophos were at BDL in fishes studied from both organic and conventional farms during the study period.

Substantial information is available in literature on accumulation of OCs in various freshwater fishes in India and abroad. However, there are only a few studies on the fate and behavior of OP, SP and Cbs in fishes in any agroecosystem. Data on the distribution of these currently-used chemicals in freshwater fishes is not only important for ecological reasons, but also for their impact on human health.

Methyl Parathion is less persistent in the atmosphere and easily absorbed by organic matter. Sublethal (1 mg/L) and lethal (7 mg/L) doses of Methyl Parathion are reported to have created structural changes of the gill lamellae, epithelial detachment, necrosis, hyperplasia and altered cellular morphology in freshwater Silver Dollar *Metynnis roosevelti* from Sao Paula, Brazil (Machado and Fanta, 2003). Paraoxon, the active metabolite of Methyl Parathion is responsible for the inhibition of several enzymatic systems (cholinesterase and mitochondrial oxidative phospharilase) in fishes. The inhibition of AChE is the most critical

toxic effect because it results in the accumulation of the neurotransmitter acetylcholine in the synapses (da Silva *et al.*, 1993). However, in the present study we have not looked at the concentrations of Paraoxon, a metabolite of Methyl Parathion in fish tissues.

According to Central Insecticide Board and Registration Committee (CIBRC, 2014) of India, Methyl Parathion is a restricted pesticide in India. The use of 2% DP and 50% EC formulations of Methyl Parathion is permitted only on those crops where honey bees are not acting as pollinators. It is produced for use in other crops in India in the trade names of Paracid, Dhanumar, Metacid, Folidol, Dhanudol, Chlophos, Metapar, Devithion and Paracrop. 50% EC of Methyl Parathion is recommended to control boll weevils and many sucking pests such as gall midge, leafhopper, hispa, leaf roller, stem borer, whorl and maggot on paddy (Arora *et al.*, 2011). Due to rains or sediment drainage, it often contaminates water bodies, affecting non-target organisms such as fish (Machado and Fanta, 2003). USEPA classified Methyl Parathion as class-I (highly toxic) insecticide. It is moderately toxic to fish and to animals that eat fish. Reported 96 h $LC_{50}$ values are from 1.9 to 8.9 mg/L in the following fish species; Coho Salman, Rainbow Trout, Lake Trout, Black Bullhead, Channel Catfish, Green Sunfish, Bluegill and Yellow Perch. Trace levels of Methyl Parathion recorded in the present study in one of the fish samples from conventional farm do not appear to cause any adverse effects.

Phosphamidon is widely used in India and manufactured as 40% soluble liquid (SL) in the trade names of Kindon Plus, Dhanucron, Sumidon and Peridon for controlling pests such as stem borer, leaf borer, green leafhopper, brown planthopper and white-backed planthopper on paddy. WHO (2004) classified this compound as extremely hazardous based on its oral toxicity. However, negligible concentration of Phosphamidon reported in fishes collected from conventional farm in the present study may not produce any effects to fish population or consumers.

Phenthoate (trade names: Cidial and Papthion 2% DP) is a non-cumulative cholinesterase inhibitor, and mainly used to control paddy case worm. It is thermally unstable; at room

temperature the shelf-life is several months; at $50^0$C it is degraded in 110 hrs. At pH 8.0 the half-life is 12 days. It is highly toxic to fish with a listed 96h $LC_{50}$ of 3.3 ng/g. Rao *et al.* (1982) revealed that tissue total proteins decreased with a raise in the free amino acid level in the fishes exposed to sublethal concentrations of Phenthoate.

Phenthoate has been implicated in atleast three large-scale accidental fish kills in freshwater containing measurable levels (0.001-0.002 mg/L). The species involved in the above referred episodes were Goby, Grey Mullet and Rainbow Trout and residues in dead fishes were about 0.03-0.09 mg/kg. 96 hr $LC_{100}$ for Goldfish, Minnows, Mosquito-fish and Guppies were reported to be 4.5, 3.4, 0.3 and 2-3.0 mg/L respectively (http://www.pesticideinfo.org accessed on 5th May 2014). Concentrations of all above-mentioned three OPs documented in the present study are well below the levels reported elsewhere. Although, considerable quantities of OPs are used in agriculture these days, as they degrade comparatively faster in the environment, we are unable to measure their residues and assess the impact. Nevertheless, it is to be noted that within a short span of time they are capable of creating deleterious effects such as reproductive failure, neurotoxicity, kidney/ liver damage and birth defects in addition to being a possible carcinogen (USEPA, 2000a&b).

### 4.4.3 Residues of synthetic pyrethroid pesticides in fishes in Padayetti village, Palakkad district

λ-Cyhalothrin was the lone synthetic pyrethroid compound detected in trace levels i.e., 4.32 ng/g in Long-snouted Barb *Puntius dorsalis* during *Rabi* 2009-10 and 2.07 ng/g in Climbing Perch *Anabas testudineus* during summer 2009-10, while the levels were at BDL in all other fishes from both organic and conventional farms. Residues of SPs were detected in less than 0.01% of the fishes analysed from conventional farms. No statistical test was done as the situation did not warrant.

λ-Cyhalothrin is the active ingredient in several brand name products such as Warrior, Karate, Demand, Icon and Matador. It mainly controls leaf folder, stem borer, green

leafhopper, gall midge, hispa and thirips in paddy. Annual agricultural use of λ-Cyhalothrin in India has been estimated at approximately 35 MT per annum from 2001 to 2002 and increased to 40.22 MT per annum between 2006 and 2007 (Arora *et al.*, 2011). But state wise consumption of λ-Cyhalothrin is not available.

Studies on the levels of pyrethroids, particularly λ-Cyhalothrin in fishes in India are very few. The $LC_{50}$ value of λ-Cyhalothrin was reported to be 7.92 ng/ml (Kumar *et al.*, 2011a), and results of the referred study suggested that even at low concentrations, these compounds may exert toxic effects. Recent development of a number of broad spectrum pesticides and their large scale outdoor and indoor applications has inevitably increased the probability of contaminating the aquatic bodies. The insecticides used earlier are now getting replaced by SPs like Cypermethrin, Fenvalerate and λ-Cyhalothrin as they are more effective for containing crop pests. However, the available reports have indicated that these compounds might be toxic to other aquatic organisms in general and the fishes in particular (Kumar, 2011a; Gu *et al.*, 2007; Amweg *et al.*, 2006; Koprucu and Aydin, 2004; Saha and Kaviraj, 2003). The reported $LC_{50}$ (96 hr) of λ-Cyhalothrin is 210 ng/L for bluegill sunfish, 240 ng/L for rainbow trout, 360 ng/L for *Daphnia magna*, 4.9 ng/L for mysid shrimp, and 0.8 ng/L for sheepshead minnow.

The lipophilicity of pyrethroids facilitates their rapid access to various tissues and thus leads to high affinity of these pesticides to the central nervous system (Anadon *et al.*, 1996). Since the fishes have poor ability to metabolize and eliminate such xenobiotics as compared to those of higher vertebrates, these pesticides become relatively more toxic (about 100 times) to several species of fishes (Demaute, 1989) as compared to that of mammals and birds (Bradbury and Coates, 1989). Concerns also have therefore been raised about the widespread use of λ-Cyhalothrin and its potential impact on aquatic ecosystems.

### 4.4.4 Residues of carbamate pesticides in fishes in Padayetti village, Palakkad district

Levels of Carbamate pesticides, namely Carbaryl and Carbofuran were below detection limit in fishes from both organic and chemical farms studied.

### 4.4.5 Dietary intake of pesticide residues to man

Based on the OP and SP residues recorded in fishes collected from conventional farms of Padayetti agroecosystem, an attempt was made to assess their suitability for human consumption. It is to be noted that the annual per capita consumption and recommended intake of fish is 9.8 and 13 kgs, respectively in India (MoSPI, 2011; www.mospi.gov.in). However, considering the local situation, assuming that a person consumes 250 g of fish per week, the average daily intake of pesticides through fishes was calculated as explained by FAO/WHO (1991). The calculated dietary intake levels represent the quantum of pesticides entering the human body through consumption of these five species of fishes, selected for the present study. The levels were compared with the Allowable Dietary Intake (ADI) concentration levels proposed by various statutory agencies across the world. Apart from this, the maximum permissible residue levels of the pesticides proposed by International Regulatories and Advisories have also been compared with the residue levels documented in the present study.

In India, although information on the residue levels of pesticides in fishes is available, information on the daily dietary intake of pesticides through consumption of fishes to man is limited, except a few (Jayanthi, 2012; Dhananjayan and Muralidharan, 2010; Muralidharan *et al.*, 2009; Amaraneni, 2002; Kumari *et al.*, 2001). Vijayan *et al.* (2004) documented many persistent contaminants in about 66 species of freshwater fishes from 173 wetlands spread across 14 states in India. The study also looked at the dietary input to human beings in contrast to the WHO guidelines.

In the present study, the calculated dietary intake (CDI) concentrations of OP pesticides, namely Methyl Parathion, Phosphamidon and Phenthoate residues were 115.36, 106.43 and 99.29 ng/g through consumption of *Channa punctatus*, *Cirrhinus mrigala* and *Puntius sophore* respectively. Intake of synthetic pyrethroid, λ-Cyhalothrin residues was 154.29 ng/g through *Puntius dorsalis* and 73.77 ng/g through *Anabas testudineus*. It is noted that pesticide use in India is regulated by the Central Insecticides Board and Registration Committee (CIBRC) and the Food Safety and Standards Authority of India (FSSAI). The CIBRC registers pesticides for crops while the FSSAI sets the MRL for pesticides for the crops it has been registered for. Bases on the concentrations of OP and SP pesticides in the fishes from conventional farm in the present study cannot be calculated as MRLs for these pesticides have not been formulated yet by FSSAI or International statutory agencies, namely United States Environmental Protection Agency (USEPA), European Commission, Health Canada, Germany, Food and Agricultural Organisation and World Health Organisation (FAO/WHO *Codex Alimentarius* commission). Further, effects that result from long-term exposure through diet to low doses are often difficult to distinguish although acute toxic effects are easily recognized. Information on the levels of presently used chemicals are of crucial importance not only to fill knowledge gaps on the status of contamination, but also to produce baseline data to handle issues that might arise in near future.

To summarise, the estimated dietary intake of select OP and SP pesticides covered in the study did not entail a serious risk to consumers. But precautions must be taken when generalizing this negative result to people with high dietary exposure level or an intake of other pesticides. Some research has been conducted on the OP and SP contamination of the abiotic environment in agroecosystem (Tse *et al.*, 2004; Gitahi *et al.*, 2002). However, no such studies have investigated the distribution of OPs, SPs and Cbs residues in aquatic organisms especially fishes from paddy agroecosystem. Hence a base-line study on pesticide contamination in fishes is needed since fishes are considered to be an important food source for human.

### 4.5 Pesticide residues in frogs in the agroecosystem in Padayetti village, Palakkad district

Amphibians are considered to be sentinels of environmental contamination. Biphasic life stages, varying reproductive strategy and highly permeable skin through which xenobiotics are readily absorbed from the surroundings may render frogs more vulnerable to chemicals than many other vertebrates (Bruhl *et al.,* 2013). During the last two decades, decline in amphibian populations in many parts of the world has been reported (Stuart *et al.,* 2004; Sparling *et al.,* 2001; Hudson *et al.,* 1984). However information about this phenomenon is scarce, particularly in India. According to global amphibian assessment (2007), of the 5,743 known species, over 1,856 (32.5%) appear on the IUCN red list as endangered, threatened or vulnerable, suggesting they are at greater risk than mammals (23%) and birds (12%). These precipitous and rapid declines are thought to be caused by one or a combination of the following factors: habitat destruction (Houlahan *et al.,* 2000); increased UV-B radiation (Blaustein *et al.,* 2003); climate change (Carey and Alexander, 2003); acid deposition (Vertucci and Corn, 1997); bacterial, viral and fungal infections (Morehouse *et al.,* 2003); introduced predators (Knapp, 2005); harvesting for food, scientific research and pet trade (Jensen and Camp, 2003); heavy metal, polychlorinated biphenyl (PCB), fertilizer and pesticide contamination (Collins and Storfer, 2003). Often deformities in amphibians have been reported in agricultural areas where breeding and larval developments of frogs occur mainly during winter season when use of chemical pesticides is the maximum (Fryday and Thompson, 2012; Taylor *et al.,* 2005). Therefore, frogs inhabiting such environments may experience pesticide exposure causing toxic effects. In the present study we addressed the following objectives, (i) to study the levels of pesticide accumulation in the frogs collected from organic and conventional farms and (ii) to assess for a potential contribution of pesticides to frog deformities.

In India, studies on the adverse effects of past and presently-used-pesticides on amphibian populations in agroecosystems are scarce, even though the role of chemical pesticides is suspected. There are only a few short-term studies on sublethal toxicity of pesticides on amphibian eggs, embryos and tadpoles. As on 20$^{th}$ October 2014, there were two hundred

and forty six molecules of pesticides registered under section 9(3) of the Indian Insecticides Act, 1968 (http://cibrc.nic.in/ retrieved on 20th October 2014). Sublethal toxicity studies of OC pesticides, namely α-HCH in *Microhyla ornata* (Pawar and Katdare, 1984); Endosulfan in *Limnonectes limnocharis* (Dey and Gupta, 2002) and Chlordane in *Bufo bufo* (Jayakumar and Kandeepan, 2009) are the available few in India. However, there are no such studies on currently-used pesticides such as OP, SP or Cbs.

In the present study, a total of 109 frogs comprising two species, namely Indian Rice Frog *Fejervarya limnocharis* (n=78) and Jerdon's Bull Frog *Hoplobatrachus crassus* (n=31) were collected seasonally (*Kharif*, *Rabi* and summer) between May 2009 and April 2012. The present study reports the levels of pesticide residues (20 OCs, 12 OPs, six SPs and two Cbs) in 72 samples of healthy (102 individuals: 52 from organic and 50 from conventional farms) and seven individual deformed frogs collected from conventional farms.

It was observed that out of 109 frogs collected, seven had deformities (6.42%). Interestingly, all the deformed frogs were collected from the conventional farm during *Rabi* (2009-10). Species with deformity were Indian Rice Frog (n=6) and Jerdon's Bullfrog (n=1). It is to be noted that we did not examine the abundance, species distribution or species richness of frogs. However, visually encountered frogs were collected and examined for any morphological disfigurement following Meteyer (2000). The mean occurrence of deformity observed in this study (6.42%) is slightly higher than the background levels of deformities (5%) reported in wild frog populations in California, USA (Johnson *et al.*, 2001) and agroecosystems (3.98%) in central Western Ghats (Gurushankara *et al.*, 2007).

Species and farm wise variation of OC, OP, SP and Cb were presented in fig. 4.5a. Of the 4 groups of pesticides analyzed, concentrations of Σ-OC (33.22 ± 16.48 ng/g) and SPs (26.91 ± 7.12 ng/g) were fairly high in deformed *F. limnocharis*, whereas in deformed *H. crassus* the levels of Σ-OC were in trace amounts (1.44 ng/g), but Σ-SP levels (42.15 ng/g) were very high (fig. 4.5a). The concentrations of Σ-OP residues observed in *F. limnocharis* (4.97 ± 1.73 ng/g)

and *H. crassus* (1.02 ± 0.29 ng/g) indicate considerable exposure to these compounds. Although, most of the OP pesticides ingested by animals are excreted slowly, they are extremely toxic to fishes, frogs, mammals and other non-target organisms including birds. Most of the OPs and SPs are more toxic but less persistent lasting only for days, weeks or months in the environment (Wania and Mackay, 1996; WHO, 2000). Hence, they have almost replaced OCs except a few. However, the OPs are potent cholinesterase (ChE) inhibitors. They can bind covalently with the serine residues in the active site of AChE enzyme, and prevent its natural function in the catabolism of neurotransmitters, and eventually lead to death. Levels of currently-used pesticides varied significantly between organic and conventional farm frogs ($p < 0.05$; F= 8.2).

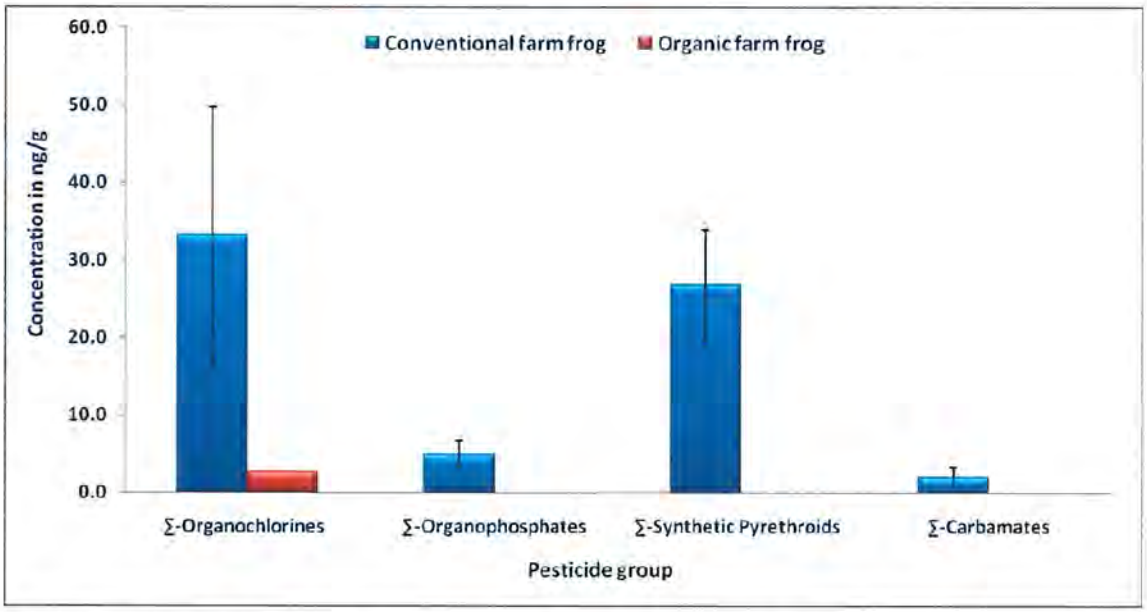



**Fig. 4.5a. Mean (± SE) levels of OC, OP, SP and Cb pesticide residues in frogs (n=74; ng/g wet wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12)**

### 4.5.1 Residues of major isomers/ metabolites of organochlorine pesticides in frogs in Padayetti village, Palakkad district

Among the OCs, residues of only HCH (29.72 ± 7.63 ng/g) and Heptachlor (2.98 ± 0.72 and 1.44 ng/g) were recorded in deformed *F. limnocharis* and *H. crassus* respectively collected from conventional farms during *Rabi* and *Kharif* seasons (2009-10). In healthy frogs residues of all the OC pesticides were below detectable limit except γ-HCH (2.12 ± 0.98 ng/g) in *F.*

*limnocharis* collected from organic farm *Kharif* (2009-10). To the Σ-OC load recorded in deformed *F.limnocharis*, Σ-HCH and Σ-Heptachlor residues contributed 89.50 and 10.50% respectively.

### 4.5.1a) HCH and its isomers

Concentrations of β- (9.36 ± 2.01 ng/g), α- (8.84 ± 1.86 ng/g), δ- (8.38 ± 1.24 ng/g) and γ-HCH (3.14 ± 0.39 ng/g) were found to be high in the deformed *F. limnocharis* (fig. 4.5b) collected from the conventional farms during *Kharif* (2009-10), but HCH isomers were at BDL in the healthy frogs collected from the same field. The isomeric forms of HCH are toxic and recalcitrant, particularly β isomer acts as an environmental estrogen. Mean concentrations of γ-HCH (3.14 ± 0.39 ng/g) and α-HCH (8.38 ± 1.24 ng/g) recorded in one of the deformed *F. limnocharis* is higher (γ-HCH: 0.29 and α-HCH: 0.31 ng/kg) than the levels reported by Fellers *et al.* (2004) in healthy yellow-legged frog *Rana muscosa* from Sierra Neveda Mountains of California, USA. Use of HCH continued unabated until it was banned in 1997 in India, but restricted use of γ-HCH was allowed till 2013.

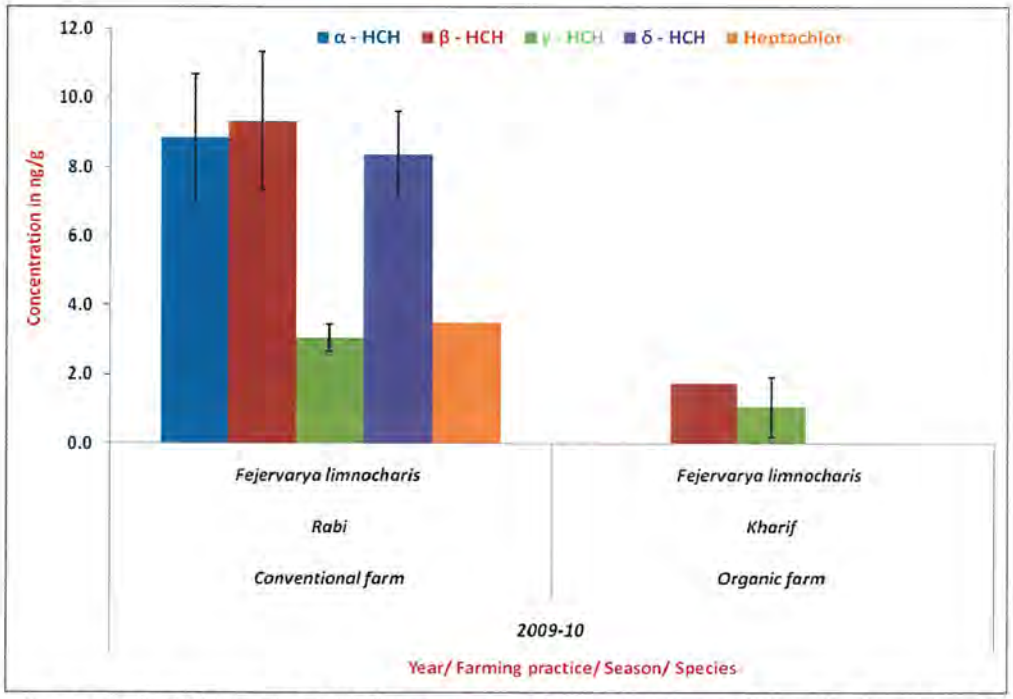



**Fig. 4.5b. Mean (± SE) levels of HCH isomers and Heptachlor residues in frogs (n=74; ng/g wet wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12)**

### 4.5.1b) DDT metabolites and Cyclodiene insecticides

Residues of Heptachlor in *F. limnocharis* (2.98 ng/g) and Heptachlor epoxide in *H. crassus* (1.44 ng/g) were detected at trace concentrations during *Kharif* season (fig. 4.5b). It has to be noted that these two frog species with deformities were collected from conventional farm and residues might indicate their use in the past in this region. Use of Heptachlor was banned in India in 1996 (CIBRC, 2014). But its metabolite Heptachlor Epoxide has the potential to enter food chain (USEPA, 1986) and cause impact. Mayer and Ellersieck (1986) from the US Department of Interior, Fish and Wildlife Service reported 435 ng/g of Heptachlor to cause 50% of tadpole mortality in *Bufo woodhousei fowleri*. Levels of Heptachlor residues recorded in the present study in both frog species are well below the sublethal toxic limits ($LC_{50}$ = 440 ng/g). DDT was identified as a POPs during the Stockholm Convention on POPs in 2001. India, China and Russia opted for exemption from its total ban (Dash *et al.*, 2007).

### 4.5.2 Residues of organophosphate pesticides in frogs in Padayetti village, Palakkad district

Among the OP pesticides, concentrations of Phorate and Quinalphos were 1.02 ± 0.29 ng/g and 2.62 ± 1.02 ng/g respectively, in deformed *F. limnocharis* during *Kharif* (2010-11). While Parathion Ethyl (1.02 ng/g) was detected in deformed *H. crassus* (Fig. 4.5c) in the same season and year, other OPs were BDL in healthy frogs from both organic and conventional farms. Normally, the OP insecticides undergo hydrolysis in surface waters with highly variable reaction rates depending upon the water quality parameters. However, OPs are more polar than the OCs and their chemical properties do not favour bioaccumulation (Fellers *et al.*, 2004).

Granular form of Phorate (10%) is most commonly used under the trade names of Jackpot, Thimate, Foril, Dahn 10G and Phoradox (Arora *et al.*, 2011) in the study area. It is reported to be moderately persistent in the soil with a half-life of 2-173 days depending on the conditions. It binds relatively well with soil and is neither highly mobile nor highly soluble in

water so it would reach water sources mainly through run-off and not leaching (PAN, 2007). Quinalphos, which is classified as a yellow label (highly toxic) pesticide in India (CIBRC, 2014) is widely used in paddy and other cash crops.

Although information on the lethal dose of Phorate (85.2 mg/L), Quinalphos (3.1 mg/L) and Parathion (8.75 mg/L) is available for *Rana* sp., (Luo *et al.*, 2003; Alam and Shafi, 1991; Hudson *et al.*, 1984) no detail is available for other species of frogs under field conditions. Both Phorate and Quinalphos are most commonly used pesticides in India (CIBRC, 2014 www.cibrc.nic.in accessed on 20$^{th}$ October 2014). Although negligible amounts of most commonly used acetylcholinesterase inhibiting pesticides such as OPs observed in the current study, they do not stay in system for long time, but they are capable of producing delayed metamorphosis and/or disrupting nervous systems (Bridges, 1999). Sublethal effects of OPs in well-known amphibian model (*Xenopus laevis*) observed motility impairment in embryonic stage, muscular disorders such as abnormal tail flexure and notochord, and contorted posture in tadpoles (Bonfanti *et al.*, 2004; Bridges 1999). The long-term effects of many OPs need to be evaluated as these chemicals create great risks to the wildlife and environment. Since agriculture has a place of prime importance in India's economy, large scale and unscrupulous use of chemical pesticides in this sector is likely to continue, which might lead to many adverse effects not only on amphibians, but also man.

### 4.5.3 Residues of synthetic pyrethroid pesticides in frogs in Padayetti village, Palakkad district

The levels of SP, Fenvalerate-II recorded in the present study in deformed *F. limnocharis* (26.91 ± 9.19 ng/g) and *H. crassus* (42.15 ng/g) are higher than the levels reported in four species of frogs (*Rana utricularia*, *R. clamitans* and *Hyla cinerea*: < 10 ng/g and *Bufo fowleri*: 20 ng/g) collected from a cotton field which had been aerially treated with Fenvalerate at 0.112 kg of active ingredient/ha (0.1 lb/acre) 5 days prior to sample collection in USA (Bennett *et al.*, 1983). But such field based assessment studies on Fenvalerate are not available in India. Hence, we compared a few studies on sublethal effects available in the literature. As stated by Materna *et al.* (1995), formulated Fenvalerate available in the market

could cause lethal effects in northern leopard frog *Rana pipiens* at 7.29 ng/g. The known effects of pyrethroids on frogs at sublethal concentrations include incoordination, lack of limb control, hyperactivity, tremors and writhing. Tadpoles of leopard frog exhibited convulsions and twitching following sublethal (1.3 µg/L) Fenvalerate exposure, with behavioral homeostasis re-established only one week after removal to clean systems (Materna *et al.,* 1995). Larsen and Sorensen (2004) reported that 150 ng/mL Fenvalerate killed 50% of the tested *Xenopus laevis* population in 96 hr. Moreover, regulatory agencies have not yet formulated any norms for protection of sensitive wildlife resources against Fenvalerate. Nonetheless, the USEPA AQUIRE database classified Fenvalerate as one of the toxic pesticides.



**Fig. 4.5c. *Hoplobatrachus crassus* - Incomplete limb with missing lower portion**

Studies have indicated that pyrethroids are highly toxic to a number of non-target organisms such as honeybees, aquatic arthropods, freshwater fishes and amphibians even at very low concentrations (Palmquist *et al.,* 2011). These behavioural aberrations, though not lethal, may increase chances of predation of affected amphibians and fish.

Pyrethroid exposure also affects early development in amphibians. Hatching success of moor frog (*Rana arvalis*) eggs was reduced following exposure to concentrations of α-Cypermethrin as low as 1.0 µg/L, and a number of exposed embryos exhibited convulsions

and tail kinking following hatch. Cypermethrin concentrations as low as 0.1 µg/L exposed during the egg stage also reduced length at metamorphosis (Greulich and Pflugmacher, 2003). Most notably, the jelly mass surrounding the amphibian eggs did not prevent embryos from absorbing Cypermethrin from the surrounding aqueous medium. For instance, although exposure to either Permethrin or Fenvalerate at 0.01 to 2 µg/L for 22 to 96 hrs caused no increase in mortality, in green frog *Rana clamitans* embryonic development was stunted and operculum growth get adversely affected at pyrethroid concentrations greater than 1 µg/L in water (Berrill *et al.*, 1998). Exposure to pyrethroids also causes reductions in tadpole biomass production (Materna *et al.*, 1995).

### 4.5.4 Residues of carbamate pesticides in frogs in Padayetti village, Palakkad district

Residues of Carbofuran (2.09 ng/g) were detected in one of the healthy *F. limnocharis* collected from conventional farm during *Rabi* (2011-12). This reflects the use of Carbofuran in the study area. Although we have no data on the total application of Carbofuran at the studied sites, farmers of the area spray up to 750 g/ha. It may be worth mentioning that around 500 MTs of Carbofuran is being used in India for various crops (http://cibrc.nic.in retrieved on 25$^{th}$ July, 2014). According to Jayatillake *et al.* (2011), application of Carbofuran between 50 and 500 g/ha had affected the growth, development and swimming activity in tadpoles of Asian common toad *Duttaphrynus melanostictus*. Hence, effects of Carbofuran needs continuous monitoring.

Some currently-used pesticides (OPs, Cbs and SPs) are acutely toxic and associated with population declines in amphibians (Broomhall, 2004). These classes of pesticides have relatively short half-lives in tissues, but nonetheless can still evoke toxicity. In the present study five out of seven deformed frogs revealed hind limb deformity i.e., missing digits or lower limb while short toe was missing in the other two. Numerous laboratory studies have shown that many different contaminants can kill or cause deformities in frogs (Palmquist *et al.*, 2008a&b). Mass mortality of eggs, larvae and adults have been reported in some areas,

and deformed amphibians only rarely survive to adulthood (Johnson *et al.,* 2001). According to Materna *et al.* (1995), commercially available Fenvalerate was found to be lethal to *Rana pipiens* at 7.29 ng/g. Hence, we suspect that high concentrations of Fenvalerate detected in the present study in deformed *F. limnocharis* (26.91 ng/g) and *H. crassus* (42.15 ng/g) might have contributed to the deformity. However, not all the frogs collected in conventional farms showed external deformities.

Moreover, since the farm belongs to several small farmers, pattern and quantities of Fenvalerate application might have varied. Therefore if pesticides play a role in amphibian decline, it could be due to sublethal effects and possible combined effects with other factors such as disease. Synergistic effects of pesticides also cannot be ruled out. The challenge is to unravel how the agents causing deformities interact with one another. A coordinated multidisciplinary approach will be necessary to find a solution for this problem. Regulatory agencies should consider imposing restrictions on the use of these pesticides, and farmers have to be provided with safe alternatives to handle pests without compromising on productivity. While submission of toxicity data on birds and mammals are necessary for registering a new molecule world over, for amphibians no reference is made in the registration process not only in India but also in many other countries. Hence, our results indicate that existing risk assessment procedures for pesticide formulation need to be strengthened to protect the amphibians.

### 4.6 Pesticide residues in paddy and fodder in the agroecosystem in Padayetti village, Palakkad district

More than two billion people (> 60%) world over consume rice as staple food, and 90% of the rice grown in the world is consumed in Asia (Singh *et al.,* 2011). In India, rice is cultivated in about 44.3 million ha producing 141 million metric tons of grains annually (Yadav *et al.,* 2010). As per the estimate of Directorate of Economics and Statistics, Govt. of Kerala (Kumari, 2014) during 2009-10, paddy was cultivated in 2.34 lakh ha and 6.25 lakh tons of grain was produced. The presence of pesticide residues in the paddy may have human health implications and hence attention has increased for the need to control use of these

organic pollutants (Nguyen *et al.,* 2008). It is also possible that detrimental levels of residues may remain in the edible portion of paddy or paddy straw (fodder) and may affect human and animal health (Bakore *et al.,* 2004). Besides fodder, hull is used as main feed for ruminants in many parts of India, and hence it was essential to determine the levels of pesticide residues in both paddy and fodder.

The main focus of the present investigation was to compare the load of pesticides in paddy and straw (fodder) grown organically and conventionally using currently available fertilizers and pesticides. Under rain fed conditions, the farmers of Padayetti village cultivate two crops (*Kharif* and *Rabi*) annually and do go for third crop if water is available during summer (March to May). Even though Malampuzha canal passes through Padayetti village, water from this cannot be used for irrigation because the farms are located at higher elevation. More over the wells in the farms are small and they dry during summer. Residue levels of OCs, OPs, SPs and Cbs in paddy and fodder have been estimated to check the overall load of pesticides with reference to farming practice and seasons. In addition to this, within a group, levels of individual pesticides have been quantified wherever possible. This could help understand the implications with reference to potential health risks associated with the exposure to these pesticides, usage pattern or policy of the government in existence at national (Food Safety and Standards Authority of India - FSSAI, 2011) and International (USEPA, 2008; EC, 2000; Health Canada, 1997; FAO/WHO *Codex Alimentarius* commission, 2010) level.

Between May 2009 and April 2012, totally 120 samples (paddy and fodder 60 each) of three common paddy (*Oryza sativa* L.) varieties, namely *Aishwarya*, *Jothi matta* and *Thavalakannan* cultivated adopting conventional and organic methods in Padayetti village, Kerala during *Kharif* and *Rabi* seasons were collected. Residues of a set of legendary and currently-used pesticide formulations were analyzed in these samples.

Residues of persistent OCs were detected in 3% of the paddy samples analysed, with the highest (6.38 ng/g) in *Thavalakannan* variety from conventional farm collected during *Rabi* (2009-10) and lowest (1.89 ng/g) in the same variety of paddy during *Kharif* (2010-11). While levels of Σ-OC residues were at BDL in samples of paddy collected from organic farm during the study period, none of the samples of fodder showed any accumulation of OCs from both the farms. Presence of trace levels of these OC compounds in paddy samples, suggests that these residues might have biomagnified through sediments/ soil and stalk of the plants (Kannan *et al.*, 1992). Similar trends in residues have also been reported by Abdullah *et al.* (1997) from Malaysia, Vietnam, Thailand, Philippines, and Bangladesh. In the present context, detection of residues of OC in a small number of samples could be considered as a good sign.

Among the various OP pesticides analysed in paddy grains, trace amounts of Chlorpyrifos (1.83 ng/g) was the lone compound detected in *Thavalakannan* variety collected from conventional farm during *Rabi* (2009-10). Whereas concentrations of all the other OPs, namely Phorate, Methyl Parathion, Malathion, Parathion Ethyl, Pirimiphos Ethyl, Quinalphos, Phenthoate, Ethion and Triazophos were not detected in any of the samples tested. But in fodder, ∑-OP residues were detected in 12 out of 30 (40%) samples of freshly harvested paddy straw from conventional farms. It ranged between 2.02 ng/g (*Aishwarya*; *Rabi*, 2011-12) and 14.62 ± 1.63 ng/g in the same variety and season during 2009-10 with the mean value of 7.34 ± 3.14 ng/g. Interestingly, Quinalphos (4.39 ng/g) was present in trace level in only one of the samples of organic fodder (*Thavalakannan* variety) collected during *Rabi* 2009-10. This could be due to the fact that a few farmers adopting organic farming applied Quinalphos (Ekalux 25% EC) to control brown planthopper, hispa/ bune beetle, leaf roller and stem borer in paddy when effective biocontrol measures to control the above-referred pests was not employed.

Interestingly, residues of one of the fast degrading pesticide groups, namely synthetic pyrethroids (SPs) (λ-Cyhalothrin, Permethrin-I and II, and Fenvalerate-I and II) were detected

in six out of thirty paddy samples (20%) analysed from conventional farms with the maximum (87.55 ng/g) in *Aishwarya* variety collected during *Rabi* (2010-11) and minimum (8.04 ng/g) in the same variety and season during 2009-10. Levels of all the SPs were at BDL in organic paddy collected during the years. Unlike paddy grains, 36.66% of freshly harvested conventional farm fodder samples had ∑-SP residues ranging between 3.58 ng/g in *Thavalakannan* (*Rabi,* 2010-11) and 6732.55 ng/g in *Jothi matta* in the same season during 2009-10. While 2% samples of paddy straw (*Aishwarya*: 930.23 ± 388.0 ng/g) collected from newly adopted organic farms had the highest level of ∑-SP, the lowest was (4.26 ng/g) in *Jothi matta* during *Rabi* (2009-10).

It should be noted that conventional farm farmers of Padayetti village applied both λ-Cyhalothrin (Karate 5% EC) and Fenvalerate (Fenvol 25% EC) to control grain sucking insects such as rice bugs *Leptocorisa* sp (Hemiptera: Alydidae) and stink bugs *Pygomenida* sp (Hemiptera: Pentatomidae) those feed on the sap of developing spikelets of paddy during flowering stages in the conventional farm. At the same time, a few newly adopted organic farmers also applied Fenvalerate on paddy to control these insects. However, they did not apply any other chemical pesticides or fertilizers during the study period. A small group of farmers adopting organic farming managed to apply neem based biopesticides to control these groups of pests. We also have noticed that the overall usage of pesticides was reduced in the Padayetti agroecosystem and even a few conventional farmers applied only the fast degrading chemicals when it was unavoidable.

Concentrations of two carbamate pesticides such as Carbaryl and Carbofuran were at BDL in paddy cultivated from both the farms during the study period (2009-2012). Since samples of both paddy and fodder with detectable levels of residues were not many, no statistical test was performed. Concentrations of OPs and SPs in fodder varied significantly between the farms ($p < 0.05$; $F = 4.15$).

### 4.6.1 Residues of major isomers/ metabolites of organochlorine pesticides in paddy and fodder in Padayetti village, Palakkad district

Among the isomers and metabolites of OCs analysed, negligible amount of both Dieldrin (6.38 ng/g) and γ-HCH (1.89 ng/g) were detected in only one sample each from conventional farm (*Thavalakannan*) collected during *Rabi* (2009-10) and *Kharif* (2010-11) seasons respectively, while levels of other OCs were at BDL. Similarly, residues of all other OCs were not detectable in paddy from organic farm as well as freshly harvested paddy straw (fodder) from both the farms. The presence of Lindane (99% γ-HCH) in paddy might be because of the fact that high purity of this molecule is more resistant to biological and chemical degradation under aerobic conditions (Ashnagar *et al.,* 2009) and it was also most commonly used pesticides in India till 1997, but restricted use of γ-HCH was allowed till 2013 (CIBRC, 2014).

The most active isomer of HCH, namely γ-HCH which has powerful insecticidal properties as well (Aslam *et al.,* 2013). It was the only HCH isomer detected at traceable levels in paddy grains collected from conventional farm, while other isomers, namely α-, β- and δ-HCH were at BDL in all the paddy and fodder from both the farms. Trace amount of γ-HCH reported in the present study is lower than the levels reported in paddy grains from various Indian cities; Bombay (11 mg/kg: Noronha *et al.,* 1980); Parbhani (5.3 mg/kg: Jadhav, 1986) and Lucknow (4 ng/g: Misra *et al.,* 1998), and different cereals and pulses (3.9-5.8 ng/g) collected from four cities in the northern (New Delhi), western (Bombay), eastern (Calcutta), and southern (Madras, Chidambaram and Parangipetti) parts of India (Kannan *et al.,* 1992); ten Basmati rice from (Mazra, Baddripur, Clinton, Pithuwalakala, Shivalkhurd, Mahiwalamafi, Buddi, Harrhawala, Jogiwala and Balawalakala: 13-113 ng/g) in Dehradun (Babu *et al.,* 2003); polished rice samples (11 ± 2 ng/g) from supermarkets of Hyderabad city, India (Ravikumar *et al.,* 2013), and had higher than the levels reported in commercial rice-grains of Karachi, Pakistan (Uddin *et al.,* 2011) and rice-grain, husk, bran and broken rice (Pareja *et al.,* 2011) from Spain.

Levels of γ-HCH (1.89 ng/g) reported in paddy from conventional farm in the current study are well below the MRLs permitted by various statutory agencies, namely Prevention of Food Adulteration Act (PFA, 1954: 10 ng/g); Ministry of Health (Govt. of India, 1986: 50 ng/g; Anonymous, 1992); WHO/FAO *Codex Alimentarius* commission, 1986: 50 ng/g (Anonymous, 1988b), and Food Safety and Standards Authority of India (FSSAI, 2011: 100 ng/g) under Food Safety and Standards (contaminants, toxins and residues) Regulations.

Similarly, traceable level of Dieldrin (6.58 ng/g) residues detected in only one of the paddy varieties, namely *Thavalakannan* from conventional farm during *Rabi* (2009-10) was slightly higher than those reported from different cereals and pulses (3.9-5.8 ng/g) of New Delhi, Bombay, Calcutta, Madras, Chidambaram and Parangipetti, India (Kannan *et al.*, 1992); lower than wheat and rice reported from West Bengal, Maharashtra, Tamil Nadu and Delhi (10 ng/g) and higher than the levels observed from Australia (1.6 ng/g) (Kannan *et al.*, 1997a). Dieldrin, after many years of use in India, it was banned because of its persistence and toxicity on living organisms. Persistence of certain OCs has already been proved by researchers (Kaushik *et al.*, 2011; Ashnagar *et al.*, 2009; Gonzalez-Rodriguez *et al.*, 2008) who found residues of Dieldrin in the paddy, even though the application was stopped. The levels of Dieldrin reported in the present study are well below the MRLs set by PFA, 1954 (10 ng/g) in paddy grains. However, since it has been banned in most of the countries, agencies such as USEPA, European Commission, Health Canada, Germany, FSSAI, FAO/WHO did not revise the MRL (Arora *et al.*, 2014). Residues of other important OCs such as DDT and Endosulfan were not detected in any of the paddy or fodder samples analysed from both the farms. The overall residue levels, both in paddy and fodder, show a tendency of decline over three-year period of monitoring.

### 4.6.2 Residues of organophosphate pesticides in paddy and fodder in Padayetti village, Palakkad district

Trace amounts of Chlorpyrifos (1.83 ng/g) was detected in conventionally grown paddy *Thavalakannan* variety collected during *Rabi* (2009-10). Generally, Chlorpyrifos applied on

paddy persists mainly on paddy, fodder and hull (Arora *et al.*, 2011). The trace level (1.83 ng/g) of Chlorpyrifos reported in the present study is lower than the levels reported in Basmati paddy grains (1-50 ng/g) collected from IPM and non-IPM field trials conducted at four regions of Haryana, Uttar Pradesh and Uttarkhand in India (Arora *et al.*, 2014); paddy field experiments conducted in Nanjing (49-230 ng/g) and Guangxi (52-385 ng/g) provinces of China (Zhang *et al.*, 2012) and slightly lower than in different cereals and cereal byproducts (2-8 ng/g) reported from Jhansi, Banda, Gwalior, Jalaun, Tikamgar, Hamirpur, Chattarpur, Panna, Sagar and Bhind in India (Nag and Raikwar, 2011); higher than the levels reported in commercial rice-grains received from exporter/ trading corporation of Karachi, Pakistan (Uddin *et al.*, 2011); polished rice collected from supermarkets of Hyderabad, India (Ravikumar *et al.*, 2013); Basmati rice analysed from Tilwari (Dehradun), Kaithal (Haryana), Modipuram (Uttar Pradesh) and Pantnagar (Uttarkhand) (Mukherjee and Arora, 2011).

Chlorpyrifos residues recorded in paddy from conventional farm in this study was compared with MRL suggested by various statutory bodies such as PFA, 1954 (50 ng/g) and Joint FAO/WHO, 1986 (100 ng/g). Only some countries have established MRL for Chlorpyrifos in some crops. In China, Korea, Taiwan and India (FSSAI), the MRL of Chlorpyrifos in rice grain is 100 ng/g. Hence, the levels reported in the present study are well below the MRL set by the above-referred countries, it is unlikely to create any adverse effects. There are a few reports available on Chlorpyrifos residues in food chain (Aysal *et al.*, 2004). There is growing concern world over about the toxicological and environmental risks associated with Chlorpyrifos residues in environmental components. Levels of all other OPs, namely Phorate, Methyl Parathion, Parathion Ethyl, Pirimiphos Ethyl and Ethion were at BDL. Further, none of the OP pesticides checked was at detectable level in organically grown paddy.

Interestingly, 24% of fodder (straw) samples from conventional farm had ∑-OP residues with the highest level (14.62 ± 1.63 ng/g) in *Aishwarya* variety collected during *Rabi* season (2009-10), followed by 12.27 ng/g in *Jothi matta* variety collected during the same season and year, and lowest (2.02 ng/g) in *Aishwarya* variety collected during the same season in 2011-12.

Other samples those had relatively high level of ∑-OPs in conventional farms include 8.24 and 6.43 ng/g in *Thavalakannan* collected during *Rabi* and *Kharif* seasons in 2009-10 respectively, and 6.72 ng/g in *Jothi matta* (6.72 ng/g) collected during *Rabi* (2010-11).

Among the individual OPs observed in paddy straw from conventional farm, Quinalphos was found to be the highest (12.27 ng/g) in *Jothi matta* collected during *Rabi* (2009-10) and Triazophos the lowest (2.02 ng/g) in *Aishwarya* (*Rabi,* 2011-12) (fig. 4.6a). While Triazophos was detected in 16% of the conventional fodder samples, Malathion, Quinalphos and Phenthoate residues were detected in 2, 10 and 3% of the samples respectively. As stated above none of the paddy samples from organic farms had any detectable level of any of the OP pesticides. "*Thavalakannan*" is a popular variety because of its taste, high recovery of grain from paddy after milling (>60%) and ability of cooked rice to stay in cold water for a long time without losing hardness and taste. Cold rice (cooked rice kept overnight in coldwater) is the preferred breakfast for many in the village and elsewhere.

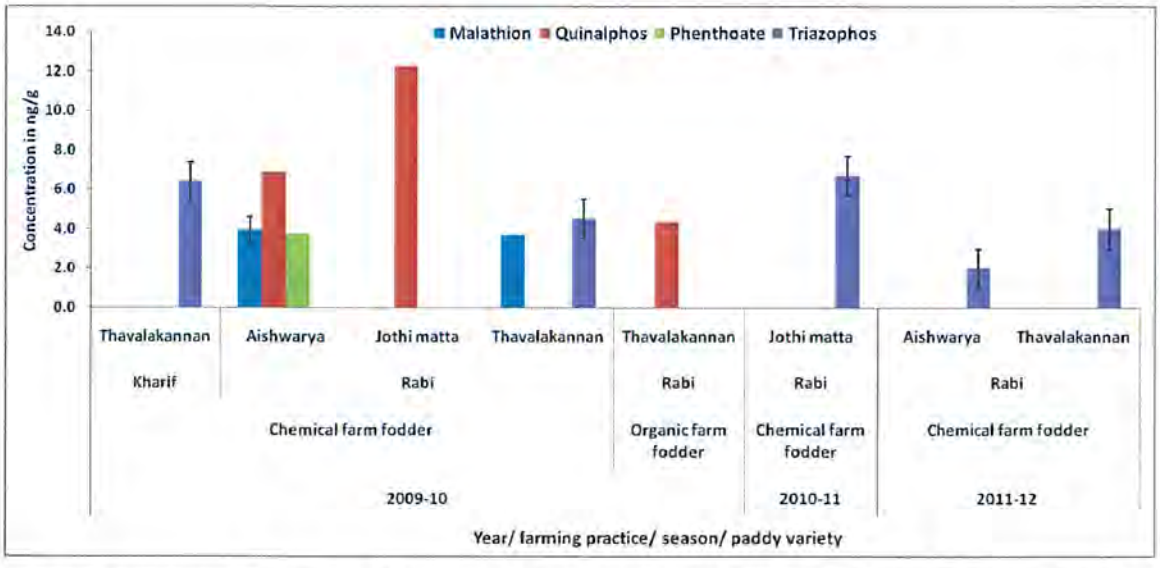



**Fig. 4.6a. Mean ( ± SE) levels of organophosphate residues in fodder (n=60; ng/g dry wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12)**

Among the varieties of paddy straw, *Jothi matta* had the highest levels of ∑-OP residues (9.50 ± 2.78 ng/g), followed by *Aishwarya* (8.32 ± 6.3 ng/g) and lowest in *Thavalakannan*

(5.77 ± 0.98 ng/g). There is a decreasing trend in ∑-OP residues observed in fodder during the study period 2009-10 (10.39 ± 1.87 ng/g) > 2010-11 (6.72 ng/g) > 2011-12 (3.02 ng/g). Trace level of Quinalphos (4.39 ng/g) was recorded in only one of the organic farm fodder samples of *Thavalakannan* variety during *Rabi* (2009-10). It should be noted that a few organic farmers applied Quinalphos (Ekalux 25% EC) during 2009-10 to control brown planthopper, hispa, leaf roller and stem borer in paddy. This might have contributed to the trace level of Quinalphos in the fodder. All other OPs were at BDL in the remaining samples of organic fodder collected during the study period. Quinalphos residue was detected in 10% of fodder samples from conventional farm. It is one of the commonly used pesticides by farmers of conventional farming in the Padayetti village. It comes in the trade names of Ekalux and Quinaltaf (1.5% DP, 5% G, 25% EC and 20% AP). The WHO ranked it as 'moderately hazardous' (http://www.pesticideinfo.org/ accessed on 2nd June 2014) and use of Quinalphos is either banned or restricted in most countries. It is classified as a yellow labeled (highly toxic) pesticide in India.

As evidenced from the survey of literature, only a few reports on the presence of certain OP pesticide residues in paddy and fodder are available from India and elsewhere (Bhattacharjee *et al.*, 2012; Arjmandi *et al.*, 2010; Chen *et al.*, 2009; Sanyal *et al.*, 2009). Several studies have been carried out on OC pesticide contamination of foodstuff from different parts of the world (Ravikumar *et al.*, 2013; Bhanti and Taneja, 2007; Gonzalez *et al.*, 2005; Hans and Farooq, 2000; Abdullah *et al.*, 1997). But limited information is available on the residues of OP group of pesticides in paddy and fodder.

Trace amounts (3.95 ± 0.68 ng/g and 3.71 ng/g) Malathion were observed in conventional fodder *Thavalakannan* (*Kharif*) and *Aishwarya* (*Rabi*) collected during 2009-10. Malathion is one of the five most commonly used pesticides in India, accounting for 65% of all OP pesticides applied in the field (Indian Chemical Statistics, 2002). Malathion products comes in the trade names of Celthion and Malatox (50% EC); Malaphos (25% and 5% WP) for controlling stem borer and leaf folders of paddy (Arora *et al.*, 2011). Malathion residues

were also frequently reported in soil, water, animal and plant tissues (Rajendran, 2003; Karanth, 2000; Harris *et al.,* 1998). Malathion is a slightly toxic compound in EPA toxicity class-III and it has not been shown to cause serious hazards to terrestrial plants or aquatic algae. However, studies support that Malathion is taken up and stored in plant tissues (USEPA, 2000a). Metabolites of Malathion were detected in new stem and leaf growth up to one-year post application (Newhart, 2006).

Similarly, Phenthoate (3.76 ng/g) residue was detected only in conventional fodder *Aishwarya* collected during *Rabi* (2009-10). Phenthoate (2% DP and 50% EC) was mainly used for controlling rice caseworm by conventional farmers. However, trace amounts of all the reported OPs are well below the tolerance limits. Hence, these levels need not create any adverse effects to any life forms. But continued exposure may harm the consumers.

### 4.6.3 Residues of synthetic pyrethroid pesticides in paddy and fodder in Padayetti village, Palakkad district

Residues of ∑-SP (87.55 ng/g) were the highest in paddy *Aishwarya* collected from conventional farm during *Rabi* (2010-11), and lowest (8.04 ng/g) in the same variety and season during 2009-10. The results showed that 20 and 36.6% of the freshly harvested paddy and fodder samples respectively from conventional farm had residues of atleast one of the six SPs, namely λ-Cyhalothrin (I and II), Permethrin (I and II) and Fenvalerate (I and II). Less than 2% of the organic fodder samples had Fenvalerate residues during the first year i.e. 2009-10 when farmers were converting from conventional to organic farming. They applied only Fenvalerate (Fenvol 20% EC) when they found difficulty in controlling rice hispa, leaf roller and stem borer when recommended biological control means failed. However, levels of all the SPs were at BDL in paddy grown organically. Among the individual SP molecules, levels of Fenvalerate-II (69.43 ng/g) was observed to be the highest in *Aishwarya* during *Rabi* (2010-11), and Permethrin-I the lowest in the same variety (2.56 ng/g) and season during 2009-10 (fig. 4.6b).

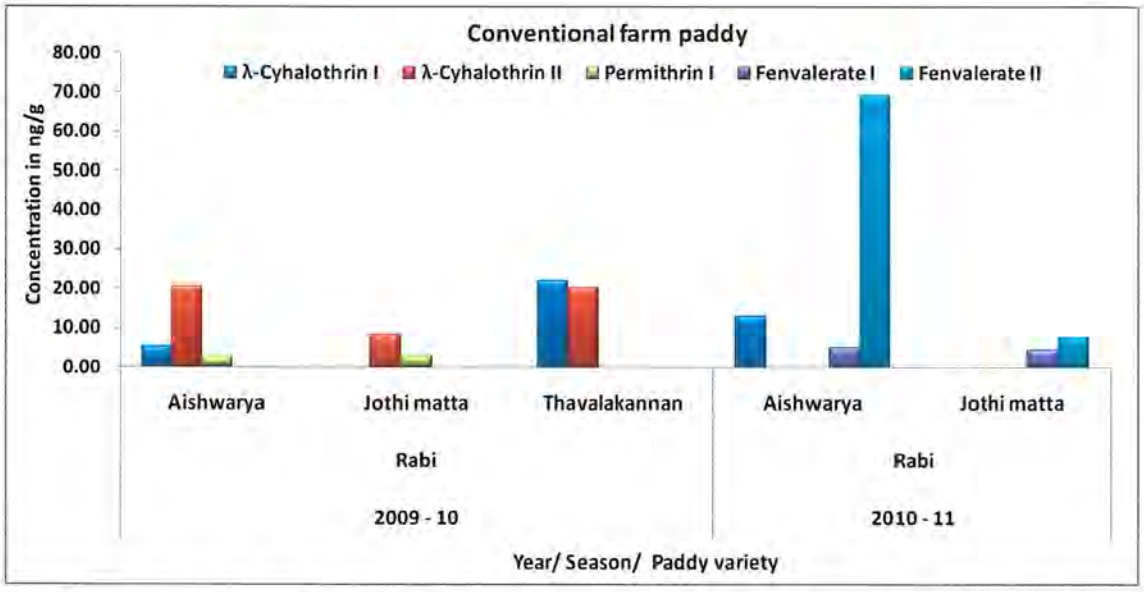



**Fig. 4.6b. Mean (± SE) levels of individual synthetic pyrethroid residues in paddy (n=60; ng/g dry wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12)**

At the same time, among the SPs analysed in fodder from conventional farm, highest concentration was recorded in *Jothi matta* (3390.60 ± 1260.08 ng/g) during *Rabi* (2009-10), and lowest (3.58 ng/g) in *Thavalakannan* during *Kharif* (2010-11). Further, fodder samples those had considerable levels of ∑-SP molecules are *Thavalakannan*: 1823.24 and *Aishwarya*: 1359.62 ± 195.05 ng/g collected during *Rabi* (2009-10). Whereas in organic straw, relatively low levels of residues were recorded in only one of the *Jothi matta* samples (4.26 ng/g) collected during *Rabi* (2009-10) and highest levels in *Thavalakannan* (275.98 ng/g) during the same season and year. This could be due to few newly adopted organic farmers applied Fenvalerate on paddy to control grain sucking insects and stink bugs. However, a small group of farmers adopting organic farming managed to apply biopesticides (mixture of plant extracts of garlic, ginger and chilli and neem oil) to control these pests.

Among the seasons, 10 out of 30 fodder samples analysed had higher SP residues during *Rabi* (winter) season with a mean concentrations of 687.92 ± 365.34 ng/g than *Kharif* (5.25 ± 1.72 ng/g). Similarly, between the farms, the residues of SPs were higher in conventional farm fodder (660.79 ± 369.72 ng/g) than organic fodder (95.67 ± 19.16 ng/g). It should be

noted that normally the study sites receive high rainfall during *Kharif* (May - September) than *Rabi* (October - February) season and it gradually decreases after November every year. Hence, there is more chance for recently applied pesticide residues to be washed off from the fodder (paddy straw) during *Kharif* than *Rabi* season.

Moreover, we have noticed that application of SPs on paddy in conventional farm when it is in flowering stage during *Kharif* season is very less than *Rabi* due to persistent rainfalls which leads to less attack of insect pests. The most part of the *Rabi* crop grows under excessive water and there is enough sunlight and cool climatic conditions during later part of paddy growth which support insect diversity. Hence, the farmers apply more SPs to control them. As we have reported elsewhere in the report, during the initial years i.e., 2009-10 a few farmers who were committed to organic farming applied λ-Cyhalothrin (Karate 25% EC) for controlling pests. Moreover, Fenvalerate (Fenvol 20% EC) was used by a few farmers to control the same insects while shifting from conventional to organic farming. This might have contributed to the trace levels of these pesticides in organic fodder during 2009-10. However, SP compounds were at BDL in all other fodder samples collected from both the farms. According to Laskowski (2002), the hot and humid climate, water temperature and pH could play important role in enhancing degradation rate of SPs in paddy straw. However, we did not study the role of these parameters in the present study.

Among the individual residues recorded in conventional fodder, Fenvalerate was found to be the maximum (3375.29 ± 1027 ng/g) in *Jothi matta* collected during *Rabi* (2009-10) and minimum (λ-Cyhalothrin: 3.58 ng/g) in *Thavalakannan* (*Kharif*, 2010-11). While λ-Cyhalothrin was detected in 4 and 0.5% of the conventional and organic fodder samples, ∑-Permethrin and ∑-Fenvalerate were detected in 1 and 2.5% of the fodder samples collected from conventional farm. Among the varieties of paddy straw, *Jothi matta* had the highest residues (851.40 ± 346.14 ng/g), followed by *Aishwarya* (457.15 ± 151.23 ng/g) and lowest in *Thavalakannan* (352.98 ± 197.37 ng/g). Similar to paddy, there was a decreasing trend in the residues of currently-used ∑-SP in paddy straw during the years i.e., 2009-10 (980.66 ±

488.28 ng/g) followed by 2010-11 (3.90 ± 0.22 ng/g) and 2011-12 (6.21 ± 1.18 ng/g). During the years, we noticed that the farmers of Padayetti minimized the overall usage of pesticides except a few in the conventional farms who used pesticides (λ-Cyhalothrin and Fenvalerate) to control grain-sucking insects during developing stages of grains.

Trace amounts of Permethrin I (3.75 ng/g) and Permethrin II (10.07 ng/g) were also observed in *Aishwarya* and *Thavalakannan* varieties of fodder collected during *Rabi* (2009-10). However, the levels of these pesticides were not detected in any of the fodder samples collected from organic farm. Pyrethroids are widely used on paddy in some countries (Zalom *et al.,* 2005; Dennett *et al.,* 2003; Lawler *et al.,* 2003; Das *et al.,* 1995), but prohibited in countries like China (Gu *et al.,* 2007) because of their high toxicity to aquatic organisms (Weston *et al.,* 2004).



**Fig. 4.6c: One of the old and popular varieties of rice in Kerala, *Thavalakannan***

*Thavalakannan* (In Malayalam means '*Thavala*' Frog and *Kannan* means 'eyed') is an indigenous variety of rice grown in Kerala. It is popular in Kerala and it is used for preparing idlies, appams and plain rice. This variety has been very popular from early days because of the richness of rice, its unique taste and use in preparations of rice based snacks. This gives normally up to four feet and because of its rich nutrients and large biomass it is used as

fodder. Normally it is been cultivated during *Viruppu* season in the study area (120 days crop) when pest attach is very minimum and it yields 1200-1500 kgs (Fig. 4.6c).

Some of the presently used OP and SP pesticides detected in the paddy and fodder are smaller molecular weight compounds, designed to breakdown rapidly in the environment (Zhang *et al.*, 2012) and may disintegrate further due to high temperature and long time storage (Babu *et al.*, 2003; Hans and Farooq, 2000).

This is the first study that documented the presence of OP and SP residues in samples of paddy and fodder in Padayetti village. The study further confirmed that the products (paddy and paddy straw) from the organic farms are safe to consume as the residues of insecticides were either below MRL or not detected. Although the products from conventional farms had residues of various pesticides below the established tolerance limits, their continued consumption by both human and ruminants can create deleterious effects in long term.

The results obtained from this study were compared with the recommended tolerance limits of the FDA, FAO/WHO, and FSSAI and Ministry of Health, Govt. of India for OC in paddy and fodder. To our knowledge there exists little information on the acceptance limits for OPs and SPs in food stuffs set forth by the Government of India. Hence, the tolerance limits suggested by the other countries were compared wherever applicable. The OC residue levels in paddy were well below the maximum acceptable limit set by FAO/WHO and MoH, government of India. However, regular monitoring of paddy crops are necessary for checking the status of residues since contaminated feed and fodder act as the main source of pesticides in animal body. Based on the results of the monitoring programme, appropriate measures should be taken so that animal products which are free from residues can be produced. The concept of healthy food and organic farming is catching up very fast world over. Consumers in the developed countries are prepared to pay a premium for the organic food. However, in India the mind set of people is yet to change.

### 4.6.4 Residues of Carbamate pesticides in paddy and fodder in Padayetti village, Palakkad district

Residues of Carbamate group of pesticides, namely Carbaryl and Carbofuran were not detected in any of the organic or conventional farm paddy and fodder samples tested.

## 4.7 Pesticide residues in cow-milk in the agroecosystem in Padayetti village, Palakkad district

The intake of contaminated feed and fodder by milch animals is the main source of entry of pesticides into their body which ultimately results in the contamination of milk, meat and other food consumed by humans. Thus the human body also gets contaminated (Nag and Raikwar, 2008). Occurrence of pesticide residues in cow-milk is a matter of public health concern, since they are widely consumed by infants, children and adults throughout the world. Pesticide monitoring studies are useful to know the level and main sources of contamination in milk (Kaushik *et al.*, 2011; Khaniki, 2007). Being on the highest levels of the trophic chain and due to its lipophilic nature (Alle *et al.*, 2009; Borga *et al.*, 2001), milk has been usually studied as an indicator of the bioconcentration process of environmental organic micropollutants (Di Muccio *et al.*, 1989). Hence, as recommended by UNEP (Leslie and Deboer, 2011) it is one of the convenient food stuffs for measuring the pesticides. In view of this and considering acute and chronic toxic effects on human caused by pesticide residues in food, this study aimed to analyze the residues of legendary and contemporary chemicals present in milk from cows which were fed with paddy straw (fodder) harvested from organic and conventional farms.

A total of 90 full-fat milk samples (each 45 from organic and conventional) were collected seasonally (*Kharif*, *Rabi* and summer) between May 2009 and April 2012. Present study reports the levels of 40 pesticide residues (20 OCs, 12 OPs, six SPs and two Cbs) in 70 cow-milk (each 35 from organic and conventional farms) samples.

### 4.7.1. Residues of OP, SP and Cbs in cow-milk in Padayetti village, Palakkad district

Out of 70 samples of cow-milk collected from conventional farm, 4.29% samples were contaminated with OP, SP and Cb pesticide residues, while one of the organic farm milk contained detectable level of OP compound i.e., Methyl Parathion (table 4.7a). But none of other compounds or groups (i.e., OCs) analysed either in organic or conventional farm milk samples had any residue of any other pesticides. The restriction and banning of OCs have resulted in reduction of their residues in the milk (Kaushik *et al.*, 2011).

**Table 4.7a Mean ( ± SE) levels of individual OP, SP and Cb residues in cow-milk (n=70; ng/mL wet wt) in the agroecosystem in Padayetti village, Palakkad district (2009-12)**

| Year/ Season | Farming Practice | Organophosphate | Synthetic Pyrethroid | Carbamate |
|---|---|---|---|---|
| | | Methyl Parathion (ng/mL ) | Fenvalerate-II (ng/mL) | Carbaryl (ng/mL) |
| 2009-10/ *Rabi* | Conventional | BDL | 3.87 | BDL |
| 2009-10/ Summer | Conventional | BDL | BDL | 5.23 ± 1.5 |
| 2010-11/ Summer | Organic | 22.64 | BDL | BDL |
| 2011-12/ Summer | Conventional | 30.09 | BDL | BDL |

Note: BDL - Below Detection Limit

The mean concentration of total pesticide residues (9.80 ng/mL) was the highest during summer seasons i.e., 2009-10 and 2011-12 in milk samples collected from cows which were fed with feeds available in the market and fodder grown in conventional farms. These residues were detected mainly during summer seasons. This might be because of the dietary habit of the cows. Since there is no fresh green grasses available during summer months due to paucity of water, farmers fed cows with dry fodder (paddy straw) and what is available in the market. But during *Kharif* and *Rabi*, the cattle get fresh grasses to eat. The residues, which are adhered on the grasses, might get washed off to some extent with rain water, reducing the overall intake of pesticides as compared to summer seasons.

Among all the OPs, Methyl Parathion (30.09 ng/mL) was the only compound which was detected in one of the conventional farm milk samples collected during summer (2011-12).

This is lesser than the levels in milk collected from organic (22.64 ng/mL) farms in the same season during 2010-11. This chemical which is used presently could have entered through misuse. We have noticed that few farmers used dustable power of Methyl Parathion to control ants, termites and ticks in their cattle yards. This might have contaminated the cow-milk. However, regulatory bodies such as Food Safety and Standards Authority of India (FSSAI, 2011), European Commission (2000), Health Canada (1997), Food and Agricultural Organisation and World Health Organisation (FAO/WHO *Codex Alimentarius* commission, 2010) have not established any limits for Methyl Parathion in milk. The residues detected in the present study were lower than the MRLs (50 ng/mL) set by Australian government (Stenhouse, 1992).

Methyl Parathion is a restricted use pesticide in India (CIBRC, 2014). Use of 2% DP and 50% EC formulations on fruits and vegetables were banned and permitted only on those crops where honey bees are not acting as pollinators. Production of Methyl Parathion may be continued in the country for use on other crops (CIBRC, 2014) mainly for controlling gall midge, leafhoppers, hispa, leaf roller, stem borer and whorl maggot (Arora *et al.*, 2011).

Traceable amount of a SP compound, Fenvalerate-II (3.87 ng/mL) was detected in one of the conventional farm cow-milk samples analysed during *Rabi*, 2009-10. Pyrethroids are of greatest interest as they are water soluble. Fenvalerate, which is used in conventional farms, could be up to 20 times more toxic than other SPs (Amweg *et al.*, 2005). It was found that Fenvalerate did not exceed the MRLs limits of 100 ng/mL for milk set by the WHO/FAO (1986) and 10 ng/mL on fat basis by FSSAI (2011). Other agencies have not established any threshold limits for Fenvalerate in milk.

Residues of Carbaryl (5.23 ± 1.5 ng/mL) were detected in two of the milk samples analysed from conventional farm during summer (2009-10). This reflects the use of Carbaryl in the study sites. Farmers in the area sprayed up to 250 g/ha for controlling all sap sucking insects of paddy. It may be worth mentioning that around 225 MT of Carbaryl is being used in India on various crops (CIBRC, 2014). The elimination of Carbaryl is rapid and takes place mainly

through urine, feces and milk in dairy animals. However, even minute quantities of its residues in food chain may produce toxicological effects following their consumption by man and animals (Rai *et al.,* 2008; Kuhr and Dorough, 1976). Even though FSSAI (2011) has not established any limit for Carbaryl in milk, the residue level recorded in the present study is lower than the MRLs (50 ng/mL) fixed by WHO/FAO (2006).

It could be concluded on the basis of residue analysis that trace amount of Methyl Parathion was detected in both organic and conventional milk, while both Fenvalerate and Carbaryl were measured only in conventional farm milk samples. However, none of the samples contained residues above the MRL set by any agency. Although Methyl Parathion, Fenvalerate and Carbaryl were used for paddy cultivation in Padayetti village in conventional farms, their residues in trace levels need not pose any adverse effects. The boiling, pasteurization or sterilization can reduce these pesticides. The study shows the need to continue monitoring of past and currently-used pesticides in milk in order to ensure food safety and evaluate effective implementation of imposed ban/ restrictions on usage (Kaushik *et al.,* 2011). Thus, monitoring of residues in milk is a helpful tool and its data can be reference to a vigilance system in the studied sites, decreasing the risks for consumers.

# SUMMARY AND CONCLUSION

# 5. SUMMARY AND CONCLUSION

- The ever-increasing human population and the corresponding demand for food have forced the producers to intensify their efforts to increase the productivity. Among the grain crops, paddy is the highest pesticide consuming crop in India. Increasing awareness on health and many environmental issues associated with the indiscriminate use of chemical fertilizers and pesticides has led to interest in alternate methods of agricultural farming which are environment-friendly and economically feasible. The Kerala State Biodiversity Board (KSBB) through Department of Environment and Climate Change (DoECC) launched a demonstration project known as *"Granary of Kerala"* in Palakkad district in 2009 to conserve biodiversity associated with agroecosystems by totally avoiding chemical inputs in agriculture.

- To protect the paddy crop from weeds, insects and diseases, pesticides such as insecticides, herbicides and fungicides are used. Moreover, determination of pesticide residues in paddy has become increasingly essential for consumers, producers and authorities responsible for quality control. Hence, this study was conducted to investigate pesticide residues in different environmental components adopting two different types of cultivation methods, namely organic and conventional.

- Although use of most of the OCPs for agricultural purposes has been banned in India, their residues still remain in the environment. An attempt has been made to evaluate the possibility of contamination of foodstuffs by analyzing the presence of these residues in organic and conventional farms in select biotic (crab, snail, fish, frog and cow-milk) and abiotic (sediment, paddy and fodder) components in Padayetti village.

- Samples of sediment, crab, snail, fish, frog, paddy, fodder and cow-milk from organic and conventional paddy farms were collected and analysed for historical (OCs) and currently-used-pesticides (OPs, SPs and Cbs) to assess the magnitude of contamination and variation between conventional and organic farms. Data have been compiled to

check the overall load of pesticides, species, taxa, variety, farming practice and season wise, with reference to different groups. In addition to this, within a group, levels of individual pesticides and their isomers or metabolites have been compiled wherever applicable to understand the implications with reference to usage pattern or policy of the government in existence.

- The highest level of OC (HCH: 30.90 ± 9.83 ng/g) residue was in the sediments of conventional farms collected during *Kharif* season (2009-10) and lowest (2.34 ng/g) during summer season (2010-11). None of the sediment samples collected from organic farms had detectable levels of any OC residues. Among the currently-used OPs, 18.33% of sediments collected from conventional farms had Chlorpyrifos or Triazophos residues with maximum of 22.07 ± 4.80 ng/g, while levels of these compounds were in trace amounts (3.26 ng/g) in one of the organic farm sediments collected during *Rabi* season (2011-12) respectively. Levels of SPs such as Fenvalerate-I and Fenvalerate-II were fairly high in only one of the sediment samples collected from conventional farms (62.80 ng/g) during *Rabi* season (2011-12), whereas levels of these compounds were below detectable in other conventional and organic sediments in all the seasons during the study period (2009-12). The levels of two carbamates, namely Carbaryl and Carbofuran were BDL in sediments collected from both the farms. Levels of currently-used pesticide residues (i.e OPs) varied significantly between organic and conventional farm sediments ($p < 0.05$; $F = 0.79$).

- About 12.16% of crabs had detectable levels of OC residues with the maximum of 72.30 ng/g in crabs collected from conventional farms during summer (2011-12) and minimum of 1.55 ng/g during the same season in 2009-10, whereas in organic farm less than 3% of crabs collected contained persistent OCs with the highest (21.32 ng/g) during *Rabi* (2011-12) and lowest (1.61 ng/g) during *Kharif* (2011-12) seasons, respectively. This could be because of historical use of OCs in this region. 14.86% of crabs collected from conventionally grown paddy farms had one or other OP residues with the highest concentrations (172.40 ng/g) during *Kharif* (2011-12), but the levels of all the OPs were at BDL in crabs from organic farms. Synthetic pyrethroid residues were fairly high in only one of the crabs collected from conventional farms (197.40 ng/g) during *Kharif* season

(2011-12). One of the dead crabs collected from the conventional farm recorded 3501.91 ng/g of Carbaryl (commonly used Carbamate) residue (*Rabi* 2011-12) suggestive of poisoning.

- Among the OCs analysed in snails, 18.75% of them collected from conventional farms had residues, with the highest (83.91 ± 4.17 ng/g) during *Kharif* season (2009-10) and lowest (3.33 ± 0.17 ng/g) during the same season in 2010-11. It should be noted that most of the persistent OCs are banned for agricultural use in India except DDT which is being used for vector control. Further, due to the past usage of these pesticides in the region snails from both the farms had OC residues. Levels of γ-HCH varied significantly among the years ($p < 0.05$; $F = 8.03$) during the study period. Among the OPs, trace amounts of Triazophos (9.96 ng/g) and Phorate (5.18 ng/g) were detected in each one of the snail samples collected from conventional farms during 2009-10 *Kharif* and *Rabi* seasons respectively. However, levels of all the OPs were BDL in all the snails collected from organic farms. ∑-SP residues were detected in two and one of the conventional and organic farm snails respectively. Presence of SP residues in snails from organic farm could be because a few farmers practicing organic farming resorted to using Fenvalerate to contain sucking insect on paddy. Unfortunately effective biological control methods to contain the above-referred pest were not adopted. It was observed that the farmers of organic farming did not apply any other pesticide. Residues of both the carbamate pesticides, namely Carbaryl and Carbofuran were at BDL in all the snails collected from both the farms during the study period.

- Concentrations of all OCs were at BDL in all the species of fishes tested from both the farms, and also the fishes had very less level of OP and SP residues. Among the OPs analysed, trace levels of Phosphamidon (2.98 ng/g) was observed during *Rabi* 2009-10, while Methyl Parathion (3.23 ng/g) and Phenthoate (2.78 ng/g) were measured during summer in the same year. None of the fishes collected from organic farm had detectable levels of any of the OPs analysed. Similarly, λ-Cyhalothrin was the only SP pesticide detected at trace levels in two species of fishes (*Puntius dorsalis*: 4.32 ng/g - *Rabi* 2009-10 and *Anabas testudineus*: 2.07 ng/g - summer 2009-10) from conventional farm, while other SPs were at BDL. Levels of Cbs were also BDL in fishes from both the

farms during the study period (2009-12). Statistical tests were not performed as the number of samples with detectable levels of residues was small.

- The study also looked at the dietary input of chemicals to human beings through consumption of these fishes. The calculated dietary intake (per week) of Methyl Parathion, Phosphamidon and Phenthoate residues were 115.36, 106.43 and 99.29 ng/g through consumption of *Channa punctatus*, *Cirrhinus mrigala* and *Puntius sophore* respectively. Intake of λ-Cyhalothrin residues was 154.29 ng/g through *Puntius dorsalis* and 73.77 ng/g through *Anabas testudineus*. Based on the concentrations of OP and SP pesticides in the fishes dietary input was not calculated as MRLs for these pesticides have not been formulated yet by FSSAI or International statutory agencies, namely USEPA, European Commission, Health Canada, Germany, Food and Agricultural Organisation and World Health Organisation.

- Seven frogs from conventional and none from the organic farms had deformities. The mean occurrence of deformity observed in this study (6.42%) is slightly higher than the background levels of deformities (5%) reported in wild frog populations in California, USA and agroecosystems (3.98%) in central Western Ghats. Species with deformity were Indian Rice Frog *Fejervarya limnocharis* (n=6) and Jerdon's Bullfrog *Hoplobatrachus crassus* (n=1). Levels of Σ-OC (33.22 ± 16.48 ng/g) and SPs (26.91 ± 7.12/ 42.15 ng/g) were fairly high in deformed *F. limnocharis* and *H. crassus* significantly greater than in healthy frogs. The concentrations of Σ-OP residues observed in *F. limnocharis* (4.97 ± 1.73 ng/g) and *H. crassus* (1.02 ± 0.29 ng/g) indicate considerable exposure to these compounds. Residues of Carbofuran (2.09 ng/g) were detected in one of the healthy *F. limnocharis* collected from conventional farm. Levels of currently-used pesticides varied significantly between organic and conventional farm frogs ($p < 0.05$; $F= 8.2$). Hence, we suspect that high concentrations of Fenvalerate detected in the present study in deformed *F. limnocharis* (26.91 ng/g) and *H. crassus* (42.15 ng/g) might have contributed to the deformity.

- The residues of persistent OCs were detected in 3% of the paddy samples analysed, with the highest (6.38 ng/g) in *Thavalakannan* variety from conventional farm collected

during *Rabi* (2009-10) and lowest (1.89 ng/g) in the same variety of paddy during *Kharif* season in 2010-11. However, levels of all the OCs were at BDL in fodder samples from both the farms. The present study revealed trace levels of these OC compounds in few paddy samples, suggestive of bioconcentration through sediments/ soil and stalk of the plants. In the present context, decreasing trend in OC residues is a good sign. Among the OPs in paddy grains, trace amounts of Chlorpyrifos (1.83 ng/g) was detected in *Thavalakannan* variety from conventional farm during *Rabi* (2009-10). But in fodder, ∑-OP residues were detected in 40% of freshly harvested paddy straw from conventional farms with the mean being 7.34 ± 3.14 ng/g. However, Quinalphos (4.39 ng/g) was present in trace level in only one of the organic fodder samples of *Thavalakannan* variety during *Rabi* 2009-10. This could be because few organic farmers applied Quinalphos (Ekalux 25% EC) during 2009-10 to control brown planthopper, hispa, leaf roller and stem borer in paddy. Interestingly, one of the fast degrading pesticide groups, ∑-SPs (λ-Cyhalothrin, Permethrin-I & II, and Fenvalerate-I & II) were detected in 20% of paddy samples analysed from conventional farms. Unlike paddy grains, 36.66% of freshly harvested conventional farm fodder samples had ∑-SP residues ranging between 3.58 ng/g in *Thavalakannan* (*Rabi*, 2010-11) and 6732.55 ng/g in *Jothi matta* in the same season during 2009-10. While 2% of samples of paddy straw (*Aishwarya*: 930.23 ± 388.0 ng/g) collected from newly adopted organic farms had the highest level of ∑-SP, the lowest was 4.26 ng/g in Jothi matta during *Rabi* (2009-10). The concentrations of OPs in fodder varied significantly between the farms ($p < 0.05$; $F = 4.15$).

- From the results of the present study, it is understood that conventional farm fodder contain residues of OPs and SPs. The level of contamination is not much and number of samples exceeding the tolerance limit are also very few. However, regular monitoring of paddy crops is necessary to know the status of contamination in feed and fodder which act as the main source of pesticides in animal body. Based on the results of the monitoring programme, appropriate measures should be taken so that animal products which are free from pesticide residues can be produced.

- The results showed that harvested produce (paddy and paddy straw) from the organic farms are safe for the consumers as the residues of insecticides were either below MRL

or BDL. However, in conventional farms, the concentration of various pesticides were well below the established tolerance limit but continued consumption of such paddy and fodder by both human and cattle even with moderate contamination level can accumulate in the receptor's body and may prove fatal for human population in the long term. By comparing with various statutory guidelines that γ-HCH, Dieldrin, Chlorpyrifos, Quinalphos, Phenthoate, Triazophos, λ-Cyhalothrin, Permethrin and Fenvalerate detected in the paddy and straw from conventional farms. It could be said that the residues do not pose direct hazard. However, exposure to these pesticides has been shown to pose some risk to human as well as animals elsewhere.

- Around 4.29% of cow-milk collected from conventional farm had detectable levels of at least one of the OP, SP and Cb pesticide residues. Among all the OPs, Methyl Parathion (30.09 ng/mL) was the only compound detected at higher concentration in one of the conventional farm milk samples collected during summer (2011-12) than in milk collected from organic (22.64 ng/mL) farms in the same season during 2010-11. This currently used pesticide could have entered through indiscriminate use. National regulatory body such as FSSAI (2011), European Commission (2000), Health Canada (1997), and FAO/WHO *Codex Alimentarius* commission (2010) have not established any limits for Methyl Parathion in milk. However, these residual concentrations were lower than the MRLs (50 ng/mL) set by Australian government (Stenhouse, 1992). Trace amount of lone SP compound, Fenvalerate-II (3.87 ng/mL) was detected in one of the milk samples from conventional farm during *Rabi*, 2009-10. Residues of Carbaryl (5.23 ± 1.5 ng/mL) were detected in two of the milk samples analysed from conventional farm during summer (2009-10). This reflects the use of Carbaryl at the studied sites. Farmers of the area spray up to 250 g/ha of Carbaryl for controlling all sap sucking insects on paddy. It may be worth mentioning that around 225 MT of Carbaryl annually is being used in India on various crops (CIBRC, 2014). The elimination of Carbaryl is rapid and it takes place mainly through the urine, faeces and milk of dairy animals. However, even minute quantities of its residues in food chains may produce toxicological effects in man and animals (Rai et al., 2008; Kuhr and Dorough, 1976). Even though FSSAI (2011) has not established limit for Carbaryl in milk, the residue level was lower than the MRLs (50 ng/mL) fixed by WHO/FAO (2006).

- Some of the conventional farm fodder samples contained detectable level of residues of OP and SP pesticides, but in cow-milk these contaminants were not detected. This is may be because pesticide residues in fodder could have undergone environmental degradation.

- On the basis of percentage of composition of individual pesticides present in each components, Fenvalerate was most frequently detected. Both Fenvalerate-I (28.11 ng/g) and Fenvalerate-II (34.70 ng/g) were detected in sediments collected from conventional farms during *Rabi* 2011-12, while it was BDL in sediment from organic farm. Among the other components, crab *Geothelphusa dehaani* (59.58 ng/g; *Kharif* 2011-12), frogs *F. limnocharis* (26.91 ± 9.19 ng/g) and *H. crassus* (42.15 ng/g) during 2010-11 *Kharif* season, snail *Pila globosa* (20.33 ng/g; 2009-10 *Kharif* season) and freshly harvested paddy straw (*Jothi matta*: 6732.55 ng/g; 2009-10 *Rabi* season), collected from conventional farms had high concentrations of ∑-Fenvalerate residues. The high concentration of these contaminants is of great concern, especially when the increasing accumulation potential of these compounds in the food chain is considered. Thus, to avoid the negative effects of agricultural pesticides on faunal diversity, we urge for atleast a minimization programme in the use of these products, and in cases where their use is considered essential, atleast for the correct use and dosage.

- To promote more judicious use of pesticides in the paddy field, adoption of IPM strategies involving a variety of biological and physical pest control methods are essential. This has resulted in varying degrees of success. We have noticed that the overall usage of pesticides was reduced in the Padayetti agroecosystem and even a few farmers adopting conventional farming applied only the fast degrading chemicals based on requirement. However, IPM strategies in conventional farms in Padayetti village were not implemented effectively. This has been attributed to the farmers' lack of understanding of pest management concepts. It recognized that effective IPM programs must be adopted to the need of local farmers.

- Data generated from this study could form baseline information for future assessment of OC, OP, SP and Cb residues through exposure levels in organic and conventional paddy farming.

- Based on the present investigation, it can be concluded that emphasis must be given on framing norms for application of pesticides in conventional farms. This may reduce the potential health hazards on non-target organisms upon their exposure to these chemicals. However, eventually organic farming is the need of the hour.

**RECOMMENDATIONS**

- Convincing farmers to switch from conventional farming practice to organic is easier said than done. Unless farmers are convinced of advantages in terms of monetary benefits and quality of produce, efforts will go futile. This requires systematic approach with the active cooperation of government agencies, farmers, agriculture scientists, ecotoxicologists and not the least, the consumers.

- Some of the currently-used pesticides are highly toxic across the food chain including man. These classes of pesticides have relatively short half-lives, but nonetheless can still evoke toxicity in non-target organisms. Hence, regulatory agencies should consider imposing restrictions on the use of these pesticides.

- While submission of toxicity data on birds and mammals are necessary for registering a new molecule world over, for amphibians no reference is made in the registration process not only in India but also in many other countries. Our results indicate that existing risk assessment procedures for pesticide formulation need to be strengthened to protect also the amphibians.

- We hope that our work will encourage further investigations on the role of agrochemicals on frog declines, given that to-date there has been relatively very little research on the role of contaminants on amphibian decline in India.

- Farmers have to be provided with safe alternatives to handle pests without compromising productivity. Agricultural subsidy should be offered to farmers towards conserving biodiversity in agroecosystem and organic farming needs to be promoted among different levels of farmers.

- Production of organic fertilizers (neem and castor oil cakes), biopesticides, bionutrients and green manures should meet the demands.

- More organic farm produce outlets need to be operated across the state and country to help farmers sell their produce at reasonable rates. This could have appropriate government regulation.

- The concept of healthy food and organic farming is catching up very fast the world over. Consumers in the developed countries are prepared to pay a premium for the organic food. The premium price of organic produce is an important factor to encourage farmers to convert from conventional to organic farming.

- It is also advisable to carryout similar studies in other parts of the state/ country to evaluate the level of exposure. Present study calls for urgent action to reduce the level of OP, SP and Cb exposure and their effects on wildlife and human health.

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# PUBLICATION

# *Pesticide Residues in Two Frog Species in a Paddy Agroecosystem in Palakkad District, Kerala, India*

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# Pesticide Residues in Two Frog Species in a Paddy Agroecosystem in Palakkad District, Kerala, India

**Ganesan Kittusamy · Chandrasekar Kandaswamy · Nambirajan Kandan · Muralidharan Subramanian**

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**Abstract** Pesticides residues were quantified in 109 frogs comprising two species (*Fejervarya limnocharis* and *Hoplobatrachus crassus*) from organic and conventional paddy farms in Kerala, India. Seven frogs from conventional but none from the organic farms revealed deformities. Levels of total Organochlorines (OCs) (33.22 ng/g) and Synthetic Pyrethroid, Fenvalerate-II (26.91/42.15 ng/g) in deformed *F. limnocharis* and *H. crassus* were significantly greater than in healthy frogs. Among OCs in healthy frogs, traces of γ (gamma)-HCH (hexachlorocyclohexane) (2.12 ng/g) were found only in *F. limnocharis* from organic farm. Among Organophosphates, Phorate (1.02 ng/g) and Quinalphos (2.62 ng/g) were present in traces in deformed *F. limnocharis*, while Parathion ethyl (1.02 ng/g) was detected in deformed *H. crassus*. The data indicate that the high level of pesticides may have contributed to the deformity of frogs. Therefore, an elaborative study will be essential to conserve amphibians in India.

**Keywords** Pesticides · *Fejervarya limnocharis* · *Hoplobatrachus crassus* · Deformity · Agroecosystem

Amphibians are considered to be sentinels of environmental contamination. Biphasic life stages, varying reproductive strategy and highly permeable skin through which xenobiotics are readily absorbed from the surroundings may render frogs more vulnerable to chemicals than many other vertebrates (Bruhl et al. 2013). During the last two decades, decline in amphibian populations in many parts of the world has been reported (Hudson et al. 1984; Sparling et al. 2001; Stuart et al. 2004). However, information about this phenomenon is scarce, particularly in India. According to the global amphibian assessment (2007), of the 5,743 known species, over 1,856 (32.5 %) appear on the IUCN red list as endangered, threatened or vulnerable, suggesting they are at greater risk than mammals (23 %) and birds (12 %). These precipitous and rapid declines are thought to be caused by one or a combination of the following factors: habitat destruction (Houlahan et al. 2000); increased UV-B radiation (Blaustein et al. 2003); climate change (Carey and Alexander 2003); acid deposition (Vertucci and Corn 1997); bacterial, viral and fungal infections (Morehouse et al. 2003); introduced predators (Knapp 2005); harvesting for food, scientific research and pet trade (Jensen and Camp 2003); heavy metal, polychlorinated biphenyl (PCB), fertilizer and pesticide contamination (Collins and Storfer 2003). Often deformities in amphibians have been reported in agricultural areas where breeding and larval developments of frogs occur mainly during winter season when use of chemical pesticides is the maximum (Taylor et al. 2005; Fryday and Thompson 2012). Therefore, frogs inhabiting such environments may experience pesticide exposure causing toxic effects. In the present study we addressed the following objectives, (i) to study the levels of pesticide accumulation in the frogs collected from organic and conventional farms and (ii) to assess for a potential contribution of pesticides to frog deformities.

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were evaporated to near dryness and reconstituted with 1 mL of HPLC grade n-Hexane and final analyses were carried out with Gas Chromatography-Mass Spectrometry (GC–MS) at the analytical facility of Agilent Technologies India Pvt. Ltd, Bangalore. Qualitative and quantitative analyses of OCs, OPs, SPs and Cb were made with GC (Agilent Model 7890A Series) equipped with MS (5,975 Quadruple) using HP-5 ms fused silica capillary column (15 m × 0.25 mm I.D × 0.25 μm film thickness) coated with 5 % phenyl and 95 % dimethyl polysiloxane. While Helium (IOLAR) was the carrier gas (flow rate: 1.0 mL/min), chromatographic operating conditions were as follows: detector temperature was set at 325°C; the injector temperature was programmed as 85°C for 12 s, increased at 600°C/min to 325°C and held for 5 min, then again increased at 10°C/min to 200°C (0 min); similarly oven temperature programmed as 70°C (1 min), increased at 25°C/min to 150°C (0 min), again increased at 3°C/min to 200°C (0 min) and then 8°C/min to 280°C (10 min). MS source temperature: 230°C; MS quadruple: 150°C; Technique: Electron ionization (EI); total run time: 40.867 min. The calibration standards with a minimum of five calibration points (1.56, 3.26; 6.25, 12.5, 25, 50 and 100 ng/g) spanning the expected concentrations in the samples were used for each analyte. All the samples were analyzed for a set of OCs, OPs, SPs and Cb (Table 2). An equivalent mixture manufactured by Sigma-Aldrich was used as standard. Concentrations of individual compounds were quantified from the peak area of the sample to that of the corresponding external standard. Recoveries of the compounds from fortified samples (50 μg/kg) ranged from 91 to 102 %. Results were not corrected for per cent recovery and expressed in wet weight basis. The limits of detection (LOD) for the method were in the range of 0.25–0.75; 0.29–0.62; 0.09–0.40 and 0.25–0.80 ng/g for the OC, OP, SP and Cb respectively. Quantification limits were set at three times the instrumental detection limit for each chemical, and they ranged from 0.25 to 0.80 ng/g. Precision for the duplicate samples were between 98 and 99.5 %. Results are presented as mean values and range (min–max). Statistical tests of pesticide levels between healthy and deformed frogs as well as organic and conventional farms were carried out using analysis of variance (ANOVA) (SPSS Ver. 16) with a significance level of 0.05. Mean values were calculated by treating the concentration of the non-detected analytes as zero. Values below the detection limits are reported as below detection limit (BDL).

## Results and Discussion

Levels of currently used pesticides (i.e. OPs and SPs) varied significantly between organic and conventional farm frogs ($p < 0.05$) but the levels of historical chemicals (i.e. OCs) did not vary between farms ($p > 0.05$) (Table 2). It was observed that out of 109 frogs collected, seven had deformities (6.42 %). Interestingly, all the deformed frogs were collected from the conventional farm. Species with deformity were Indian Rice Frog (n = 6) and Jerdon's Bullfrog (n = 1; Fig. 2). It is to be noted that we did not examine the abundance, species distribution or species richness of frogs. However, visually encountered frogs were collected and examined for any morphological disfigurement following Meteyer (2000). The mean occurrence of deformity observed in this study (6.42 %) is slightly higher than the background levels of deformities (5 %) reported in wild frog populations in California, USA (Johnson et al. 2001) and agroecosystems (3.98 %) in central Western Ghats (Gurushankara et al. 2007).

Concentrations of β (beta)–(9.36 ng/g), α (alpha)–(8.84 ng/g), δ (delta)–(8.38 ng/g) and γ (gamma)–HCH (3.14 ng/g) were found to be high in the deformed *F. limnocharis* collected from the conventional farms, but HCH isomers were BDL in the healthy frogs collected from the same field. Technical HCH is a broad spectrum OC insecticide used throughout the world for agricultural and non-agricultural purposes due to its effectiveness in pest control and low cost (Raina et al. 2008). The isomeric forms of HCH are toxic and recalcitrant, particularly the β isomer acts as an environmental estrogen. Mean concentrations of the γ-HCH (3.14 ng/g) and α-HCH (8.38 ng/g) recorded in one of the deformed *F. limnocharis* is higher (γ-HCH: 0.29 and α-HCH: 0.31 ng/kg) than the levels reported by Fellers et al. (2004) in healthy yellow-legged frog *Rana muscosa* from Sierra Neveda Mountains of California, USA. Use of HCH continued unabated until it was banned in 1997 in India, but restricted use of lindane was allowed till 2011. Although the levels of other chemicals, namely DDT, Heptachlor, Endosulfan, Aldrin,



**Fig. 2** *Hoplobatrachus crassus* with missing lower limb

**Table 2** continued

| S·No | Chemical compound | Normal frogs | | | | Deformed frogs | |
|---|---|---|---|---|---|---|---|
| | | Organic farm | Conventional farm | Organic farm | Conventional farm | Conventional farm | |
| | | *Fejervarya limnocharis* (*$p$ = 6) | *Fejervarya limnocharis* (*$p$ = 6) | *Hoplobatrachus crassus* (*$p$ = 4) | *Hoplobatrachus crassus* (*$p$ = 4) | *Fejervarya limnocharis* (n = 6) | *Hoplobatrachus crassus* (n = 1) |
| | *Synthetic pyrethroids* | | | | | | |
| 32. | λ-Cyhalothrin-I | BDL | BDL | BDL | BDL | BDL | BDL |
| 33. | λ-Cyhalothrin-II | BDL | BDL | BDL | BDL | BDL | BDL |
| 34. | Permethrin-I | BDL | BDL | BDL | BDL | BDL | BDL |
| 35. | Permethrin-II | BDL | BDL | BDL | BDL | BDL | BDL |
| 36. | Fenvalerate-I | BDL | BDL | BDL | BDL | BDL | BDL |
| 37. | Fenvalerate-II | BDL | BDL | BDL | BDL | 26.91 (BDL-71.38) | 42.15 |
| | ∑-SPs | BDL | BDL | BDL | BDL | 26.91 (BDL-71.38) | 42.15 |
| | *Carbamate* | | | | | | |
| 38. | Carbofuran | BDL | 2.09 (BDL-6.21) | BDL | BDL | BDL | BDL |

Number of samples collected: Indian Rice Frog *Fejervarya limnocharis* (n = 78) and Jerdon's Bullfrog *Hoplobatrachus crassus* (n = 31)
Number of individual deformed frogs analysed: *Fejervarya limnocharis*–6 and Jerdon's Bullfrog *Hoplobatrachus crassus*—1
*p*" pooled samples, an average of 4 individuals, *n* No. of individuals, *BDL* Below detection limit

Dieldrin, Endrin, Chlordane and Mirex were below detectable levels (BDL) in healthy frogs collected from both farms, trace levels of γ-HCH (2.12 ng/g) was recorded only in *F. limnocharis* collected from organic farm (Table 2). This could be because of its historical use. Residues of the cyclodiene insecticide, Heptachlor in *F. limnocharis* (2.98 ng/g) and Heptachlor epoxide in *H. crassus* (1.44 ng/g) were detected at trace concentrations. It has to be noted that these two frog species with deformities were collected from conventional farm and residues might indicate their use in the past in this region. Use of Heptachlor was banned in India in 1996. But its metabolite Heptachlor epoxide has the potential to enter the food chain (USEPA 1986) and cause adverse effects. Mayer and Ellersieck (1986) reported 435 ng/g of Heptachlor to cause 50 % of tadpole mortality in *Bufo woodhousei fowleri*. Levels of Heptachlor residues recorded in the present study in both frog species are well below the sublethal toxic limits ($LC_{50}$ = 440 ng/g). Among the OCs, residues of only HCH (29.72 ng/g) and Heptachlor (2.98 and 1.44 ng/g) were recorded in deformed *F. limnocharis* and *H. crassus* respectively. In healthy frogs residues of all the OC pesticides were BDL except γ-HCH (2.12 ng/g).

Among the OP pesticides, concentrations of Phorate and Quinalphos were 1.02 and 2.62 ng/g, in deformed *F. limnocharis* respectively. While Parathion ethyl (1.02 ng/g) was detected in deformed *H. crassus*, other OPs were BDL in healthy frogs from both organic and conventional farms (Table 2). Normally, the OP insecticides undergo hydrolysis in surface waters with highly variable reaction rates depending on the water quality parameters. However, OPs are more polar than the OCs and their chemical properties do not favour bioaccumulation (Fellers et al. 2004). Although information on the lethal dose of Phorate (85.2 mg/L), Quinalphos (3.1 mg/L) and Parathion (8.75 mg/L) is available for *Rana* sp. (Hudson et al. 1984; Alam and Shafi 1991; Luo et al. 2003) no detail is available for other species of frogs under field conditions. Both Phorate and Quinalphos are among the most commonly used pesticides in India (CIBRC 2013). Although negligible amounts of OPs were observed in the current study, they do not stay in system for long time (half-life: 1–7 days), but they are capable of producing delayed metamorphosis and/or disrupting nervous systems (Bridges 1999). Sublethal effects of OPs in well-known amphibian model (*Xenopus laevis*) with include motility impairment in embryonic stage, muscular disorders such as abnormal tail flexure and notochord, and contorted posture in tadpoles (Bridges 1999; Bonfanti et al. 2004). The long-term effects of many OPs need to be evaluated as these chemicals create great risks to the wildlife and environment. Since agriculture has a place of prime importance in India's economy, large scale and unscrupulous use of chemical pesticides in this sector is likely to continue, which might

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**Abstract** Pesticides residues were quantified in 109 frogs comprising two species (*Fejervarya limnocharis* and *Hoplobatrachus crassus*) from organic and conventional paddy farms in Kerala, India. Seven frogs from conventional but none from the organic farms revealed deformities. Levels of total Organochlorines (OCs) (33.22 ng/g) and Synthetic Pyrethroid, Fenvalerate-II (26.91/42.15 ng/g) in deformed *F. limnocharis* and *H. crassus* were significantly greater than in healthy frogs. Among OCs in healthy frogs, traces of γ (gamma)-HCH (hexachlorocyclohexane) (2.12 ng/g) were found only in *F. limnocharis* from organic farm. Among Organophosphates, Phorate (1.02 ng/g) and Quinalphos (2.62 ng/g) were present in traces in deformed *F. limnocharis*, while Parathion ethyl (1.02 ng/g) was detected in deformed *H. crassus*. The data indicate that the high level of pesticides may have contributed to the deformity of frogs. Therefore, an elaborative study will be essential to conserve amphibians in India.

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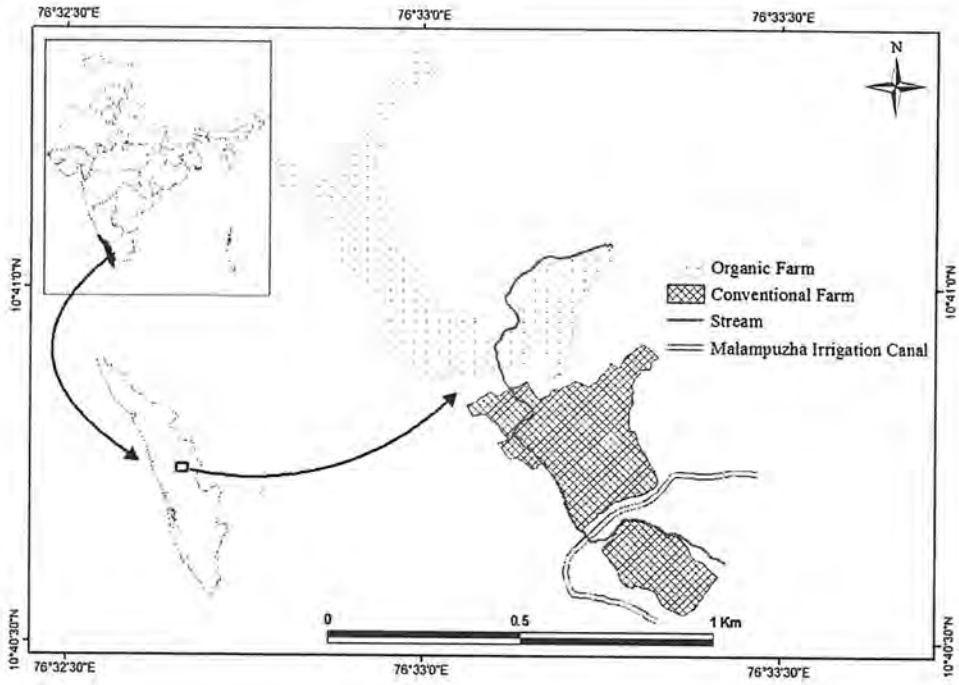



**Fig. 1** Digital map of the study sites at Padayetti village, Kerala, India

**Table 1** List of currently used pesticides on conventional paddy cultivation in Padayetti village and their relevant details

| Common name | Trade name | Formulation | Dose/ha |
|---|---|---|---|
| Phorate | Thimate | 10 % G | 750–1,250 g |
| Methyl parathion | Folidol powder | 2 % DP | 250–750 g |
| Triazophos | Hostathion | 40 % EC | 1,000–1,250 mL |
| Quinalphos | Ekalux | 25 % EC | 325–375 g |
| Lambda (λ) Cyhalothrin | Karate | 5 % EC | 400 mL |
| Fenvalerate | Fenvol | 20 % EC | 500–1,500 mL |
| Carbofuran | Furadan 3G | 3 % G | 750 g |

*EC* Emulsifying Concentration, *G* Granule, *DP* Dustable Powder

## Materials and Methods

The study was conducted in Padayetti village, Palakkad district of Kerala which is known as "Granary of Kerala". The area is around 100 acres of paddy field adopting both organic and conventional farming (50 acres each) situated in a valley sandwiched between hills. The organic farming area lies within the longitude of 10° 41′106″ N and latitude of 76° 32′ 900″ E and conventional farming lies within the longitude of 10° 40′ 615″ N and latitude of 76° 32′ 289″ E (Fig. 1). The organic farming area is elevated slightly relative to the conventional farming area, but contiguous (Fig. 1). Human settlements are 300–500 m away from the farms on a higher elevation. Questionnaire survey was conducted among the farmers to know the farming practice and chemicals used. Forty-nine out of 69 households in Padayetti village are engaged in paddy cultivation. Pesticides used in the farms at present and in the past, and their relevant details are furnished in the Table 1. A total of 109 frogs comprising two species, namely Indian Rice Frog *Fejervarya limnocharis* (n = 78) and Jerdon's Bull Frog *Hoplobatrachus crassus* (n = 31) were collected seasonally (*Kharif*, *Rabi* and summer) between May 2009 and April 2012. The present study reports the levels of pesticide residues {20 Organochlorines (OCs), 11 Organophosphates (OPs), six Synthetic Pyrethroids (SPs) and one Carbamate (Cb)} in 23 pooled samples of healthy (102 individuals: 54 from organic and 50 from conventional farms) and seven individual deformed frogs collected from conventional farms.

Frog samples were collected by nocturnal visual encounter survey (VES) which yields greater number of individuals and species per unit area (Crump and Scott 1994). Observations were made fortnightly between 1800 and 2030 h. Belt transects (50 × 4 m) for VES were placed randomly and four observers walked inside the flooded paddy fields with light-emitting diode (LED) torch (Britelite) and each visually searched the entire 4 m width of the transect. During each survey, 150 min were spent in an area of 2.5 ha. Identity of the frogs was confirmed referring to Daniels (2005). Samples were cleaned, morphometric measurements recorded, labelled and packed in clean polythene bags, transported on thermal insulated box with ice to the laboratory and stored at −20°C until processing. Multi-residue extraction method was followed for extracting pesticide residues (Anastassiades et al. 2003). Extracts

were evaporated to near dryness and reconstituted with 1 mL of HPLC grade n-Hexane and final analyses were carried out with Gas Chromatography-Mass Spectrometry (GC–MS) at the analytical facility of Agilent Technologies India Pvt. Ltd, Bangalore. Qualitative and quantitative analyses of OCs, OPs, SPs and Cb were made with GC (Agilent Model 7890A Series) equipped with MS (5,975 Quadruple) using HP-5 ms fused silica capillary column (15 m × 0.25 mm I.D × 0.25 μm film thickness) coated with 5 % phenyl and 95 % dimethyl polysiloxane. While Helium (IOLAR) was the carrier gas (flow rate: 1.0 mL/min), chromatographic operating conditions were as follows: detector temperature was set at 325°C; the injector temperature was programmed as 85°C for 12 s, increased at 600°C/min to 325°C and held for 5 min, then again increased at 10°C/min to 200°C (0 min); similarly oven temperature programmed as 70°C (1 min), increased at 25°C/min to 150°C (0 min), again increased at 3°C/min to 200°C (0 min) and then 8°C/min to 280°C (10 min). MS source temperature: 230°C; MS quadruple: 150°C; Technique: Electron ionization (EI); total run time: 40.867 min. The calibration standards with a minimum of five calibration points (1.56, 3.26; 6.25, 12.5, 25, 50 and 100 ng/g) spanning the expected concentrations in the samples were used for each analyte. All the samples were analyzed for a set of OCs, OPs, SPs and Cb (Table 2). An equivalent mixture manufactured by Sigma-Aldrich was used as standard. Concentrations of individual compounds were quantified from the peak area of the sample to that of the corresponding external standard. Recoveries of the compounds from fortified samples (50 μg/kg) ranged from 91 to 102 %. Results were not corrected for per cent recovery and expressed in wet weight basis. The limits of detection (LOD) for the method were in the range of 0.25–0.75; 0.29–0.62; 0.09–0.40 and 0.25–0.80 ng/g for the OC, OP, SP and Cb respectively. Quantification limits were set at three times the instrumental detection limit for each chemical, and they ranged from 0.25 to 0.80 ng/g. Precision for the duplicate samples were between 98 and 99.5 %. Results are presented as mean values and range (min–max). Statistical tests of pesticide levels between healthy and deformed frogs as well as organic and conventional farms were carried out using analysis of variance (ANOVA) (SPSS Ver. 16) with a significance level of 0.05. Mean values were calculated by treating the concentration of the non-detected analytes as zero. Values below the detection limits are reported as below detection limit (BDL).

## Results and Discussion

Levels of currently used pesticides (i.e. OPs and SPs) varied significantly between organic and conventional farm frogs ($p < 0.05$) but the levels of historical chemicals (i.e. OCs) did not vary between farms ($p > 0.05$) (Table 2). It was observed that out of 109 frogs collected, seven had deformities (6.42 %). Interestingly, all the deformed frogs were collected from the conventional farm. Species with deformity were Indian Rice Frog (n = 6) and Jerdon's Bullfrog (n = 1; Fig. 2). It is to be noted that we did not examine the abundance, species distribution or species richness of frogs. However, visually encountered frogs were collected and examined for any morphological disfigurement following Meteyer (2000). The mean occurrence of deformity observed in this study (6.42 %) is slightly higher than the background levels of deformities (5 %) reported in wild frog populations in California, USA (Johnson et al. 2001) and agroecosystems (3.98 %) in central Western Ghats (Gurushankara et al. 2007).

Concentrations of β (beta)–(9.36 ng/g), α (alpha)–(8.84 ng/g), δ (delta)–(8.38 ng/g) and γ (gamma)–HCH (3.14 ng/g) were found to be high in the deformed *F. limnocharis* collected from the conventional farms, but HCH isomers were BDL in the healthy frogs collected from the same field. Technical HCH is a broad spectrum OC insecticide used throughout the world for agricultural and non-agricultural purposes due to its effectiveness in pest control and low cost (Raina et al. 2008). The isomeric forms of HCH are toxic and recalcitrant, particularly the β isomer acts as an environmental estrogen. Mean concentrations of the γ-HCH (3.14 ng/g) and α-HCH (8.38 ng/g) recorded in one of the deformed *F. limnocharis* is higher (γ-HCH: 0.29 and α-HCH: 0.31 ng/kg) than the levels reported by Fellers et al. (2004) in healthy yellow-legged frog *Rana muscosa* from Sierra Neveda Mountains of California, USA. Use of HCH continued unabated until it was banned in 1997 in India, but restricted use of lindane was allowed till 2011. Although the levels of other chemicals, namely DDT, Heptachlor, Endosulfan, Aldrin,



**Fig. 2** *Hoplobatrachus crassus* with missing lower limb

**Table 2** Concentration of ∑-OC, OP, SP and Cb (ng/g, wet wt.) in frogs collected from organic and conventional farms in Padayetti village, Kerala

| S·No | Chemical compound | Normal frogs | | | | Deformed frogs | |
|---|---|---|---|---|---|---|---|
| | | Organic farm | Conventional farm | Organic farm | Conventional farm | Conventional farm | |
| | | *Fejervarya limnocharis* (*$p$ = 6) | *Fejervarya limnocharis* (*$p$ = 6) | *Hoplobatrachus crassus* (*$p$ = 4) | *Hoplobatrachus crassus* (*$p$ = 4) | *Fejervarya limnocharis* (n = 6) | *Hoplobatrachus crassus* (n = 1) |
| | *Organochlorines* | | | | | | |
| 1. | α–HCH | BDL | BDL | BDL | BDL | 8.84 (BDL -25.04) | BDL |
| 2. | β–HCH | BDL | BDL | BDL | BDL | 9.36 (BDL-25.97) | BDL |
| 3. | γ–HCH | 2.12 (BDL-5.60) | BDL | BDL | BDL | 3.14 (BDL-8.42) | BDL |
| 4. | δ–HCH | BDL | BDL | BDL | BDL | 8.38 (BDL-21.71) | BDL |
| | ∑-HCH | 2.63 (BDL-5.60) | BDL | BDL | BDL | 29.72 (BDL-81.14) | BDL |
| 5. | o, p–DDT | BDL | BDL | BDL | BDL | BDL | BDL |
| 6. | p,p'–DDT | BDL | BDL | BDL | BDL | BDL | BDL |
| 7. | o,p'–DDE | BDL | BDL | BDL | BDL | BDL | BDL |
| 8 | p,p'–DDE | BDL | BDL | BDL | BDL | BDL | BDL |
| 9. | o,p'–DDD | BDL | BDL | BDL | BDL | BDL | BDL |
| 10. | p,p'–DDD | BDL | BDL | BDL | BDL | BDL | BDL |
| | ∑- DDT | BDL | BDL | BDL | BDL | BDL | BDL |
| 11. | α -Endosulfan | BDL | BDL | BDL | BDL | BDL | BDL |
| 12. | β-Endosulfan | BDL | BDL | BDL | BDL | BDL | BDL |
| 13. | Endosulfan sulphate | BDL | BDL | BDL | BDL | BDL | BDL |
| | ∑-Endosulfan | BDL | BDL | BDL | BDL | BDL | BDL |
| 14. | Heptachlor | BDL | BDL | BDL | BDL | 2.98 (BDL-16.69) | BDL |
| 15. | Heptachlor epoxide | BDL | BDL | BDL | BDL | BDL | 1.44 |
| | ∑-Heptachlor | BDL | BDL | BDL | BDL | 3.50 (BDL-16.69) | 1.44 |
| 16. | Aldrin | BDL | BDL | BDL | BDL | BDL | BDL |
| 17. | Dieldrin | BDL | BDL | BDL | BDL | BDL | BDL |
| 18. | Endrin | BDL | BDL | BDL | BDL | BDL | BDL |
| 19. | Chlordane | BDL | BDL | BDL | BDL | BDL | BDL |
| 20. | Mirex | BDL | BDL | BDL | BDL | BDL | BDL |
| | ∑-OCs | BDL | BDL | BDL | BDL | 33.22 (BDL-97.83) | 1.44 |
| | *Organophosphates* | | | | | | |
| 21. | Phorate | BDL | BDL | BDL | BDL | 1.02 (BDL-3.35) | BDL |
| 22. | Methyl parathion | BDL | BDL | BDL | BDL | BDL | BDL |
| 23. | Chlorpyrifos methyl | BDL | BDL | BDL | BDL | BDL | BDL |
| 24. | Malathion | BDL | BDL | BDL | BDL | BDL | BDL |
| 25. | Chlorpyrifos | BDL | BDL | BDL | BDL | BDL | BDL |
| 26. | Parathion ethyl | BDL | BDL | BDL | BDL | BDL | 1.02 |
| 27. | Pirimiphos ethyl | BDL | BDL | BDL | BDL | BDL | BDL |
| 28. | Quinalphos | BDL | BDL | BDL | BDL | 2.62 (BDL-7.19) | BDL |
| 29. | Phenthoate | BDL | BDL | BDL | BDL | BDL | BDL |
| 30. | Ethion | BDL | BDL | BDL | BDL | BDL | BDL |
| 31. | Triazophos | BDL | BDL | BDL | BDL | BDL | BDL |
| | ∑-OPs | BDL | BDL | BDL | BDL | 4.97 (BDL-12.44) | 1.02 |

**Table 2** continued

| S·No | Chemical compound | Normal frogs | | | | Deformed frogs | |
|---|---|---|---|---|---|---|---|
| | | Organic farm | Conventional farm | Organic farm | Conventional farm | Conventional farm | |
| | | *Fejervarya limnocharis* (*$p$ = 6) | *Fejervarya limnocharis* (*$p$ = 6) | *Hoplobatrachus crassus* (*$p$ = 4) | *Hoplobatrachus crassus* (*$p$ = 4) | *Fejervarya limnocharis* (n = 6) | *Hoplobatrachus crassus* (n = 1) |
| | *Synthetic pyrethroids* | | | | | | |
| 32. | λ-Cyhalothrin-I | BDL | BDL | BDL | BDL | BDL | BDL |
| 33. | λ-Cyhalothrin-II | BDL | BDL | BDL | BDL | BDL | BDL |
| 34. | Permethrin-I | BDL | BDL | BDL | BDL | BDL | BDL |
| 35. | Permethrin-II | BDL | BDL | BDL | BDL | BDL | BDL |
| 36. | Fenvalerate-I | BDL | BDL | BDL | BDL | BDL | BDL |
| 37. | Fenvalerate-II | BDL | BDL | BDL | BDL | 26.91 (BDL-71.38) | 42.15 |
| | ∑-SPs | BDL | BDL | BDL | BDL | 26.91 (BDL-71.38) | 42.15 |
| | *Carbamate* | | | | | | |
| 38. | Carbofuran | BDL | 2.09 (BDL-6.21) | BDL | BDL | BDL | BDL |

Number of samples collected: Indian Rice Frog *Fejervarya limnocharis* (n = 78) and Jerdon's Bullfrog *Hoplobatrachus crassus* (n = 31)
Number of individual deformed frogs analysed: *Fejervarya limnocharis*–6 and Jerdon's Bullfrog *Hoplobatrachus crassus*—1
*p''* pooled samples, an average of 4 individuals, *n* No. of individuals, *BDL* Below detection limit

Dieldrin, Endrin, Chlordane and Mirex were below detectable levels (BDL) in healthy frogs collected from both farms, trace levels of γ-HCH (2.12 ng/g) was recorded only in *F. limnocharis* collected from organic farm (Table 2). This could be because of its historical use. Residues of the cyclodiene insecticide, Heptachlor in *F. limnocharis* (2.98 ng/g) and Heptachlor epoxide in *H. crassus* (1.44 ng/g) were detected at trace concentrations. It has to be noted that these two frog species with deformities were collected from conventional farm and residues might indicate their use in the past in this region. Use of Heptachlor was banned in India in 1996. But its metabolite Heptachlor epoxide has the potential to enter the food chain (USEPA 1986) and cause adverse effects. Mayer and Ellersieck (1986) reported 435 ng/g of Heptachlor to cause 50 % of tadpole mortality in *Bufo woodhousei fowleri*. Levels of Heptachlor residues recorded in the present study in both frog species are well below the sublethal toxic limits ($LC_{50}$ = 440 ng/g). Among the OCs, residues of only HCH (29.72 ng/g) and Heptachlor (2.98 and 1.44 ng/g) were recorded in deformed *F. limnocharis* and *H. crassus* respectively. In healthy frogs residues of all the OC pesticides were BDL except γ-HCH (2.12 ng/g).

Among the OP pesticides, concentrations of Phorate and Quinalphos were 1.02 and 2.62 ng/g, in deformed *F. limnocharis* respectively. While Parathion ethyl (1.02 ng/g) was detected in deformed *H. crassus*, other OPs were BDL in healthy frogs from both organic and conventional farms (Table 2). Normally, the OP insecticides undergo hydrolysis in surface waters with highly variable reaction rates depending on the water quality parameters. However, OPs are more polar than the OCs and their chemical properties do not favour bioaccumulation (Fellers et al. 2004). Although information on the lethal dose of Phorate (85.2 mg/L), Quinalphos (3.1 mg/L) and Parathion (8.75 mg/L) is available for *Rana* sp. (Hudson et al. 1984; Alam and Shafi 1991; Luo et al. 2003) no detail is available for other species of frogs under field conditions. Both Phorate and Quinalphos are among the most commonly used pesticides in India (CIBRC 2013). Although negligible amounts of OPs were observed in the current study, they do not stay in system for long time (half-life: 1–7 days), but they are capable of producing delayed metamorphosis and/or disrupting nervous systems (Bridges 1999). Sublethal effects of OPs in well-known amphibian model (*Xenopus laevis*) with include motility impairment in embryonic stage, muscular disorders such as abnormal tail flexure and notochord, and contorted posture in tadpoles (Bridges 1999; Bonfanti et al. 2004). The long-term effects of many OPs need to be evaluated as these chemicals create great risks to the wildlife and environment. Since agriculture has a place of prime importance in India's economy, large scale and unscrupulous use of chemical pesticides in this sector is likely to continue, which might

lead to adverse effects including but not limited to amphibians.

The levels of SP, Fenvalerate-II recorded in the present study in deformed *F. limnocharis* (26.91 ng/g) and *H. crassus* (42.15 ng/g) are higher than the levels reported in four species of frogs (*Rana utricularia*, *R. clamitans* and *Hyla cinerea*: <10 ng/g and *Bufo fowleri*: 20 ng/g) collected from a cotton field which had been aerially treated with Fenvalerate at 0.112 kg of active ingredient/ha (0.1 lb/acre) 5 days prior to sample collection in USA (Bennett et al. 1983). But such field based assessment studies on Fenvalerate are not available in India. Hence, we compared a few studies on sublethal effects available in the literature. As stated by Materna et al. (1995), formulated Fenvalerate available in the market could cause lethal effects in northern leopard frog *Rana pipiens* at 7.29 ng/g. The known effects of pyrethroids on frogs at sublethal concentrations include incoordination, lack of limb control, hyperactivity, tremors and writhing. Tadpoles of Leopard frog exhibited convulsions and twitching following sublethal (1.3 μg/L) Fenvalerate exposure, with behavioral homeostasis re-established only 1 week after removal to clean systems (Materna et al. 1995). Larsen and Sorensen (2004) reported that 150 μg/mL Fenvalerate killed 50 % of the tested *X. laevis* population in 96 h. Moreover, regulatory agencies have not yet formulated any norms for protection of sensitive wildlife resources against Fenvalerate. Nonetheless, the USEPA AQUIRE database classified Fenvalerate as one of the toxic pesticides.

Residues of Carbofuran (2.09 ng/g) were detected in one of the healthy *F. limnocharis* collected from the conventional farm. This reflects the use of Carbofuran in the study area. Although we have no data on the total application of Carbofuran at the studied sites, farmers of the area spray up to 750 g/ha. It may be worth mentioning that around 500 metric tons of Carbofuran is being used in India for various crops (CIBRC 2013). According to Jayatillake et al. (2011), application of Carbofuran between 50 and 500 g/ha affects growth, development and swimming activity in tadpoles of Asian common toad *Duttaphrynus melanostictus*. Hence, effects of Carbofuran needs continuous monitoring.

Some currently used pesticides (OPs, Cbs and SPs) are acutely toxic and associated with population declines in amphibians (Broomhall 2004). These classes of pesticides have relatively short half-lives in tissues, but nonetheless can still evoke toxicity. In the present study five out of seven deformed frogs revealed hind limb deformity i.e. missing digits or lower limb while short toe was missing in the other two. Numerous laboratory studies have shown that many different contaminants can kill or cause deformities in frogs (Palmquist et al. 2008a, b). Mass mortality of eggs, larvae and adults has been reported in some areas, and deformed amphibians only rarely survive to adulthood (Johnson et al. 2001). According to Materna et al. (1995), commercially available Fenvalerate was found to be lethal to *Rana pipiens* at 7.29 ng/g. Hence, we suspect that high concentrations of Fenvalerate detected in the present study in deformed *F. limnocharis* (26.91 ng/g) and *H. crassus* (42.15 ng/g) might have contributed to the deformity. However, not all the frogs collected in conventional farms showed external deformities. Moreover, since the farm belongs to several small farmers, pattern and quantities of Fenvalerate application might have varied. Therefore if pesticides play a role in amphibian decline, it could be due to sublethal effects and possible combined effects with other factors such as disease. Synergistic effects of pesticides also cannot be ruled out. The challenge is to unravel how the agents causing deformities interact with one another. A coordinated multidisciplinary approach will be necessary to find a solution for this problem. Regulatory agencies should consider imposing restrictions on the use of these pesticides, and farmers have to be provided with safe alternatives to handle pests without compromising on productivity. Moreover, while submission of toxicity data on birds and mammals are necessary for registering a new molecule, for amphibians no reference is made in the registration process not only in India but also in many other countries. Our results indicate that existing risk assessment procedures for pesticide formulation need to be strengthened to protect the amphibians.

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