

INTERACTION AND PERSISTENCE OF PESTICIDES IN TOMATO (*Lycopersicon esculentum* Mill.)

DEB PRASAD RAY



T- 7128

**DIVISION OF AGRICULTURAL CHEMICALS
INDIAN AGRICULTURAL RESEARCH INSTITUTE
T7128 NEW DELHI-110 012**



IARI

2003

INTERACTION AND PERSISTENCE OF PESTICIDES IN TOMATO (*Lycopersicon esculentum* Mill.)

By

DEB PRASAD RAY

A thesis submitted to the faculty of Post-Graduate School,
Indian Agricultural Research Institute, New Delhi
in partial fulfilment of the requirements
for the award of the degree

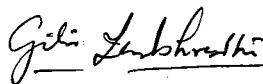
of

DOCTOR OF PHILOSOPHY
IN
AGRICULTURAL CHEMICALS

2003

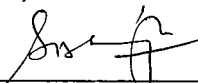
Approved by :

Advisory Committee : Chairperson



(Dr. (Mrs.) Gita Kulshrestha)

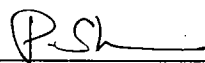
Members:



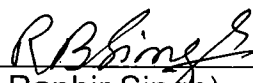
(Dr. (Mrs.) Shashi Bala Singh)



(Dr. S. C. Datta)



(Dr. (Mrs.) P. Sharma)



(Dr. Ranbir Singh)



for (Dr. S. Chander)

T- 7128

Dr. (Mrs.) Gita Kulshrestha
Professor

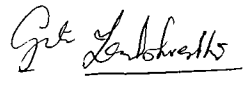
Division of Agricultural Chemicals
Indian Agricultural Research Institute
New Delhi-110 012, India

CERTIFICATE

This is to certify that the thesis entitled "**Interaction and persistence of pesticides in tomato (*Lycopersicon esculentum* Mill.)**" submitted in partial fulfilment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY IN AGRICULTURAL CHEMICALS**, to the Faculty of the Post Graduate School, Indian Agricultural Research Institute, New Delhi, embodies the results of ***bona fide*** research work carried out by **Mr. Deb Prasad Ray**, in the laboratories of the Division of Agricultural Chemicals, IARI, New Delhi under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received by him during the course of investigation and the source of literature drawn are duly acknowledged.

Place : New Delhi
Date : 7th March, 2003


(Gita Kulshrestha)
Chairperson
Advisory Committee

Dedicated to
my
beloved parents
Sri Kiran Chandra Ray
Smt. Sushama Ray
and
my wife
Prajna

ACKNOWLEDGEMENT

It is my pride privilege to express my sincere and heartfelt gratitude to Dr. (Mrs.) Gita Kulshrestha, Professor, Division of Agricultural Chemicals, IARI, New Delhi, Chairperson of my advisory committee for her judicious and precious advice, constructive criticism, constant encouragement, concrete suggestion and painstaking supervision throughout the course of investigation and preparation of the manuscript.

I convey my profound gratitude to my Co-Chairperson Dr. (Mrs.) Shashi Bala Singh, Senior Scientist, Division of Agricultural Chemicals, IARI, New Delhi for her inspiring guidance , generous advice cooperating attitude and pleasant discussion throughout the investigation.

I also express my sincere thanks to Dr. S.C. Datta, Principal Scientist, Division of Soil Science and Agricultural Chemistry, Dr. (Mrs.) P. Sharma, Principal Scientist, Division of Plant Pathology, Dr. Ranbir Singh, Senior Scientist, Division of Agronomy and Dr. S. Chander, Senior Scientist, Division of Entomology, members of my advisory committee for their valuable advise and help endowed during the course of my research work.

I feel immense pleasure to convey my heartfelt thanks to Dr. B.S. Parmar, Head, Division of Agricultural Chemicals for providing me necessary facilities required during the entire spans of the study.

I avail this opportunity to thank Dr. S.K. Handa, Dr. N.K. Roy, Dr. N.P. Agnihotri, Dr. R.P. Singh, Dr. P. Dureja, Dr. M. Gopal, Dr. A.K. Dikshit, Dr., V.T. Gajbhiye, Dr. (Mrs.) Irani Mukherjee and Dr. (Mrs.) Suman Gupta, Dr. Rajesh of the Division of Agricultural Chemicals for their kind help and suggestion during the course and research work.

I deem it to be humble privilege to express my sincere thanks to Dr. B.K. Goswami and Dr. D. Prasad of the Division of Nematology and Dr. T. K. Das, Division of Agronomy for their kind gesture and candid help at the time of need.

I am grateful to Prof. N.A. Choudhury and Dr. Ashim Chowdhury of Bidhan Chandra Krishi Viswavidyalaya, whose blessing, affection and encouragement has always been a treasure for me.

I wish to thank Pawanji, Tulsi ma'm and Mohinderji for their timely help and encouragement.

My heartfelt thanks are due to Birenderji, Yajuluji, Maheshji and other technical staff members of the Division of Agricultural Chemicals and Mangu Singhji of Division of Agronomy for their valuable technical assistance.

I do not find words to convey my gratitude to Shashi, Nirmali, Davinder, Ramesh, Atul, Praveen, Suresh, Amit, Vandana, Shailja and Shalini for their constant inspiration and mental support during the entire course of study.

My heartiest thanks to Supradip and Debashis for their kind help at the time of real need.

I express my heartfelt thanks to all my seniors, friends and juniors in IARI. In particular I wish to thank Dr. S. Srivastava, Kajal, Kali, Tashi, Tarun, Anirban, Anup, Rajib, Sugata, Bijul, Aarti, Priya, Anshu, Bikram, Chandra, Jaikishan, Asit, Nadu, Arup, Arnab, Pratap, Mrinal, Manish, Reza and Tuna for their encouragement and ready help throughout the research work.

I express my heartfelt thanks to Sushovan, Pradipda, Bani, Kashinath Dibyendu, Amitava, Nirmal, Naresh Sir, Sivakumar and Sumathy for their encouragement and constant inspiration.

Steady patience, constant encouragement, encompassing love and affection bestowed on me by Thamma, Maa, Baba, Dadamoni, Bara Boudi, Mejda, Mejo Boudi, Didi, Shibuda, Neeli, Dolly, Shiuli, Tulip, Bihan and Priya and all other relatives bring me to this pursuit of studies. Their eternal fountain of blessings never ending sacrifice and constant backup kept me enthusiastic throughout my educational career.

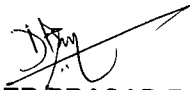
The warm blessings, constant support and inspiration of my father-in-law Professor Hrishikesh Halder and mother-in-law Smt. Sima Devi worth a lot for me without which I would have not been able to climb this point. I also wish to express my heartiest thanks and admiration to Rinku for her inspiration and encouragement.

The endless love, sacrifice and perpetual source of encouragement from my wife Prajna, who has been my morale booster and has enabled me to reach the footsteps of my long cherished aspiration. Thank you Sonai, for all that you are to me.

I am thankful to Mr. Susheel and Suresh Prasad, Computer Centre (BIC), Central Library, IARI for statistical analysis and typing my thesis promptly and neatly.

Finally, the financial assistance provided by the authorities of P.G. School, IARI and ICAR in the form of Senior Research Fellowship during the tenure is gratefully acknowledge.

New Delhi


(DEB PRASAD RAY)

Dated : 7th March 2003

CONTENTS

Sl. No.	Title	Page No.
1.	INTRODUCTION	1-4
2.	REVIEW OF LITERATURE	5-56
3.	MATERIALS AND METHODS	57-92
4.	RESULTS AND DISCUSSION	93-216
5.	SUMMARY AND CONCLUSIONS	217-227
	BIBLIOGRAPHY	i-xxi
	APPENDIX	

LIST OF TABLES

Table No.	Titles	Page No.
1.	Multiresidue technique adapted to estimate pesticide in tomato	57
2.	Pesticide used in the experimental and their supplier	58
3.	Commercial product, type and rate of application of various experimental pesticides	60
4.	Different treatments used for the root dip/foliar spray in the tomato field	61
5.	Physico-chemical properties of IARI farm soil	62
6.	Composition of different media used for microbial population count	68
7.	Absorbent used for the clean-up of pesticides	73
8.	Solvents used for the preparation of standard solutions of different pesticides	78
9.	Level of pesticide fortification of substrates for recovery study	80
10.	Extraction and clean up of different pesticides	81
11.	GLC condition used for different pesticides	83
12.	Column temperature suitable for different pesticides and their respective retention time (Rt) values	93
13a.	Calibration of pendimethalin by GLC technique	93
13b.	Calibration of endosulfan by GLC technique	94
13c.	Calibration of chlorothalonil by GLC technique	94
13d.	Calibration of triazophos by GLC technique	94
13e.	Calibration of β -cyfluthrin by GLC technique	95
14.	Calibration of imidacloprid by HPLC techniques	96
15.	Sensitivity of the GLC/HPLC method for different pesticides	96
16.	GLC temperature programming suitable for multiresidue analysis of pesticides	98
17.	Solvent used for extraction of pesticides and their mixture from soil and tomato	100
18a.	Recoveries of pendimethalin from various substrates	101
18b.	Recoveries of chlorothalonil from various substrates	102
18c.	Recoveries of endosulfan from various substrates	102
18d.	Recoveries of triazophos from various substrates	103
18e.	Recoveries of β -cyfluthrin from various substrates	103
18f.	Recoveries of imidacloprid from various substrates	103

LIST OF TABLES

Table No.	Titles	Page No.
19a.	Recoveries of pesticide mixture from tomato fruits	106
19b.	Recoveries of pesticide mixture from soil	107
19c.	Recoveries of pesticide mixture from culture media	108
20.	Recoveries of pesticide mixture (ET β) from tomato fruits	109
21a.	Residues and per cent dissipation of imidacloprid in tomato fruits.	110
21b.	Residues and per cent dissipation of endosulfan in tomato fruits.	113
21c.	Residues and per cent dissipation of chlorothalonil in tomato fruits.	115
22.	Residues and per cent dissipation of pesticides in/on tomato fruit when applied in sequence of imidacloprid-chlorothalonil-endosulfan	117
23.	Residues and per cent dissipation of pesticides in tomato fruits when applied as mixture	119
24.	Regression equations, rate constant, correlation co-efficients and half-lives of pesticides in sequential and mixture application on tomato	121
25.	Residues and per cent dissipation of triazophos in tomato fruits	121
26.	Residues and per cent dissipation of β -cyfluthrin in tomato fruits	122
27.	Persistence of pesticides (in module-II) when applied as mixture	124
28.	Regression equation, rate constant, correlation coefficient and half-lives of pesticides in mixture application in tomato in module II	125
29a.	Residues and per cent dissipation of pendimethalin in the tomato field soil	126
29b.	Residues and per cent dissipation of imidacloprid in the tomato field soil	128
29c.	Residues and per cent dissipation of chlorothalonil in the tomato field soil	130
29d.	Residues and per cent dissipation of endosulfan in the tomato field soil	131
30.	Residues and per cent dissipation of pesticides in tomato field soil when applied in sequence of pendimethalin-imidacloprid-chlorothalonil-endosulfan	133
31.	Residues and per cent dissipation of pesticides in tomato field soil when applied as mixture	135
32.	Regression equation, rate constant, correlation coefficient and half-lives of pesticides applied to soil as sequential and as a mixture	137

LIST OF TABLES

Table No.	Titles	Page No.
33a.	Residues and per cent dissipation of pendimethalin in soil under laboratory condition	138
33b.	Residues and per cent dissipation of imidacloprid in soil under laboratory condition	139
33c.	Residues and per cent dissipation of endosulfan in soil under laboratory condition	140
33d.	Residues and per cent dissipation of chlorothalonil in soil under laboratory condition	141
34a.	Dissipation of pendimethalin under field and laboratory conditions	142
34b.	Dissipation of imidacloprid under field and laboratory conditions	142
34c.	Dissipation of endosulfan under field and laboratory conditions	143
34d.	Dissipation of chlorothalonil under field and laboratory conditions	143
34e.	Half-lives of different pesticides in soil under field and laboratory conditions	144
35a.	Initial concentration, equilibrium concentration and pendimethalin adsorbed on IARI alluvial soil	145
35b.	Initial concentration, equilibrium concentration and imidacloprid adsorbed on IARI alluvial soil	146
35c.	Initial concentration, equilibrium concentration and chlorothalonil adsorbed on IARI alluvial soil	147
35d.	Initial concentration, equilibrium concentration and pesticide mixture adsorbed on IARI alluvial soil	149
35e.	Freundlich adsorption isotherm constants for individual and mixture components of pesticides in soil	150
36a.	Residues and per cent dissipation of pendimethalin in sterile alluvial soil.	152
36b.	Residues and per cent dissipation of pendimethalin in non-sterile alluvial soil.	153
36c.	Residues and per cent dissipation of imidacloprid in sterile alluvial soil.	155
36d.	Residues and per cent dissipation of imidacloprid in non-sterile alluvial soil.	155
36e.	Residues and per cent dissipation of endosulfan in sterile alluvial soil.	156
36f.	Residues and per cent dissipation of endosulfan in non-sterile alluvial soil.	156
36g.	Residues and per cent dissipation of chlorothalonil in sterile alluvial soil.	157
36h.	Residues and per cent dissipation of chlorothalonil in non-sterile alluvial soil.	158

LIST OF TABLES

Table No.	Titles	Page No.
36i.	Regression equation, slope, rate constant, correlation-coefficient and half-life of pesticide under sterile and non-sterile soil	158
36j.	Residues and per cent dissipation of pesticide mixture (PICE) in sterilized soil	160
36k.	Residues and per cent dissipation of pesticide mixture in non-sterile alluvial soil.	161
36l.	Regression equation, slope, rate constant, correlation-coefficient and half-lives of pesticide under sterile and non-sterile soil	162
36m.	Biological degradation of pendimethalin in soil	164
36n.	Biological degradation of imidacloprid in soil	164
36o.	Biological degradation of endosulfan in soil	165
36p.	Biological degradation of chlorothalonil in soil	165
36q.	Biological degradation of pesticide mixture in soil	166
37a.	Rate kinetics of pendimethalin in broth	168
37b.	Rate kinetics of imidacloprid in broth	169
37c.	Rate kinetics of endosulfan in broth	170
37d.	Rate kinetics of chlorothalonil in broth	170
37e.	Rate kinetics of pesticide mixture in broth	171
37f.	Regression equation, slope, rate constant, correlation-coefficient and half-lives of pesticide in broth	172
38.	Mass fragmentation pattern and retention times (Rt) of various metabolites of pendimethalin	173
39.	Mass fragmentation pattern and retention times (Rt) of various metabolites of imidacloprid	175
40.	Fungal species capable of degrading pesticide alone and in combination.	177
41a.	Effect of pendimethalin on fungal and nematode population in tomato field soil	180
41b.	Effect of imidacloprid residues on fungal and nematode population in tomato field soil	181
41c.	Effect of chlorothalonil residues on fungal and nematode population in tomato field soil	182
41d.	Effect of endosulfan residues on fungal and nematode population in tomato field soil	182
41e.	Effect of sequential application of pesticides on fungal and nematode population in tomato field soil	183
41f.	Effect of pesticide mixture application on fungal and nematode population in tomato field soil	184

LIST OF TABLES

Table No.	Titles	Page No.
42a.	Effect of pesticide treatment (module I) on predominant nematode species in soil	185
42b.	Effect of pesticide treatments (module II) on predominant nematode species in soil	186
42c.	Effect of pesticide treatment (module I) on tomato against root knot nematode and soil mycoflora	188
43a.	Effect of pesticide treatments on different insect pests pest infecting tomato crop in the field	192
43b.	Effect of pesticide treatments on different diseases infecting tomato crop in the field	196
43c.	Effect of root-dip treatment on insect-pests infecting tomato crop in the field	199
43d.	Effect of root-dip treatment on disease infecting tomato crop in the field	199
43e.	Effect of treatment on weed population (No./m ²) at different growth stages	202
43f.	Effect of treatments on weed dry weight (g m ⁻²) at different growth stages	204
44.	Effect of treatments on yield of tomato.	207
45a.	Stability of pesticides mixtures in organic solvent on 10 th day of incubation at 25±1°C.	210
45b.	Concentration (µg mL ⁻¹) of pesticides in mixture of analytical solution on 10 th day of incubation at 25±1°C	210
45c.	Concentration (µg mL ⁻¹) recovered from formulation mixture of pesticides following incubation at 25±1°C	212

LIST OF FIGURES

Fig. No.	Titles	After page
1.	Meteorological observations of the period during field experiments	63a
2a.	GLC chromatogram of pendimethalin	93
2b.	GLC chromatogram of endosulfan	93
2.c	GLC chromatogram of chlorothalonil	93
2d.	GLC chromatogram of β -cyfluthrin	93
2e.	GLC chromatogram of triazophos	93
3a.	Calibration of pendimethalin by GLC	94
3b.	Calibration of endosulfan by GLC	94
3c.	Calibration of chlorothalonil by GLC	94
3d.	Calibration of triazophos by GLC	95
3e.	Calibration of β -cyfluthrin by GLC	95
4a.	Calibration of imidacloprid by HPLC	95
4b.	HPLC chromatogram of imidacloprid	95
5a.	GLC of chromatogram of the multiresidue in the module I	97
6a.	Persistence of imidacloprid in tomato fruits	111
6b.	Dissipation of imidacloprid in tomato fruits	111
6c.	Logarithmic plots of imidacloprid residues in tomato fruits	111
7a.	Persistence of alpha and beta endosulfan in tomato fruits	114
7b.	Dissipation of endosulfan in tomato fruits	114
7c.	Logarithmic plots of endosulfan residues in tomato fruits	114
8a.	Persistence of chlorothalonil in tomato fruits	115
8b.	Dissipation of chlorothalonil in tomato fruits	115
8c.	Logarithmic plots of chlorothalonil residues in tomato fruits	115
9.	Persistence pattern of pesticides in tomato fruits when applied sequentially	117
10a.	Persistence pattern of pesticides in tomato fruits when applied as mixture	117
10b.	Dissipation of pesticides when applied as mixture	117
10c.	Logarithmic plots of pesticides residues in tomato fruits when applied as mixture.	117
11a.	Persistence of triazophos in tomato	121
11b.	Per cent dissipation of triazophos in tomato fruits	121
11c	Logarithmic plots of triazophos residues in tomato fruits	121

LIST OF FIGURES

Fig. No.	Titles	After page
23a.	Freundlich adsorption isotherm of pendimethalin in soil	147
23b.	Logarithmic plot of adsorption of pendimethalin in soil.	147
24a.	Freundlich adsorption isotherm of imidacloprid in soil	147
24b.	Logarithmic plot of adsorption of imidacloprid in soil.	147
25a.	Freundlich adsorption isotherm of chlorothalonil in soil	147
25b.	Logarithmic plot of adsorption of chlorothalonil in soil.	147
26a.	Freundlich adsorption isotherm of pendimethalin in soil	149
26b.	Logarithmic plot of adsorption of pendimethalin in soil.	149
27a.	Freundlich adsorption isotherm of imidacloprid in mixture	149
28b.	Logarithmic plot of adsorption of imidacloprid in mixture	149
28a.	Freundlich adsorption isotherm of chlorothalonil in mixture	149
28b.	Logarithmic plot of adsorption of chlorothalonil in mixture	149
29a.	Persistence of pendimethalin in sterile and non-sterile soil	154
29b.	Dissipation of pendimethalin in sterile and non-sterile soil	154
29c.	Logarithmic plots of pendimethalin in sterile and non-sterile soil	154
30a.	Persistence of imidacloprid in sterile and non-sterile soil	155
30b.	Dissipation of imidacloprid in sterile and non-sterile soil	155
30c.	Logarithmic plots of imidacloprid in sterile and non-sterile soil	155
31a.	Persistence of endosulfan in sterile and non-sterile soil	156
31b.	Dissipation of endosulfan in sterile and non-sterile soil	156
31c.	Logarithmic plots of endosulfan in sterile and non-sterile soil	156
32a.	Persistence of chlorothalonil in sterile and non-sterile soil	157
32b.	Dissipation of chlorothalonil in sterile and non-sterile soil	157
32c.	Logarithmic plots of chlorothalonil in sterile and non-sterile soil	157
33a.	Persistence of pendimethalin in sterile and non-sterile soil when applied as mixture.	162
33b.	Logarithmic plots of pendimethalin in sterile and non-sterile soil when applied as mixture.	162
33c.	Persistence of imidacloprid in sterile and non-sterile soil when applied as mixture.	162
33d.	Logarithmic plots of imidacloprid in sterile and non-sterile soil when applied as mixture.	162
33e.	Persistence of endosulfan in sterile and non-sterile soil when applied as mixture.	162

LIST OF FIGURES

Fig. No.	Titles	After page
34a.	Microbial degradation of pendimethalin in broth	169
34b.	Logarithmic plots of pendimethalin residues in broth	169
34c.	Microbial degradation of imidacloprid in broth	169
34d.	Logarithmic plots of imidacloprid residues in broth	169
34e.	Microbial degradation of endosulfan in broth	170
34f.	Logarithmic plots of endosulfan residues in broth	170
34g.	Microbial degradation of chlorothalonil in broth	170
34h.	Logarithmic plots of chlorothalonil residues in broth	170
35a.	Microbial degradation of pendimethalin in broth as mixture component	171
35b.	Logarithmic plots of pendimethalin in broth as mixture component	171
35c.	Microbial degradation of imidacloprid in broth	171
35d.	Logarithmic plots of imidacloprid in liquid broth as mixture component	171
35e.	Microbial degradation of endosulfan in broth	171
35f.	Logarithmic plots of endosulfan residues in broth as mixture component	171
35g.	Microbial degradation of chlorothalonil in broth	171
35h.	Logarithmic plots of chlorothalonil in broth as mixture component	171
35i.	Postulated degradation products of pendimethalin in broth	174
35j.	Postulated degradation products of imidacloprid in broth	175
36a.	Effect of treatment on different insect-pests infecting tomato crop in the field 7 days after treatment.	193
36b.	Effect of treatment on different insect-pests infecting tomato crop in the field 10 days after treatment.	193
37a.	Effect of treatment on different diseases infecting tomato crop in the field 7 days after treatment.	197
37b.	Effect of treatment on different diseases infecting tomato crop in the field 10 days after treatment.	197
38a.	Effect of treatment on different weed population (No.m ⁻²) at different growth stages of tomato.	204
38b.	Effect of treatment on weed dry matter (g m ⁻²) at different growth stages of tomato	204
39.	Effect of different treatment on the yield of tomato crop.	204

LIST OF PLATES

Plate No.	Titles	After page
1.	Pesticide applied in tomato field	60
2.	Fruit borer larvae and the damaged tomato fruits	189
3.	White fly (<i>Bemisia tabaci</i>) nymphs feeding on tomato leaf	189
4.	Leaf curl infestation in tomato due to white fly infestation	190
5.	Early blight (<i>Alternaria solani</i>) and late blight infestation on tomato leaf	194
6.	Weed infestation in tomato field	199

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) one of the most popular vegetable belonging to Lycopersicace family is under extensive cultivation in all agroclimatic zones of India. This vegetable is rich source of vitamin C, amino acids and minerals. This is often consumed raw and/or as cooked and for the preparation of ketchup, sauce, chutney, soup, puree, paste etc. In India it is cultivated in 0.5 million ha with total production of around 8.4 million tons (Economic Survey, 2002).

However, the crop suffers devastation due to infestation by insect pests, diseases, nematodes and weeds. Among the insects infesting this vegetable viz., fruit borer (*Helicoverpa armigera*), white fly (*Bemisia tabaci*), aphids (*Aphis gossipy*), tobacco caterpillar (*Spodoptera litura*), jassids (*Amrasca biguttula biguttula*) and serpentine leaf miner (*Liriomyza trifolii*) and epilachna beetle (*Henosepilachna viginti octopunctata*) are the major pests of tomato from the extent of damage in the field and require effective management.

Many fungal, bacterial and viral diseases viz., early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), leaf curl, buck eye rot or fruit rot (*Phytophthora parasitica*), wilt (*Ralstonia solanacearum*), tomato spotted wilt virus and mosaic diseases affecting this crop require frequent application of fungicides.

The major weed flora observed in the tomato field comprise *Cynodon dactylon*, *Euphorbia hirta*, *Cyperus rotundus*, *Boerhavia diffusa*

T-7128

etc. which cause tremendous yield losses due to crop-weed competition, unless controlled effectively.

To prevent these huge crop losses, farmers inadvertently apply insecticides, fungicides, nematicides and herbicides. The pesticide applications are often made sequentially one after the other to combat the attack of insect pests or diseases, either with the some pesticide or some other pesticides. This not only enhances load of pesticides in the environment but also causes, several times, development of pest/disease resistance to that pesticide.

If applied simultaneously as mixture instead of sequential application, the combination will not only improve biological performance but will also reduce the cost of application. Use of pesticide mixture can combat a broad spectrum of pests. Different species of insects, pathogens of plant disease and weeds can be controlled in a single application.

The pesticide mixture may show synergism or additive effect of two or more pesticides. Use of pesticide mixture can also minimize the selection pressure, which may delay the dominance of resistant strains developed as a result of long-term use of some particular pesticides.

The basic prerequisite for high effectiveness of a pesticide mixture is the compatibility of its components. That is, all the component of the mixture must remain in harmony with one another. There should not be any disadvantageous interaction between them which could decrease the expected efficacy of the mixture and/or cause phytotoxicity.

The chemical or physical interactions as well as biological incompatibility can reduce the performance of a mixture. Chemical incompatibility often arises from hydrolytic degradation and interaction with pesticide components including solvents, surfactants, fillers, etc. The physical

properties such as melting and boiling points, volatility, solubility etc. also adversely affect the handling properties, application and performance of mixtures if the ingredients are not compatible which may be revealed by the function of agglomerates, crystals, phase separation of formulations and thickening of spray liquids affecting adversely accurate application.

The chemical and physical compatibility of the components of a mixture together with the high biological compatibility are required for high performance of the mixture. If one component of a mixture is able to block the biological activity of the other, the mixture can be less effective or even completely inactive. The phytotoxic incompatibility may cause toxicity to desirable plants and could be due to chemical or physical incompatibility.

The studies were, therefore, undertaken to investigate interaction and residual fate of pesticides when applied sequentially or simultaneously to suggest a mixture of pesticides which will be effective against desired pests and diseases and reduce the dose of application .

The objectives of the study were as follows :

A. To investigate interaction, residual and metabolic fates of pesticides applied as mixtures, either simultaneously or sequentially in tomato (*Lycopersicon esculentum* Mill.).

1. To study persistence, residual and metabolic fate of pesticides/mixture when applied sequentially or simultaneously in tomato crop.
2. To evaluate interaction of pesticides/mixtures with soil/microbes.
 - 2.1. Adsorption of pesticides/mixtures in soil
 - 2.2. Effect of soil microbes on pesticides.
 - (i) Degradation of pesticides
 - (ii) Identification of pesticide degrading microorganisms. /
 - 2.3. Effect of pesticides on microorganisms

3. To investigate biological interaction/compatibility of pesticides/mixtures.
 4. To examine chemical interaction/compatibility of pesticides/mixture.
- B. To standardize parameters for separation, identification, quantification of pesticides/transformation products in tomato fruits and soil.**

Standardization of the accuracy and precision of the analytical methods for pesticides/mixtures in tomato and soil.

REVIEW OF LITERATURE

The review of literature related to the investigation carried out is presented under the following heads :

2.1. Pesticides

2.1.1. *Pendimethalin*

2.1.2. *Imidacloprid*

2.1.3. *Endosulfan*

2.1.4. *Chlorothalonil*

2.1.5. *β -cyfluthrin*

2.1.6. *Triazophos*

2.2. Persistence/mixture of pesticides in crop and soil

2.3. Interaction of pesticides/mixture with soil and microbes

2.3.1. Adoption of pesticides/mixture

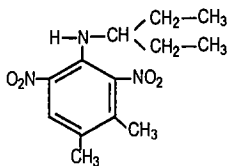
2.3.2. Degradation of pesticides

2.4. Chemical compatibility of pesticides and mixtures

2.5. Microquantitative analysis of the pesticides and their mixtures

2.1.1. Pendimethalin – A dinitroaniline herbicide

Pendimethalin is a dinitroaniline herbicide having following physical, chemical and biological properties.



Molecular formula	C ₁₃ H ₁₉ N ₃ O ₅
Molecular weight	281.3
Chemical name	N-(1-ethylpropyl)-2,6-dinitro-3,4-dinitro-3,4-xylidine

Trade name(s)	Tricel, Stomp
Manufacturer	Cynamid
Form	Orange yellow crystals
Melting point (m.p.)	54-58°
Vapour pressure (v.p.)	4 mPa (25°C)
Specific gravity	1.19 at 25°C
K _{ow} log p	5.18
Solubility	Water solubility, 0.3 mg L ⁻¹ at 20°C. Soluble in acetone 700, isopropanol 77, hexane 138, xylene 628 (all in gL ⁻¹ at 26°C)
Stability	Very stable in storage (5-13°C). Stable to acids and alkalis. Slowly decomposed by light
Acute oral toxicity	LD ₅₀ 1250 mg kg ⁻¹ (rats)
Formulation	Formulated as EC, GR
Compatibility	Compatible with many other herbicides and most liquid fertilizers
Recommended application	1.0 – 1.5 kg a.i. ha ⁻¹

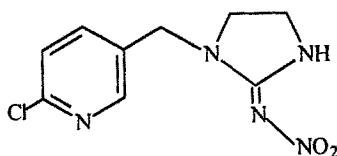
Biological activity

Pendimethalin is a pre-emergence or post emergence incorporated herbicide for the control of annual grasses and many broad-leaved weeds in onion, garlic, maize, rice, sorghum, soybean, groundnut, brassica, carrot, peas, beans, potato, cotton, capsicum, tomato, sunflower and tobacco. It control many weeds like *Echinocloa* sp. (in rice), *Fimbristylis* sp. (in wheat), *Ludwigia* sp., *Cyperus iria*, *Phalaris minor*, *Avena* sp., *Poa* sp., *Chenopodium* sp.

This is a selective herbicide, absorbed principally by the roots and leaves. It acts on inhibiting cell division and cell elongation. Affected plant die shortly after germination or following emergence.

2.1.2. Imidacloprid - a chloronicotinoid insecticide

Imidacloprid is a chloronicotinoid insecticide having following physical, chemical and biological properties.



Molecular formula	$C_9H_{10}ClN_5O_2$
Molecular weight	255.7
Chemical name	1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine
Trade name(s)	'Admire', 'Confidor', 'Gaucho'
Manufacturer	Bayer
Form	Colourless crystals with a characteristic odour
Melting point (m.p.)	143.8°C (crystal form 1); 136.4°C (crystal form 2)
Vapour pressure (v.p.)	0.2 μ Pa (20°C)
Specific gravity/density	1.543 (20°C)
K_{ow} log p	0.57 (22°C)
Solubility	In water 0.51 g L ⁻¹ (20°C) In dichloromethane 50-100, isopropanol 1-2, toluene 0.5-1, n-hexane < 0.1 (all in g L ⁻¹ , 20°C)
Stability	Stable to hydrolysis at pH 5-11
Acute oral toxicity	LD ₅₀ 450 mg kg ⁻¹ for rats, 150 mg kg ⁻¹ for mice
Toxicity class	WHO II; EPA II
Formulation	Slurry for seed treatment; FS; GR; WP; SL; SC; WG; DP
Recommended application	20g a.i. ha ⁻¹

Applications

i) Mode of action

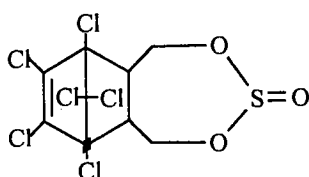
Systemic insecticide, with a very good root-systemic and contact and stomach action. Acts on the central nervous system, causing irreversible blockage of post synaptic nicotinic acetylcholine receptors.

ii) Uses

Control sucking insects, including ricehoppers, aphids, thrips and white flies. Also effective against soil insects, termites and some species of biting insects, such as rice water weevil and colorado beetle. Has no effects on nematodes or spider mites. Used as a seed dressing, as soil treatment and as foliar treatment in different crops, e.g., rice, cotton, cereals, maize, sugar beet, potatoes, vegetable, citrus fruit, pome fruit and stone fruit.

2.1.3 Endosulfan – the most popular organochlorine insecticide

Endosulfan is most popular amongst organochlorine insecticides because of its favourable toxicological parameters and low persistence (Agnihotri *et al.*, 1996). The physical, chemical and biological properties of the insecticide is enumerated below :



Molecular formula	C ₉ H ₆ Cl ₆ O ₃ S
Molecular weight	406.9
Chemical name	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo-dioxathiepine-3-oxide
Trade name(s)	Beosit, Agcel 35, Dawn 35, Endocel, Speed, Thiodan etc.

Manufacturer	Bharat Pulversing, Excel, Hindustan Insecticide Ltd.
Form	Colourless crystals
Melting point (m.p.)	$\geq 80^{\circ}\text{C}$
Vapour pressure (V_P)	0.83 m Pa (20°C)
Specific gravity/density	1.8 (20°C)
K_{ow} log p	4.74
Solubility	In water 0.32 mg L^{-1} (25°C) Soluble in ethanol 5, ethyl acetate 200, dichloromethane 200, hexane 24 (all in g L^{-1} at 20°C)
Stability	Stable to light, hydrolysed in aqueous acid and alkalies
Acute oral toxicity	LD_{50} 70 mg kg^{-1} for rats
Toxicity class	WHO II, EPA I
Formulation	EC; DP; WP; GR; UL; FT; SC; EO
Compatibility	Compatible with many other pesticides, but incompatible with alkaline materials
Recommended application	$312\text{-}656 \text{ g a.i. ha}^{-1}$ (for vegetables)

Applications

i) Mode of action

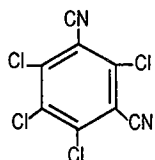
Non-systemic insecticide and acaricide with contact and stomach action

ii) Use

Control sucking, chewing and boring insects and mites on a very wide range of crops, including fruit vines, vegetables, ornamentals, potatoes, cucurbits, cotton, tea, coffee, sugarcane, tobacco, glass house crops etc.

2.1.4. Chlorothalonil

Chlorothalonil (tetrachloroisophthalonitrile) is a widely used non-systemic fungicide with protective action. The fungicide possesses the following physical, chemical and biological properties.



Molecular formula	C ₈ Cl ₄ N ₂
Molecular weight	265.9
Chemical name	Tetrachloroisophthalonitrile
Trade name(s)	Bravo, Daconil, Kavach, Bombardier, Chlortosop
Manufacturer	Sandoz
Form	Colourless crystals; technical grade has a slightly pungent odour
Melting point (m.p.)	250°
Boiling point (b.p.)	350°C / 760 mm Hg
Vapour pressure (v.p.)	0.076 m Pa (25°C)
Specific gravity/density	1.8
K _{ow} log p	2.89
Solubility	In water 0.9 mg L ⁻¹ (25°C) Soluble in most of the organic solvents. In acetone 20, xylene 80 (all in gL ⁻¹ at 25°C)
Stability	Thermally stable at ambient temperature. Stable to UV light in aqueous media and in crystalline state. Stable in acidic and moderately alkaline aqueous solutions; slow hydrolysis at pH 9
Acute oral toxicity	LD ₅₀ > 10,000 (for rats)
Toxicity class	WHO V, EPA IV
Formulation	SC; WG ; WP, Fogging concentrate
Compatibility	Compatible with most of the pesticides
Recommended application	0.66-0.937 kg ha ⁻¹ against early and late blight

Biological activity

i) Mode of action

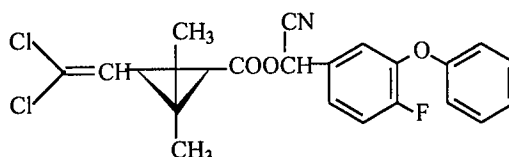
Non-systemic foliar fungicide with protective action

ii) Use

Control many fungal disease in a wide range of crops, including pome fruits, stone fruit, citrus fruit, bush and canefruit, cranberries, straw-berries, bananas, mangoes, coconut, palms, oil palms, rubber, piper, vines, hops, vegetables, cucurbits, tobacco, coffee, tea, rice, soybean, peanuts, sugar beet, cotton, maize, ornamental, mushrooms and turf.

2.1.5. β -cyfluthrin – A synthetic pyrethroid insecticide

β -cyfluthrin is a synthetic pyrethroid insecticide having following physical, chemical and biological properties.



Molecular formula	$C_{22}H_{18}Cl_2FNO_3$
Molecular weight	434.3
Chemical name	α -cyano-4-fluoro-3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate
Composition	Material is a reaction mixture of 2-enantiomeric pairs Pair II (s))- α -cyano-4-fluoro-3-phenoxy-benzyl (1R)-cis-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate and the corresponding (R) = (1s)-cis isomer, Pair IV (S) (1R)-trans isomer in the ratio 1:2
Trade name(s)	Bulldock, Responsar
Manufacturer	Bayer
Form	Whitish to yellowish powder with a weak characteristic odour

Melting point (m.p.)	81°C (enantiomer pair II), 106°C (enantiomer pair IV)
Vapour pressure (v.p.)	10 n Pa (enantiomer pair II at 20°C) 90 n Pa (enantiomer pair IV at 20°C)
K _{ow} log p	5.94 (e.p. II), 5.91 (e. p. IV)
Specific gravity / density	1.349 g cm ⁻³ (22°C)
Solubility	Both enantiomer pairs : in water 2 µg L ⁻¹ (20°C) In dichloromethane and toluene > 200 g L ⁻¹ (20°C) Enantiomer pair II in hexane 2-5, isopropanol 5-10 (both g L ⁻¹ , 20°C), Enantiomer pair IV in hexane 1-2, isopropanol 2-5 (both gL ⁻¹ , 20°C)
Stability	Thermally stable at room temperature. Hydrolysis DT ₅₀ (22°C) for enantiomer pair II : 117 d (pH 4), 20d (pH 7), 6d (pH p); for enantiomer pair IV : 25 d (pH 4), 11d (pH 7), 5d (pH 9)
Acute oral toxicity	LD ₅₀ 500 mg kg ⁻¹ (for rats, in polyethylene glycol)
Toxicity class	WHO II; EPA II
Formulation	EC, SC, EW, UL, GR
Recommended application	12.5g a.i. ha ⁻¹

Applications

i) Mode of action

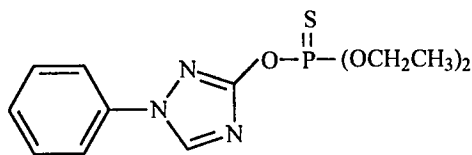
Non-systemic insecticide with contact and stomach action. Acts on the nervous system, with rapid knockdown effect and long residual activity.

ii) Uses

Insecticide effective against Lepidoptera, Coleoptera, Hemiptera and Homoptera on cotton, fruits, vegetables, cereals and other crops; also against migrating locusts and grasshoppers and animal health.

2.1.6. Triazophos

An organophosphorus insecticide, acaricide and nematicide. Triazophos having the following physical, chemical and biological properties.



Molecular formula	$C_{12}H_{16}N_3O_3PS$
Molecular weight	313.3
Chemical name	O,O-diethyl-O-1-phenyl-1-H-2,4-triazol-3-ylphosphorothioate
Trade name(s)	'Hostathion'
Manufacturer	AgrEvo
Form	Pale yellow oil
Melting point (m.p.)	2-5°C
Boiling point (b.p.)	Decomposes on distillation
Vapour pressure (v.p.)	0.39 mPa (30°C); 13 mPa (55°C)
K_{ow} log p	2200
Specific gravity /density	1.247 (20°C)
Solubility	In water 30-40 mg L ⁻¹ (20°C). In ethyl acetate, acetone > 1000, ethanol, toluene > 330, hexane 9 (all in g kg ⁻¹ , 20°C)
Stability	Stable to light. Hydrolysed by aqueous acids and alkalies
Flash point	135 ± 2°C (Pensky-Marten, closed cup); 193 ± 5°C (Cleveland, open cup)
Acute oral toxicity	LD ₅₀ 57-68 (for rats)
Formulation	EG, GR, UL, WP, DS, MG
Mixture	[triazophos +] deltamethrin
Recommended application	0.7 kg a.i. ha ⁻¹

Applications

i) Mode of action

Broad-spectrum insecticide and acaricide with contact and stomach action. Cholinesterase inhibitor. Non-systemic, but penetrates deeply into plant tissues. Also possess some nematicidal properties.

ii) Uses

Control aphids, thrips, midges, beetles, lepidopteran larvae, cut worms soil insects, spider mites and different species of mites in ornamentals, fruit trees (including citrus), vines, bananas, strawberries, vegetables, oilseed rape, cereals, sugar beet, sugarcane, maize, soybeans, pea nut, guavas, mangoes, oil palms, olives, cotton, coffee, rice, grassland and in forestry. Also controls some free-living nematodes, particularly in ornamentals and strawberries and as a bulb dip for tulips and garlic.

2.2 Persistence of pesticides in crops and soil

2.2.1. Pendimethalin

Pendimethalin (N-1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine is a dinitroaniline herbicide used selectively as pre-emergence for control of grasses and dicot weeds in crops such as cotton (*Gossypium hirsutum*), wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.), maize (*Zea mays* L.), peas (*Pisum sativum* L.) and several vegetable crops (Kulshrestha *et al.*, 1999). In India it is also recommended for *kharif* maize and winter wheat cropping system.

Savage and Jordan (1978) reported the persistence of three dinitroanilines herbicide as affected by delayed incorporation. Gas chromatographic analysis showed that dissipation of trifluralin and fluchoralin

in sandy loam was very rapid during the first 3 days and amounted to 70-75% of the applied herbicide. Pendimethalin dissipation was less rapid initially, though a total 62% was lost after 12 days.

Barrett *et al.* (1981) reported pendimethalin dissipation under various cropping and irrigation systems approximated to first order kinetics. The dissipation rate was rapid than predicted by the model for the first 10-14 days for non-flooded and flooded rice. The half-life of pendimethalin ranged from 19 to 27 days in soil sown to soybeans or rice with flush irrigation, compared to only 8-16 days in flooded rice-plots. Bioassays gave at least 20% estimates of herbicide concentration than did GLC analysis, though there was a highly significant correlation between both methods.

The effect of soil water content on the dissipation of pendimethalin was investigated by Barrett (1983). He found that pendimethalin dissipation rates were 30, 59 and 63 days in alternately flooded, dried and continuously flooded soil, respectively. The half-lives for its dissipation were 3-6 days for low land rice, 4-7 days for upland rice and 5-28 days for irrigated soybean cultivation.

Stahnke *et al.* (1991) studied the pendimethalin dissipation following annual 1.7 kg ha^{-1} application to 3-year old Kentucky bluegrass (*Poa pratensis*) cv. Touchdown turf growing on a sharpsburg silty clay loam and an 85:15 v/v sand sharpsburg soil mixture in 150 cm deep rhizoton containers. Plant tissue, thatch and soil were sampled periodically between application and 168 days after treatment (DAT). Soil and leachate were collected to monitor pendimethalin movement. Most of the herbicide appeared to remain within the turfgrass system. Pendimethalin concentration was highest in plant tissues and thatch. The 4-hydroxymethyl pendimethalin metabolite was detected in

turfgrass tissue up to 42 DAT. No pendimethalin was detected at 30, 60 or 120 cm depths of rhizoton containers.

Zimdahl *et al.* (1994) evaluated the empirical equations to describe dissipation of metribuzin and pendimethalin in soil. They found that a biexponential equation was superior to the first order equation for metribuzin and pendimethalin under 5 moisture levels and 3 temperature in laboratory and field studies (The latter conducted on clay loam and loam soils during 1986-87).

Smith *et al.* (1995) evaluated the dissipation of pendimethalin (1.1 kg ha^{-1}) in two Saskatchewan field soils following applications of an emulsifiable concentrates (EC) formulations in spring. Persistence of pendimethalin applied as a granular formulation in the fall was also investigated. Over the winter there was little loss of pendimethalin at the two site of their investigation. Some over winter dissipation of pendimethalin occurred from a granular formulation ($1.51 \text{ kg a.i. ha}^{-1}$) applied on October 4, 1993 with $1.01 \pm 0.15 \text{ kg ha}^{-1}$ ($67 \pm 10\%$ of the initial treatment) remaining in May 1994. By the third week in September 1994 (350 days after application), $0.45 \pm 0.08 \text{ kg ha}^{-1}$ ($30 \pm 5\%$ of the initial herbicide) remained.

When persistence of pendimethalin and anilophos was studied in six tropical soils of varying physiochemical properties (2 Alfisols, Aridisols, Inceptisol, Vertisol and Mollisol) using gas liquid chromatography, Alfisol-1 exhibited greatest persistence, where as Mollisol was least (Pai *et al.*, 1999).

Diwan *et al.* (1999) studied the degradation of pendimethalin (Stomp) and fluchloralin (Basalin) in sandy loam soil in an onion field during rabi seasons of 1989-90 and 1990-91 at Gujarat, India. There study revealed that the rate of dissipation of both the compounds in soil showed first order kinetics.

Pendimethalin dissipated with a half-life varying from 28.1 to 31.7 days, when applied at 0.75, 1.00, 1.25, 1.50 and 2.00 kg ha⁻¹. About 85-90% of applied pendimethalin had dissipated by harvest time.

Effects of environmental factors on bioactivity, adsorption and persistence of pendimethalin was studied in the laboratory and green house using rice (*Oryza sativa* L.) as the bioassay species (Ismail and Kalithasan, 1999). Herbicide bioactivity was inversely correlated with the organic matter (OM) content of the soil and directly correlated with herbicide concentrations. Pendimethalin adsorption increased proportionately at higher content of soil organic matter and with increasing herbicide concentration. The apparent half-life of pendimethalin was shorter at a moisture level of 70% gravimetric water content than at 30% moisture level in both soils. Under field conditions, the apparent half-life was 6.3 and 12.3 days of application rate of 1 kg and 3 kg ha⁻¹, respectively.

2.2.2. Imidacloprid

Following the discovery of insecticidal properties of the heterocyclic nitromethylenes (Soloway *et al.*, 1978), chemists of Nihon Bayer Agrochem, Japan started to optimise these structures in 1979. In 1985, the coupling of the chloropyridyl moiety to the N-nitro substituted imidazolidine ring system enabled the synthesis of highly active insecticide imidacloprid. Its insecticidal activity is ~ 10,000 fold higher than natural insecticide 1-nicotine (Tomizawa and Yamamoto, 1993 and Yamamoto *et al.*, 1995). Imidacloprid is a commercial example of the chloronicotiny insecticides acting on nicotine acetylcholine receptor (Bai *et al.*, 1991 and Leicht, 1993). Recently, studies have demonstrated the excellent activity of imidacloprid on pest species of different orders.

2.2.2.1. Crops

Rouchaud *et al.* (1994) studied the transfer of imidacloprid to sugar beet leaf when applied as seed dressing @ 90 g a.i. ha⁻¹ and reported high concentration decreased due to plant growth and metabolism in the leaf as the time elapsed. The imidacloprid concentration was greater in the field recently treated with organic fertilizers and was greater than equal to 1 mg kg⁻¹ upto 80 days after sowing. No residues were detected in the roots and leaves at harvest.

Ishii *et al.* (1994) and Iwaya *et al.* (1998) studied the behaviour of imidacloprid residues in rice when treated with granules (2%) @ 200 g a.i. ha⁻¹ at 2-3 leaf stage and reported their rapid decreased with a half life of less than three days. However, the treatment controlled the brown plant hopper (BPH) in rice up to 60 days after application with the marginal concentration of 0.01 mg kg⁻¹ for practical control. The final residues in rice grains were only 0.002 mg kg⁻¹. Since residues concentration was 2-8 times higher in the leaves than in sheath during the testing period, the insecticide moved to leaves acropetally.

Westwood *et al.* (1998) studied the presence of ¹⁴C-imidacloprid in sugarbeet plant following application to pelleted sugarbeet seeds. He found that at 21 days after application, parent imidacloprid was the main compound found in leaves and its concentration averaged 15-20 µg g⁻¹ fresh weight. At 97 days after sowing, the concentration of parent compound in leaves reduced to an average of 0.5 µg g⁻¹, the metabolites and parent compound in leaves represented 44.5 and 4.5 per cent of total applied radioactivity, respectively while in the roots parent imidacloprid and its metabolites together accounted for only 0.1 per cent of the applied activity and in the soil there was 23 per cent

parent compound and 4 per cent as metabolites. The persistence of imidacloprid and olefinic metabolite which has recently been shown to have higher aphicidal activity than the imidacloprid, explained the prolonged control of aphids.

2.2.2.2. Soil

Scholz and Spiteller (1992) reported half-lives of 48 days in simulated practical conditions with vegetation and 190 days without vegetation in standardised laboratory tests while studying the influence of ground cover on degradation of imidacloprid in soil.

Rouchaud *et al.* (1994) studied the imidacloprid soil biodegradation in sugar beet crop and reported soil half lives as 124, 105 and 108 days for cow manure, pig slurry and cow manure + pig slurry treated field compared to 40 days for control. They also reported a half life of 85 days for field containing high concentration of soil organic matter (4-3%) treated with cow manure compared to 44 days for control. After two months of crop period, its concentration in soil diminished at increasing rates leaving no residues in the soil at harvest of the crop. No residues were detected in 10-20 cm soil layer under and around sugarbeet and hence no apparent leaching in soil.

In a separate study, Rouchaud *et al.* (1996) investigated the persistence of imidacloprid and its metabolites, 1-[6-chloro-3-pyridinyl) methyl]-2-imidazolidone, 6-chloronicotinic acid, 6-hydroxynicotinic acid and 2-imidazolidone in sugarbeet following seed dressing application @ 90 g a.i. ha⁻¹ and reported no residues of imidacloprid or its metabolites in 10-20 cm on soil layer under and around sugarbeet in organic fertilizer treated and untreated fields. There was thus no leaching of these compounds in soil. No residues of imidacloprid and its

metabolites were detected in roots and leaves at harvest. During the first three months, the concentration of metabolites progressively increased, attained a maximum and then decreased. The recently organic fertilizer treated soil showed down the imidacloprid disappearance and its metabolite formation in soil.

Sarkar *et al.* (2001) conducted a laboratory experiment to study the persistence of imidacloprid from two formulations (Confidor® 200 g L⁻¹ SL and Gaucho® 700 g kg⁻¹ WS), and its metabolism in three different soils (Gangetic alluvial soil of Kalayni, lateritic soil of Jhargram and coastal alkaline soil of Canning) of West Bengal, India following application of at 0.5 kg and 1.0 kg a.i. ha⁻¹. Dissipation of imidacloprid in soil followed first order kinetics and DT₅₀ values ranged from 28.7 to 47.8 days. The shortest half-lives (28.7 and 35.8 days) were observed in the lateritic soil of Jhargram for both liquid and powder formulations. The formation of two metabolites of imidacloprid, imidacloprid-urea and imidacloprid-olefin was first detected after 30 days of degradation at 28 (±1)°C in all three soils.

2.2.4. Chlorothalonil

Chlorothalonil (2,5,6-tetrachloroisophthalonitrile) is a broad-spectrum fungicide effective against many economically important plant pathogens affecting vegetables; agronomic tree and small fruit crops; turf; and ornamentals. The diverse fungicidal activity of chlorothalonil is characteristics of its mode of action, the alkylation of cellular thiol-glutathione and thiol dependent glycolytic and respiratory enzymes (Vincent and Sisler, 1968; Tillman *et al.*, 1973). The selective activity of chlorothalonil against fungi is correlated with selective uptake by fungal cells commensurate with oil-water partition value

(Turner and Battershell, 1969, 1970). In the presence of moisture, chlorothalonil undergoes a breakdown in soil. High moisture and temperature accelerate the rate of breakdown. In temperate regions with moderate levels of moisture, chlorothalonil has a half life of 1½ -3 months, depending upon type of soil.

Agnihotri *et al.* (1995) studied the persistence of chlorothalonil residues on potato, tomato crops and soil following foliar application of chlorothalonil 40 SC and 75 WP at recommended as well as higher dose used to control late blight. No residues of chlorothalonil were detected either in soil or in potato tubers at harvest even when treated with 40 SC @ 1125 g ha⁻¹. In case of tomato, the initial deposits on fruits were 0.620 and 1.264 ppm which persisted up to 20 days but were below the maximum residue limit of 5 ppm. No residues were detected in soil in tomato field after 20 days.

The dissipation rate of chlorothalonil applied @ 4 mg kg⁻¹ dry weight compared among four soils receiving different fertilizing practices (Katayama *et al.*, 1995). The dissipation of chlorothalonil was accelerated in soil amended with FYM at 200 t ha⁻¹ twice a year. The acceleration was mainly due to abiotic action. The abiotic dissipation rate apparently followed first-order kinetics. They suggested that the accelerated abiotic dissipation of chlorothalonil in FYM soil was mainly due to chemical reactions with soil humus.

Malathi and Jeyarajan (1995) also studied persistence of chlorothalonil in potato foliage for late blight control. They showed that the dissipation of chlorothalonil was linear with time and the persistence was lowest in the top leaves of treated plants.

Kar *et al.* (2000) reported that when chlorothalonil (Kavach 75 WP) was applied at 1.7 and 3.4 kg a.i. ha⁻¹ to mango at 50% stone formation stage, the dissipation of chlorothalonil was about 90-94% within 15 days after application, regardless of application rate. The half-life value was calculated in the range of 3.81-4.56 days.

No residues was found in samples of peel or pulp taken at harvest. The safe waiting period was 17.78 and 25.65 days for the 1.7 and 3.4 kg a.i. ha⁻¹ application rates, respectively.

2.2.5. Endosulfan

Endosulfan I (alpha) and II (beta) dissipated in simple aqueous media (water or sterile soil extract yeast mannitol medium) in glass incubation vessels peaked with Teflon and showed apparent half-lives of 151 and 88 days, respectively (Gurein and Kennedy, 1992). Endosulfan sulphate, the major oxidation product of endosulfan, was much more persistence than any of the parent isomers under the same incubation conditions.

The dissipation of endosulfan residues from foliar application of at 0.07 and 0.14% a.i. to cowpea foliage and pods showed the residue persisted in foliage and pods upto 15 days (Singh and Mukherjee, 1994). Residues in both foliage and pods were below the FAO/WHO maximum residues level of 2 mg kg⁻¹ 10 days after treatment (FAO, 1989).

Antonious and Bayers (1997) studied the impact of soil mulches on the movement of endosulfan under field conditions. Cultivation of turf between cropping rows and pepper incorporated with tomato reduced run off water and sediments loss and endosulfan movement from the application site to surface water run-off.

Kaur *et al.* (1998) investigated the effect of temperature, pH and organic matter content on the rate of dissipation of endosulfan in soil and water. In water endosulfan (alpha and beta) decayed at a faster rate with increasing temperature and pH. The persistence of both (alpha and beta-) endosulfan decreased with increasing soil pH. Endosulfan persisted longer in soil than in water.

The residues of endosulfan persisted for over 21 days on both leaves and curds when the insecticides sprayed at their recommended concentration of 0.05% (Sheikh and Tikoo, 1998). The half-life, and waiting period of the pesticide was calculated as 4.14 and 14.72 days for leaves and 4.25 and 10.26 days for curds, respectively.

The spraying of endosulfan 35EC at the rate of 0.05, 0.07 and 0.10 per cent concentrations on lady's finger crops resulted on average initial deposit of 3.35, 5.06 and 6.95, respectively (Neog and Goswami, 1999), which came down to maximum residue limit of 2 ppm (FAO/WHO, 2000) after 3 days of spray treatment at lower and medium concentration. The half-life values were 3.42, 3.81 and 4.32 days for lower (0.05%), medium (0.07%) and higher concentrations, respectively. The safety intervals for endosulfan on lady's finger were 2, 4 and 6 days, respectively at tested concentrations.

Navarro *et al.* (2000) studied the dissipation rate of endosulfan isomers (alpha and beta) in sea water and sediment. The disappearance rate of both isomers from sea water and pure water was compared, and the same measurements were made in both sterile and unsterile marine sediments. The dissipation of endosulfan occurred more rapidly in sea water than in pure water. The concentration of alpha-endosulfan in sterile sediment was four times

greater than unsterile sediment, while the dissipation of beta-endosulfan in unstable sediment was approximately double that observed in sterile sediment.

Fritz *et al.* (1992) conducted a field trial to study the volatilization pesticides from agricultural ecosystems. Volatilization trials were carried out with pesticides using appropriate target crops (wheat and barley) and at commercial application times to ensure typical conditions for the experiment. The formulated active ingredients of β -cyfluthrin and other few compounds showed either no or little volatilization (< 20%).

Battu *et al.* (1999) estimated the residues of β -cyfluthrin in cotton seed and cotton lint of after six application of β -cyfluthrin (as Bulldock[®] EC) at 7-10 days intervals @ 12.50 and 18.75 g a.i.ha⁻¹. The residues of β -cyfluthrin were detected only in cotton lint samples, with average values of 0.30 and 0.40 mgkg⁻¹ for the 2 doses. No residues were detected in cotton seed.

Dikshit and Pachauri (2000) conducted a study to determine the persistence and bioefficacy of β -cyfluthrin and imidacloprid in tomato (*Lycopersicon esculentum* cv. Pusa Sheetal). They gave two sprays of β -cyfluthrin at 18.75 and 37.50g a.i. ha⁻¹ and imidacloprid at 20 and 40 g a.i. ha⁻¹ at 15 days intervals. When the first sprays was given at the time of flowering, β -cyfluthrin residues in tomato fruits declined as time elapsed and on the tenth days residues from the recommended rate 18.75 g a.i. ha⁻¹ were undetectable. Residues from twice the recommended rate were undetectable on day15. They concluded that the residues of β -cyfluthrin and imidacloprid in tomato fruits reaches the safe level at the seventh day when applied at the recommended rates. Fruit borer (*Helicoverpa armigera*) damage was drastically reduced to 22-24% by β -cyfluthrin treatment.

Gajbhiye *et al.* (2000) studied the residues of β -cyfluthrin (applied 4 times in a spray at 18.75 and 37.5 g a.i. ha⁻¹ at 10 days intervals) in cotton plants, seeds, lint and soil in 1997-99. They found that the persistence in the plant followed first order kinetics with a half-life of 1.7-2.1 days. They detected some residues on lint (up to 0.03 mg kg⁻¹), but none were detected in seeds or soil.

In the year 2001, Dikshit *et al.* again conducted an experiment on persistence and bioefficacy of β -cyfluthrin and other insecticides on Okra (*Abelmoschus esculentus*) and sponge gourd (*Luffa cylindrica*). They found that residues of β -cyfluthrin was undetectable after 7 days in okra and sponge gourd fruits.

2.2.6. Triazophos

Raj *et al.* (1999) reported the dissipation of 350 or 700 g a.i. triazophos ha⁻¹ on/in brinjal and okra fruits following its application. The residue persisted up to the 10th day on both the fruits, but dissipation was faster in brinjal fruits. They observed the comparatively higher initial deposits of 2.549 and 6.617 μ g g⁻¹ on okra dissipated with a half-life of 1 day. Unlike okra, low initial level of 0.165 and 0.218 μ g g⁻¹ on brinjal dissipated with half-life of 1 and 2 days for the lower and the higher doses, respectively. Considerable reduction of triazophos residue was noticed with washing and washing + cooking treatment for both the fruits.

Sharma *et al.* (1998) studied the persistence pattern of triazophos on field grown okra and brinjal. They reported that when the crops were sprayed with 350 or 700 g a.i. ha⁻¹ of triazophos, residue level in the fruit reached the prescribed MRL (maximum residue limit) after 7-9 and 7-10 days after spraying, respectively.

Rani *et al.* (2001) conducted a laboratory study on canal water samples (pH 8.3) spiked with triazophos 40 EC at 0.25 and 0.50 $\mu\text{g a.i. ml}^{-1}$ to study the persistence, dissipation and kinetics of triazophos residues in canal water. The dissipation of triazophos in canal water was quite fast and independent of the initial concentration.

Chahal *et al.* (2000) conducted field trial in Ludhiana, Punjab to study the persistence of triazophos in cauliflower. They reported the average residues from the 700 g a.i. ha^{-1} treatment were 0.5, 0.2 and 0.01 mg kg^{-1} at 3, 5 and 7 days after treatment. Triazophos residues were not detected after 7 days the lower application rate. The half-lives for triazophos applied at 350 and 700 g a.i./ha were 2.5 and 1.7 days, respectively.

2.3. Interaction of pesticides/mixtures with soil and microbes

2.3.1. Adsorption of pesticides in soil

2.3.1.1. Adsorption /desorption of pendimethalin in soil

Adsorption is the bonding of chemical to the sites on soil mineral or organic surface. Intermolecular forces of adsorption are high and low energy bonds. Ionic and ligands bond are high energy and charge dipole, dipole-dipole, hydrogen, charge transfer, van der Waals London, entropy generation and magnetic bonds are low energy (Calvett, 1980 and Jury, 1987). Adsorption is influenced by soil organic matter, clay and water contents; ion exchange capacity; bulk density, solution composition, pH, temperature and tillage practices and the structure and properties of the herbicide.

Adsorption of the herbicide by soil is important in determining their fate in environment, biological activity, persistence in soil and transport of the herbicide. Adsorption is also governed by physico-chemical processes which

control the quantity of pesticides in soil solution thus determine their biological activity and mobility in soil water as well as volatility (Green, 1994). The persistence of dinitroaniline group of herbicides in field is mainly affected by their photodecomposition, volatility and adsorption (Helling, 1976). Hamaker and Thomson (1972) proposed that mineral phases play significant role in the adsorption, and adsorption is also related to the soil organic matter content (Jacques and Harvey, 1979). Strongly adsorbed herbicides are less susceptible to leaching and thereby the risk of ground water pollution is reduced.

Raj *et al.* (1999) studied the adsorption–desorption of pendimethalin, fluchloranlin and oxadiazon on sandy loam soils. Pendimethalin showed the greatest adsorption on both the soils. Organic matter content of the soils was responsible for adsorption of the herbicides, which was evident by the low K_{oc} values for clay soils.

Pendimethalin adsorption increased proportionately at higher content of soil organic matter and with increasing herbicide concentration (Ismail and Kalithasan, 1999). The apparent half-life of pendimethalin decreased from 23.9 days at 25°C to 19.4 days at 35°C in Selangor series soil and from 24.4 days at 25°C to 18.5 days at 35°C in Serdang series soil.

Rai *et al.* (2000) studied the adsorption of pendimethalin on six different soils samples using batch and shake technique. Their study revealed that the adsorption isotherm fitted well in Freundlich equation ($r^2 > 0.98$) and Freundlich's constant 'K' and '1/n' values were in the range of 10.98-24.41 and 0.706-0.854, respectively. The amount adsorbed was related to clay and organic carbon content ($R^2 = 0.89$). In different soil adsorption were in the order : Mollisol > Vertisol > Inceptisol > Alfisol-1 > Alfisol-2 > Aridisol. Removal of

organic matter by H_2O_2 oxidation caused drastic reduction in adsorption of pendimethalin. The sorption property of soil system was not reversible and only 29.6 to 40.1% pendimethalin could be desorbed.

2.3.1.2. Adsorption/desorption of imidacloprid in soil

Sorption-desorption processes are important in determining the fate and distribution of agrochemicals in the soil/water environment. Since they determine the amount of pesticide that can reach the target organisms and the amounts that can be volatilized, degraded and leached. Very little information is published concerning the fate of imidacloprid and its metabolites in soil. It does not appear to leach below the surface soil (Rouchaud *et al.*, 1994, 1996). Degradation half-lives of 40 days in field experiments (Rauchaud *et al.*, 1994) and 48 days in standardized laboratory tests (Scholz and Spiteller, 1992) have been reported. The lack of leaching observed in soil for imidacloprid and its metabolites and the decreased degradation in soil with the addition of organic amendments (Rouchaud *et al.*, 1996) are presumably due to sorption-desorption characteristics of the chemicals.

Sorption-desorption studies of imidacloprid using the batch equilibration method with different soil properties showed that sorption co-efficients (K_f) and the soil properties, organic carbon and CEC gave the higher single correlation co-efficient; the latter was due to the positive correlation between OC and CEC (Cox *et al.*, 1998).

The same authors earlier (Cox *et al.*, 1997) compared the sorption-desorption of imidacloprid with its three metabolites imidacloprid-urea, imidacloprid-guanidine and imidacloprid guanidine olefine. The order of sorption (K_f) was imidacloprid-guanidine>imidacloprid guanidine olefin>imidacloprid>

imidacloprid-urea in three soils. Desorption was hysteretic for all chemicals in all soils. Greatest hysteresis was observed with imidacloprid-guanidine and imidacloprid-guanidine-olefin.

Time-dependent sorption of imidacloprid was investigated in two German soils (sandy loam and silt loam) (Oi, 1999). Soil batches containing 0.33 mg kg^{-1} a.i. were incubated for up to 100 days. Calculated sorption coefficients K_d and K_{oc} increased by a factor of 3.2-3.8 during 100 days of aging. Time-dependent sorption was verified by a column leaching experiment with the aged soil. The amount of imidacloprid in column eluates (0.01 M CaCl_2) decreased compared to total recovered by a factor of 2.

Imidacloprid sorbed on both montmorillonite and humic acid, whereas sorption is very low on iron oxide (Cox, 1998). The sorption distribution coefficient, K_d , on montmorillonite increased from 15 to 180 when pH was decreased from 3.5 to 1.0, suggesting protonation of the $-\text{NH}$ group of imidazole ring.

When the soil is incubated at 25°C and 35% soil water content, sorption is influenced by organic carbon content and increased with time (Capri, 2001). Over a period of 14 days, the distribution coefficient between solid (soil) and solution (water) phase increased twice.

The sorption/bio availability of imidacloprid metabolites changes in soil with incubation time (Koskinen *et al.*, 2001). Imidacloprid-urea sorption is highest in the soil with highest organic carbon content and increased by an average factor of 2.6 in three soils during 8 weeks incubation period. Imidacloprid-guanidine sorption increased by a factor of 2.3 in the some soils. These results revealed that increase in sorption during pesticide aging should

be taken into account during characterisation of the sorption process of mathematical models of pesticide degradation and transport.

2.3.1.3. Adsorption /desorption of chlorothalonil in soil

Chlorothalonil is often used as a foliar spray, its biological efficiency and persistence in soil mainly depends upon the process of adsorption-desorption, the capacity of which depends on the organic matter, clay and cation exchange capacity of the soil (Dios *et al.*, 1992).

Redeucker *et al.* (1988) reported the significant relationship between adsorption of chlorothalonil and organic matter content in New Jersey soils.

In their separate study, Singh and Thapilyal (1987) and Ververde *et al.* (1988) reported that organic matter content of soil provides higher binding sites and control the adsorption-desorption of chlorothalonil in soil.

Balasubramanian and Mathan (1996) observed that adsorption was highest in Udisol followed by Vertisol soil, Inceptisol and Alfisol. The isotherm obtained in the study was L-type of Giles classification indicating the soil possessed average affinity for adsorption for chlorothalonil. Similar results were reported by Ganesan and Lalithakumari (1992).

2.3.2. Degradation of pesticides

2.3.2.1. Degradation of pendimethalin

2.3.2.1.a Chemical degradation

Pendimethalin is very stable under normal conditions and it is one of the more photostable dinitroaniline herbicides. Only 10% photodecomposition of pendimethalin occurred on air-dried clay loam thin layer plates in 7 days in Maryland, USA in July (Parochetti and Dec, 1978).

Dureja and Walia (1989) had identified photodecomposition products arising in solvent (methanol) thin films and soil thin layers. The nitro reduction sequence occurs and cyclisations to afford the ethyl benzimidazole via intermediates are common reactions in the dinitroaniline series. It is interesting to note that the methyl benzimidazoles are not formed, supporting the proposed involvement of N-acetylation in rat liver (enzyme catalysed) in the formation of these metabolites (Zulalian, 1990). Benzimidazoles (not the methyl or ethyl derivatives) have been noted under the reaction with titanium dioxide in the system (Pandit *et al.*, 1995). However, these are considered to arise via formylation of an amine and cyclisation. Formylation occurs via titanium dioxide-photocatalysed hydrogen abstraction from the methanol solvent. It should be stressed that photodecomposition in solution such as methanol is much faster in the presence of titanium dioxide than in its absence (Moza *et al.*, 1992).

Irradiation of pendimethalin in methanol without titanium dioxide by Pal *et al.* (1991) afforded, in addition to N-dealkylated products, the benzyl alcohol and the benzaldehyde. Irradiation on a sandy loam soil surface for 48 hours with a low pressure mercury lamp gave 2, 5, 9 and 10 with 78% pendimethalin unchanged (Dureja and Walia, 1989).

Thus the photodecomposition of pendimethalin involves oxidative dealkylation, nitro reduction and cyclisation with one report of methyl hydroxylation and further oxidation to the aldehyde.

2.3.2.1.b Degradation in soil

Pendimethalin is strongly adsorbed to soil; high organic matter content, rather than pH; is important in this process. Thus losses by volatilization and run-off are slight (Cynamid, 1993).

The initial DT₅₀ values of pendimethalin on the surface of silt loam and clay were 4 and 6 days, respectively (Savage and Jordan, 1980, Nelson, 1980). This loss was due to photodegradation. The DT₅₀ of pendimethalin in sandy loam soil was inversely related to temperature (98 days at 30°C and 409 days at 10°C) and followed first order kinetics (Walker and Bond, 1977). At 25°C the DT₅₀ increased with decreased soil moisture content.

In 7 different soils there was a trend to lower rate of loss with increasing soil organic matter content and DT₅₀ values of 72-172 days were reported. In a later study using clay, clay loam and sandy loam, for a given temperature and moisture content the influence of soil type was small. An average DT₅₀ of 47 days was reported (Zimdahl *et al.*, 1984).

The distribution characteristics of soil bound residues have been described (Nelson *et al.*, 1983) in comparison with those of trifluralin and oryzalin. Each herbicide behave similarly.

The degradation of pendimethalin in soil is extremely complex and the pathways have not been elucidated (Cynamid, 1993). Pendimethalin is degraded rapidly *in vitro* by several species of soil fungi (*Aspergillus*, *Fusarium* and *Penicillium*). Degradation by *Fusarium solani* was studied in the greatest detail (Barua *et al.*, 1990).

2,6-dinitro-3,4-xylidene, N-(1-ethyl-propyl)-2-amino-6-nitro-3,4-xylidene and N-propyl-3-methyl-4-hydroxy-2,6-dinitro aniline were isolated and identified, although the last metabolite appears to be an unlikely structure. The first two metabolites and 15 [N-(1-ethyl propyl)-6-amino-2-nitro-3,4-xylidene] were identified in similar *in vitro* studies using *Fusarium oxysporium* and species *Paecilomyces* and *Rhizoctonia* (Singh and Kulshreshta, 1991). A study of the effect of

moisture content on degradation rate in a sandy loam (Kulshrestha and Singh, 1992) afforded 2,6-dinitro-3,4-xylidene under non-flooded conditions and the benzimidazole under flooded conditions. Under flooded and non-flooded conditions the half-lives of pendimethalin were 33 and 52 days, respectively. Some degradation occurred in sterile soil (in spite of conducting the experiments in the dark). The conversion of pendimethalin to 20 under flooded conditions was virtually quantitative within 24 hours.

Another *in vitro* study using soil bacterium *Azotobacter chroococcum* (Kole *et al.*, 1994) has provides most information on the fate of pendimethalin. Four metabolites were identified and their herbicidal activity (phytotoxicity to wheat seedlings) was investigated. With pendimethalin classed as biologically active, than any other metabolites.

The effect of long term application of pendimethalin in maize-wheat rotation was investigated when it was applied at 1.5 kg a.i. ha⁻¹ separately as one or two annual applications for five consecutive years (Kulshrestha *et al.*, 2000). Harvest time residues of the herbicide decreased gradually over the years and at the end of five years less than 3% of applied pendimethalin was recovered from soil as against 18% in the first year. Dealkylated pendimethalin derivative, partially reduced derivative and cyclised product were the main metabolites traced in surface and sub-surface soils up to 90 cm.

2.3.2.2. Degradation of imidacloprid

The metabolism and degradation of methylene ¹⁴C-imidacloprid investigated in laboratory studies with and without vegetation. The most important metabolic steps in both experiments were oxidation at imidazolidine

ring, reduction or loss of nitro group, hydrolysis to 6-chloronicotinic acid and mineralisation (Pesticide Manual, 10th edition, 1994).

The influence of vegetation on the degradation of imidacloprid was investigated in a laboratory experiment in which conditions likely to occur in the field were simulated (Scholz and Spiteller, 1992). The half-life was 48 days if vegetation was present and 190 days if there was no ground cover. The imidazoline ring was degraded stepwise to the key metabolites 6-chloronicotinic acid which led ultimately to the formation of considerable amounts of CO₂.

Rauchaud *et al.* (1994) described the degradation of imidacloprid in soil and accumulation of the compound in the sugarbeet.

The photochemical degradation of imidacloprid was investigated by differential pulse polarography (DPP) and micellar electrokinetic chromatography (Cacho *et al.*, 1999). The electrochemical study of imidacloprid by DPP at different pH values demonstrated the occurrence of two different reduction processes; at pH 6.8, two peaks at -0.90 and -1.38V, respectively, were obtained, which are related to the photochemical reduction processes. The photochemical degradation of imidacloprid caused by sunlight was polarographically monitored and its degradation products elucidated.

Aguera *et al.* (1998) oxidised imidacloprid in water under photocatalytic TiO₂ in the presence of formulating agents. Analysis of oxidised solutions with both GC-ion trap-MS using either EI and CI as ionization modes and LC-Atmospheric Pressure Chemical Ionisation-Mass Spectrometry (APCI-MS) was performed in order to confirm the evaluate the decay of imidacloprid and the presence of degradation products. LC-APCI-MS techniques allowed the monitoring the imidacloprid and 6-chloronicotinic acid along the degradation

process. GC-MS techniques allowed the detection of five DPs, three of which were unequivocally identified.

Influence of pesticide imidacloprid and its metabolites on catalase activity in soils was investigated by Zheng *et al.*, (2000). The activity of catalase was influenced by concentration of imidacloprid, with the stronger influence observed at higher concentration.

The effect of metabolites of imidacloprid through hydrolysis and photolysis on catalase activity in soil was less than that of imidacloprid, and the effect decreased with the prolonging times of photolysis.

The technical feasibility, mechanisms, and performance of degradation of aqueous imidacloprid have been studied at pilot scale in two week defined photocatalytic system (Malato *et al.*, 2001). Heterogenous photocatalysis was done with titanium dioxide whereas homogenous photocatalysis was done by photo-fenton. Ninety five per cent mineralization (< 2.0 mg /L) was reached after 250 minutes of photocatalytic treatment with Fenton and 450 minute with TiO_2 .

2.3.2.3. Degradation of endosulfan

2.3.2.3.a. Degradation in soil

Degradation was studied in test culture and in different soil types in laboratory experiments using ^{14}C -labelled endosulfan in concentration upto 1000 ppm (Martens, 1972). Endodiol, endosulfan, alpha-and beta-endosulfan, endohydroxyether, chlorendic acid and endolactone were identified as metabolites. Endosulfate was the main product of fungi, endodiol of bacteria, and actinomycetes produced all at similar rates. Under aerobic conditions,

endosulfate in quantities of 30-60% of the applied endosulfan was detected as the major metabolite in soil under different conditions (Martens, 1977).

Miles and Moy (1979) used a mixed culture of soil microorganisms study the degradation of endosulfan and its metabolites. Alpha, beta endosulfan and the sulphate, diol, ether, alpha-hydroxy ether and lactone of endosulfan were incubated separately with the mix culture. All the metabolites conversion rates, except that lactone, were greater in samples inoculated with micro-organisms than in controls.

The degradation of ^{14}C endosulfan was studied after incubation in sterilized and non-sterilized samples of three oxisols frequently to cotton, in Brazil (Monteiro, 1989). In sterilized soils the extracted residues were about 80%, the bound residues varied from 6 to 14% and the total residues, from 90 to 102%, presenting small variation during 160 days of incubation. The non-sterilized soils, after 160 days, showed 38, 45 and 43% of extracted residues; 34, 23 and 30% of bound residue, and 72, 68 and 73% of total residue.

A laboratory culture of *Trichoderma harzianum* 2023, originally isolated from soil in a banana plantation in Costa Rica, was found to be able to degrade endosulfan and some other organochlorine pesticides (Katayama and Matsumara, 1993) endosulfan sulfate and endosulfan diol were reported to be the major metabolites. Microbial degradation in minimal media containing 50 ppm either endosulfan or a metabolite (alpha, beta, endodiol or endosulphate) was studied by Awasthi *et al.* (1997) in which they observed that after 15 days nearly all the pesticide had been degraded with the simultaneous increase of bacterial mass. Degradation rates of β -endosulfan was slower than alpha-isomer.

Singh *et al.* (1999) undertook a laboratory studies to evaluate the persistence of alpha-endosulfan beta-endosulfan and endosulfan sulfate in four diverse soils under non-flooded and flooded conditions. Maximum persistence was observed in Alfisol and least in Mollisol having near neutral pH and higher organic matter. The fungus *Mucor Thermo-hyalospora* MTCC 1384 was shown to transform endosulfan molecules (Shetty *et al.*, 2000). The identification of endosulfan metabolites revealed the formation of a major non-toxic metabolites, endosulfan-diol and production of a small amount of endosulfan sulfate. The authors concluded that the fungus was involved in both oxidative and hydrolytic pathways for degradation of endosulfan.

Endosulfan could be degraded by *Phanerochaete chrysosporium* under non ligninolytic conditions (Kim, 2001). The major metabolites formed were endosulfan sulphate and endosulfan diol. The degradation was described by using a non linear rate expression which was similar to Michaelis-Menten equation.

2.3.2.3.b. Degradation endosulfan in crop

The persistence and degradation of endosulfan has been studied extensively in various crops. Gopal *et al.* (1988) reported the residues of stereoisomers of endosulfan and its toxic metabolites endosulfan sulphate in aubergin and chickpea. The residues did not exceed the maximum tolerance level on the edible plant parts at the time of harvest.

Ceron *et al.* (1995) investigated the degradation of endosulfan residues in carnation plants in a green house and in carnation plant by product for animal feeds. Higher rates of degradation were measured in the greenhouse cultivated plants. Endosulfan (β -endosulfan) half-lives were greater than those for endosulfan I (α -endosulfan).

Jaglan and Yadav (1988) studied the persistence and degradation of endosulfan in and on unprocessed and processed cauliflower curds. In the case of unprocessed curd, the initial deposits were 14.50-24.15 ppm and at the time of final sampling the insecticide persisted to level of 0.27-0.69 ppm and the half life was reported to be 2.74 days.

Singh *et al.* (1993) reported the degradation of endosulfan residues on sponge gourd and radish. The residues reached the tolerance level in 3.03 days on sponge gourd and in 4.7 days on radish.

2.3.2.4. Degradation of chlorothalonil

Chlorothalonil is a non-systemic foliar fungicide with protective action. It controls many fungal diseases in a wide range of crops, including pome fruit, stone fruit, citrus fruit, vegetables, maize, ornamentals, apples and grapes. In plants chlorothalonil metabolizes to 4-hydroxy-2,5,6-trichloroisophthalonitrile. In aerobic and anaerobic soil studies DT_{50} of chlorothalonil is 5 to 36 days (Pesticide Manual, 10th edition, 1994).

Thirty seven strains of bacteria belonging to various taxonomic positions were examined for the degrading ability of a fungicide chlorothalonil in nutrient broth (Katayama *et al.*, 1997). All of the bacteria degraded chlorothalonil, except *Pimelobacter* sp. A3. On the chlorothalonil-suspended agar, most of the gram positive bacteria grew but many gram-positive bacteria did not grow. No production of $^{14}CO_2$ was observed in the degradation of [1-nitrile- ^{14}C]- chlorothalonil. Methyl thiotrichloroisophthalonitrile and hydroxytrichloro-isophthalonitrile was detected as a minor metabolite.

Mori *et al.* (1998) evaluated the contributions of bacteria and fungi the degradation of chlorothalonil by a selective inhibition method. A long term change in biodegradation of a chlorothalonil in upland soils of Japan were

carried out by Takagi and Wada (1990). They found that in the initial year of field experiment, chlorothalonil disappeared rapidly from the plough layer. In the later period the rate of degradation decreased rapidly and much of the applied chlorothalonil accumulated in the plough layer. However, the addition of manure maintained the high rate of chlorothalonil degradation.

Balasubramanian and Mathan (1996) showed that the rate of chlorothalonil degradation followed a logarithmic pattern and differed between soil types in the order : red soil > alluvial soil > black soil > laterite soil. The chlorothalonil half-lives in alluvial, black, laterite and red soils were 13.0, 12.8, 17.4 and 10.7 days, respectively, when applied at a concentration of $1.0 \mu\text{g g}^{-1}$ and 8.86, 9.36, 8.30 and 8.47 days, respectively at $10.0 \mu\text{g g}^{-1}$.

2.4. Chemical compatibility of pesticides and mixtures

Applying a tank mix pesticides, or a pesticide and a liquid fertilizer can save time, labour, energy and equipment costs. Pesticide combinations usually alter plant absorption and translocation as well as metabolism and toxicity at the site of action of one or more the mixed products. Not all changes are for the better. Negative effect can occur such as reduced pest control, increased damage to non-targets plant phytotoxicity and incompatibility problems between materials.

There are basically four types of interactions that change the efficacy of pesticide combinations.

- a) **Additive effects** : Occurs when mixing two pesticides provide the same response as the combined effects of each material when applied alone. The products neither hurt nor enhance each other. Such mixes save time, labour and equipment use.

- b) **Synergistic responses** : are often confused with additive effects and occur when two pesticides provide a greater response than the added effects of each material when applied separately. Unlike additive effects, the chemicals in a synergistic combination are not neutral toward each other, rather, they interact in some way that increases their effect and may increase control. With true synergism one can often reduce pesticide application rate without sacrificing control. An example would be the addition of piperonyl butoxide (PBO) with the pyrethrum insecticides.
- c) **Antagonism** : When two pesticides applied together produce less control than if applied each materials separately, is called antagonism. In addition to reducing control, antagonistic response also may increase phytotoxicity to plants. For examples, when the herbicides Assert[®] (Imzamehabenz- methyl) and Banverl[®] (dicamba) are mixed together, they negatively affect each others performance.
- d) **Enhancement** is another type of interaction, but not between two pesticides. Enhancement occurs when a pesticide is mixed with an additive to provide a greater response than if applied the pesticide alone. A common example of enhancement is mixing an adjuvant with a pesticide.

Two or more pesticides are compatible if no adverse effects occur as a result of mixing them together. The deactivation of an active ingredient often occurs with chemical incompatibility. This is most affected by temperature, tank pH and length of time that hold a spray mixture in the tank before use. Physical incompatibilities usually involve the inert ingredients of a formulation. The

mixture may become unstable, forming crystals, flakes or sludge that may clog spray equipment.

The possible effects of mixing incompatible pesticides

1. Reduce effectiveness of one or both compounds
2. Precipitate in the tank, clogging screens and nozzles in the sprayer.
3. Plant phytotoxicity, stunting or reducing seed germination
4. Excessive residues
5. Excessive runoff.

As early as 1975, Lovato reported the compatibility of the herbicide propanil with insecticide CL-43064 (cyclic ethylene (diethoxy phosonothiol) dithiocarbamate) in a field trial of rice. He reported the yield 2.45 t grain ha⁻¹ with CL-43064 only, 2.55t with propanil only 2.32 t with CL-42064 + propanil and 2.64 t in the control without insecticide or herbicide. Rates of propanil > 2 l ha⁻¹ mixed with insecticide showed plant toxicity and decreased yields.

The efficacy of different combinations of Dimlin (diflubenzuron) Primicarb and Bayleton (triadimefon) against oriental armyworm (*Mythimna separata*), wheat aphid (*Sitobion avenae*), wheat rust (*Puccinia* sp.) and wheat powdery mildew (*Erysiphe graminis*) on wheat was evaluated in Hennan province of China (Ni *et al.*, 1989). One application of pesticide mixture was sufficient to control the wheat pests and diseases and increased the weight of grain kernels.

To develop a pesticide mixture strategy for managing pyrethroid resistance in the noctuid *Heliothes virescens*, several insecticides (Chlordimeform, methomyl, sulprofos, permethrin, fenvelarate, cypermethrin, EPN, methyl parathion and chlorpyrifos) were tested alone or 2-or 3- way

combinations at full and reduced rates in the laboratory (on cotton terminals) and small plot field experiment on cotton (Luttrell *et al.*, 1991). While several 2- and 3-way mixtures (especially chlordimeform + methomyl + permethrin, and chlordimeform in combination with several pyrethroids) were as effective at reduced rates as full rates of pyrethroids alone, the residual activity of the pyrethroids was often longer than that for the other compounds tested. However, residual insecticidal activity in terminal bud region of cotton is reduced as a result of plant growth which minimizes the effects of differences in pesticide decay rates for insecticides deposited in this region of the plant, the target area for most insecticide applications directed against *H. virescens*, a factor which supported the role of mixtures in resistance management. Although differences in residual activity were observed for the different insecticides studies, some insecticide mixtures still showed good potential for control of pyrethroid resistant populations of *H. virescens*.

To develop pesticide-fertilizer mixture formulations for labour-saving cultivation of paddy rice Lee *et al.* (1997) formulated 6 mixtures with various combinations of active ingredients (isoprothiolane, pyroquilon, flutolanil and hexaconazole), inert materials (binder, white carbon) and N-K fertilizer. A series of laboratory and field tests were carried out to confirm the chemical and biological performance of each. The stability of pesticide active ingredients in the mixture formulations was acceptable and showed as 3.1% to 5.2% degradation rates after 90 days of storage at $50 \pm 2^\circ\text{C}$ under sealed conditions. Rice neck blast (*Magnaportha grisea*) control by mixtures was acceptable, while rice sheath blight (*Rhizoctonia solani*) control as lower than that of conventional practices. When rice was treated with the mixture at the young panicle stage in

paddy in paddy rice, growth and yield of rice were significantly different to that in traditional cropping practices. No phytotoxicity in paddy rice was observed at standard and double dose rates.

Beauvais *et al.* (1999) used cholinesterase activity to monitor exposure of *Chironomus riparius* (Diptera, Chironomidae) to a mixture of insecticide chlorpyrifos, and the herbicides, atrazine and metolachlor in hypoxic wetland mesocosms and in normoxic conditions in the laboratory. Larvae exposed to hypoxia for up to 35 h had no change in cholinesterase (ChE) activity nor did atrazine or metolachlor cause ChE inhibition. There was no significant difference in ChE inhibition between larvae exposed to a mixture of chlorpyrifos, atrazine and atrazine and metolachlor and larvae exposed to chlorpyrifos alone.

Fahmy and Miyata (1998) conducted an experiment to show the joint action of chlorfluazuron + Teflubenzuron and chlorfluazuron + pyriproxifen against two strains of diamond back moth in cotton. They expressed the joint action in terms of co-toxicity coefficient (CTC). In 1994 Dhawan and Simwat observed that polytrin C (mixture of 40 g cypermethrin + 400 g profenophos/L) at 44 g a.i. ha⁻¹ was as effective as cypermethrin alone at 50 g a.i. ha⁻¹ against cotton boll worm.

Ranganathan and Govindan (1996) compared insecticidal mixture polytrin C 44 EC with a few other conventional insecticides viz., cypermethrin 10EC, profenophos 50 EC and endosulfan 35EC against american boll worm in cotton. Their study revealed the superiority of the mixture polytrin in controlling the boll worm infestation and producing more seed cotton yield over other insecticides which were applied individually.

Walnuj *et al.* (1997) reported that combination product Spark 36 EC (Triazophos 35% + Deltamethrin 1%) was significantly superior even at low dose of 1250 mLha⁻¹ than higher doses (2500 mL ha⁻¹) of each of separate insecticide in controlling larval population of diamond back moth. In another experiment the bioefficacy of insecticide mixture in comparison with individual insecticides against american boll worm it was revealed that the treatment with alphamethrin 0.004% + chlorpyrifos 0.08% most effective against registering 93.33% mortality of larvae (Pawar and Mali, 1997). However, the treatment with deltamethrin 0.0025% + endosulfan 0.09% and cypermethrin 0.008% + Chlorpyrifos 0.08% was *at par* with it. Sundararaj *et al.* (1998) reported that the combination product of neem formulation (Fortune aza and Neemgold) with chlorpyrifos gave maximum reduction in larval and adult mortality of *Patialus teomella*. The result showed that Neemgold @ 0.05% had better synergistic effect with 0.1% chlorpyrifos against *P. teomella* than aza.

Gaynor *et al.* (1993) conducted a field trial on sandy loam soil and evaluated the efficacy of metolachlor (1.5-3.0 kg ha⁻¹) alone or in combination with metribuzin (0.375 kg) and /or trifluralin (0.5 kg) or chloramben (3.0 kg) for the control of *Solanum ptycanthum* and annual grasses in tomato. Metolachlor applied pre-plant incorporated (PPI) resulted in excellent (88%) control of both *S. ptycanthum* and annual grasses. Metolachlor + metribuzin + trifluralin tank mix applied PPI or soil incorporated, results in grater weed control than metolachlor + metribuzin. Metolachlor dissipated from the soil throughout the growing season and residues in the fruit were hydrolysed or deacylated or hydrolysed conjugates.

Foy (1993) reported as many as 26 herbicide combination and recommended for use in corn, 23 for soybeans, 13 for peanuts, 4 for sorghums,

4 for small grains, 5 for cotton, 2 for permanent pasture, 1 for tobacco, 13 for free fruits, 8 in turf and 3 in nursery crops.

Kwon and Penner (1995) reported the interaction of insecticides with herbicides activity. They conducted a green house study to evaluate interaction of herbicide and insecticides with /without protectant on corn and weed species. Uesugi (1989) gave a good usage pattern of fungicide and insecticide mixture in Japan for the multiple control of concurrent diseases and insect pests. Etebrian (1992) investigated the symptoms of *Fusarium* with disease and its morphology. He tested 13 fungicides against the pathogen at a concentration of 100 ppm on PDA iprodione + carbendazim, benomyl and carbendazim totally inhibited fungal growth after 10 days. Iprodione + carbendazim, carbendazim and benomyl were most effective at concentration of 10 and 100 ppm.

2.5. Microquantitative analysis of pesticides and their mixture

The principal requirements for chemical analysis of pesticide residues are :

- i) Quantitative extraction
- ii) Efficient clean up
- iii) Sensitive estimation
- iv) Identification of transformation product in soil and plants

The literature on different analytical techniques for the estimation of pesticides encompassing the above steps have been reviewed and presented here :

2.5.1. Extraction and clean up

Use of suitable solvent a mixture of solvents is required to separate quantitatively the minute amount of pesticide and its transformation products, if any, from the substrate. This is then followed by clean up to isolate the toxicant

and its metabolites to make it as free as possible from extraneous co-extractive interfering in quantification.

2.5.1.1. Pendimethalin

Pendimethalin was extracted by shaking it with water (5 mL) and methanol (50 mL) on a wrist-action shaker for 1 h. (Walker and Bond, 1977). The extracts were filtered and water (20 mL) and hexane (10 mL) were added to 25 ml of the clear supernatant. The mixture was shaken vigorously for 2 min, anhydrous Na_2SO_4 (5g) was added to aid separation of the layers and the mixture shaken for a further 30 s. The hexane layers extracts the pendimethalin.

Kulshrestha and Singh (1992) used hexane and ethyl acetate (1:1) for the extraction of pendimethalin and its degradation products. Anhydrous Na_2SO_4 (20g) were added to the soil with the solvent mixture and were shaken for 1h for the extraction.

Smith *et al.* (1995) Raj *et al.* (1999) used acetonitrile, water and glacial acetic acid (80:20:2.5, v/v/v) for the extraction of pendimethalin from soil. The filtrate was diluted with 5% Na_2CO_3 solution and the residue were partitioned with n-hexane.

Kulshrestha *et al.* (2000) extracted the herbicide with methanol + concentrated HCl (98+2 by volume) by shaking on a horizontal shaker. The mixture was then filtered, methanol evaporated and the filtrate was partitioned with hexane for pendimethalin and with hexane + ethyl acetate (1+1 by volume) for pendimethalin degradation products.

2.5.1.2. Imidacloprid

Gajbhiye and Agnihotri (1999) gave the extraction method of imidacloprid from water, plant and soil samples. For water, the samples were

acidified by adding 1 ml concentrated hydrochloric acid. The acidified aqueous phase was partitioned with n-hexane. After discarding the hexane phase, the aqueous phase was made alkaline which was finally partitioned with dichloromethane.

For the extraction of plant samples, acetone was added to the sample and macerated in a waring blender. The acetone was then filtered, concentrated and diluted with 5% aqueous sodium chloride solution and then made it acidic which was then processed similar to water sample.

Soil samples were extracted by dipping it in a mixture of acetone, water and concentrated HCl (80:20:1). The supernatant clear solution was decanted and extraction was repeated twice. The extract was concentrated to remove acetone. The remaining aqueous phase was processed similar to plant samples.

Kumar *et al.* (2000) treated the plant sample and homogenized in a mixer grinder and extracted with acetonitrile + water (80+20 mL v/v, 3 x 25 mL). The extract was centrifuged and solvent was evaporated. The samples after processing were cleaned up by preparative thin layer chromatography on silica gel plates (0.25 mm, 20 x 20 cm) eluting with ethyl acetate. Iodine was used as visualizing agent.

Gajbhiye *et al.* (2000) extracted the imidacloprid from tomato with acetone acidified with acetic acid. After blending, the sample was filtered and the aqueous phase was diluted with 10% NaCl solution and made acidic by adding HCl. It was then made alkaline by adding NaOH and imidacloprid was extracted by partitioning with chloroform which was concentrated to dryness using Kuderna Danish Evaporator (KDE).

Dikshit *et al.* (2001) extracted the imidacloprid from okra fruit with acetone. The extracts were filtered, combined, concentrated and diluted with saturated NaCl solution. The contents were partitioned first with hexane and then hexane-ethyl acetate (98:2). The organic phase was discarded each time and the aqueous phase was extracted with methylene chloride. The methylene chloride was then washed with aqueous potassium carbonates solution and methylene chloride was evaporated. The residue was dissolved in 2-3 ml of ethyl acetate and passed through column containing silica gel over anhydrous Na_2SO_4 using acetonitrile as eluent.

2.5.1.3. Endosulfan

Endosulfan was extracted from plant samples by extracting it with the mixture of n-hexane and isopropanol (2:1) (Singh and Mukherjee, 1994; Tanwar and Handa, 1998; Malathi *et al.*, 1999; Gopichand *et al.*, 1999). The filtered extract was washed with distilled water. The hexane layer was passed through anhydrous Na_2SO_4 . A drop of keeper was added and extract was concentrated. The concentrated extract was dissolved in mixture of 45-50 mL of hexane-acetone (9:1) and a little quantity of Darco G 60 (activated charcoal) was added with occasional shakings. This was filtered through filter paper and residues were washed with 3 x 15 mL hexane : acetone (9:1) mixture.

2.5.1.4. Chlorothalonil

For the extraction of chlorothalonil from the plants the samples are chopped and a representative sample was extracted with acetone. The extract was concentrated in Kunderna Danish Evaporator (KDE) and residues were transferred into methylene chloride. The extract were cleaned by passing through silica gel column (Agnihotri *et al.*, 1995).

Chouhdury and Awasthi (2000) reported the extraction procedure of chlorothalonil from soil in which they used acidified acetone. The supernatant was concentrated using rotary vacuum evaporator at 35°C. To this 120 mL of 0.4 M NaHCO₃ in water was added and pH of the medium was adjusted to 4.5 by 1:1 concentrated H₂SO₄-water and 0.4 M NaHCO₃ solution. The aqueous mixture was partitioned using dichloromethane.

2.5.1.5. Triazophos

Karageorgiev (1981) reported the extraction and analysis of triazophos in apple and pears. The limit of detection was 0.03 mg/kg and recovery ranged 88 to 99%. Raj *et al.* (1999) extracted triazophos from brinjal and okra fruit with acetone. The filtrate was concentrated under reduced pressure below 35°C and the residue were partitioned in dichloromethane layer by treating with 5% aqueous sodium chloride solution. The extract was made moisture free by passing through bed of anhydrous Na₂SO₄.

2.5.1.6. β -cyfluthrin

Philstrom *et al.* (1997) developed a new solid phase extraction method for β -cyfluthrin and same pesticides in water. The sample were extracted using polymer divinyl styrene extraction column and eluted with ethyl acetate.

Mukherjee *et al.* (2002) extracted the β -cyfluthrin insecticides from cotton lint and seed using acetone as solvents and analysed in GLC using ECD detector.

Sinha *et al.* (2002) extracted the pesticide from fungal mycelia with dichloromethane which was dried over anhydrous Na₂SO₄ layer. The extract was evaporated to dryness and taken in hexane-acetone mixture for final analysis.

2.5.1.7. Multiresidue

Beena Kumari *et al.* (2001) reported a cost effective, less time consuming and effective analytical technique for estimation of multiresidues of 30 insecticides. For the extraction of the residues acetone was used as solvent which was further diluted with 10% NaCl solution and finally extracted with ethyl acetate. For the clean up of organochlorine and synthetic pyrethroids the extract was passed through a mixture of florisil (60-100 mesh) and charcoal (5:1 w/w) in between two layers of anhydrous Na₂SO₄. Ethyl acetate and hexane mixture (3:7 v/v) was used as eluting solvent. For organophosphorus pesticides, the clean up was done by passing through a silica gel column using acetone and hexane mixture (3:7 v/v) as the elutant.

2.5.2. Analytical techniques

2.5.2.1. Pendimethalin

The metabolism of AC 92,553 (pendimethalin) in soil, plants and animals (Barringer *et al.*, 1976; Marei *et al.*, 1976; Zulalian *et al.*, 1976) proceeds by both oxidation and reduction to a variety of products AC 92,553 in the predominant residues with the 4-([1-ethylpropyl]amino)-2-methyl-3,5-dinitrobenzyl alcohol the only metabolites of toxicological significance in residue analysis.

Tondreau *et al.* (1977a) developed an automated gas chromatographic procedure that utilizes instrumentation equipped with a liner ⁶³Ni electron capture detector to measure AC 92,553 and its 4-hydroxymethyl metabolite in a variety of agricultural crops. Tondreau *et al.* (1977b) described a gas chromatographic procedures to measure AC 92,553 in environmental samples. Clean up procedures are described for soil, water and fish. After isolation form

the sample matrix, AC-92,553 is qualified by gas chromatography using an electron capture detector.

Bogus *et al.* (1990) utilized solid-phase extraction and reversed-phase ion-pair chromatography (HPLC). The analysis of seven agrochemicals including pendimethalin.

Stan and Linkerhanger (1993) conducted a study to demonstrate the capability of element-specific atomic emission detection (AED) for the screening of plant food stuffs residues by capillary GC. Pendimethalin and several other compounds were suitably analysed by this method. False positive signal interpretation as a result of high concentration levels of eluting matrix compounds was prevented by examination of the compounds was prevented by examination of the elemental composition of peaks by observing the partial emission spectra recorded during GC-runs.

Garcia-Valcarcel *et al.* (1996) determined simultaneously several dinitroaniline herbicides by gas chromatography with ion trap and nitrogen phosphorus detector. The concentrations of these compounds were determined on a HP-1 capillary column and helium was used as carrier gas.

Gas chromatography (GC) with nitrogen-phosphorus detection (NPD) and mass spectrometry (MS), in full scan and tandem (MS-MS) modes to determine 11 pesticides including pendimethalin in aqueous samples (Garrido-Frenich *et al.* 2000). Preconcentration of 500 mL water with C₁₈-catridges enabled the determination of pesticides at ng L⁻¹ levels. The best precision and sensitivity were obtained from GC-NPD and GC-MS-MS. The limit of detection were between 4 and 44 ng L⁻¹ for NPD and 1 and 40 ng L⁻¹ for MS-MS. The application of this methodology to the determination of pesticides in real

T- 7128

samples was tested by analysing wetland samples. The results obtained are compared and the advantage of MS-MS over NPD for highly selective quantitative determination of pesticides in complex matrices was discussed.

2.5.2.2. Imidacloprid

Being a relatively new compound very few reports are available on the residue analysis of this insecticide on the crops. Following methods are reported for determination of imidacloprid residues and its metabolites.

- i) High performance liquid chromatographic (HPLC) method
- ii) Gas liquid chromatographic (GLC) method

2.5.2.2.1. High performance liquid chromatography (HPLC)

Bachlechner (1989) described a method which allow the determination of active ingredient (a.i.) of NTN-33893 (imidacloprid) in soil. The method consisted by extracting the soil with acetonitrile water (80:20, v/v). The extracts were cleaned up by liquid-liquid partitioning on extube columns, eluting the a.i. with dichloromethane. The quantitation was done by high performance liquid chromatography with UV-detection. The mean recoveries of the method were 82 per cent with standard deviation of 10 per cent. The lower limit of detection was 0.01 mg kg⁻¹.

Blab (1990) reported the HPLC method for determination of imidacloprid residues in apple, potatoes, sugarbeets (including leaves), corn, barley. The compound was extracted from non-fatty materials using acetonitrile and from materials of low water and high fat contents, the water-acetonitrile mixture was used. Aqueous extract was partitioned with n-hexane and n-hexane-ethylacetate mixture to remove chlorophyll type material and dichloromethane extract was partitioned with aqueous KOH solution to remove the acidic co-

extractives. The final clean-up was done over florisil column and quantitation of residues by reverse-phase (RP)-HPLC with UV-detection at 270 nm using acetonitrile-water gradient. The recoveries ranged from 77-102 per cent at fortification levels of 1.0, 0.04, 0.02 and 0.01 mg kg⁻¹ depending on the materials to be analysed.

A method for determining residues of imidacloprid (parent compound and total residues) was described by Placke and Weber (1993). The method involved three step cleanup with XAD resin, Chemelut and florisil columns.

A rapid, sensitive and reliable HPLC method was developed for the determination of imidacloprid residues in nine crops (Ishii *et al.*, 1994). The method consisted of extraction with acetonitrile-water (80:20, v/v), prewashing of concentrated extracts with cyclohexane and alkaline solution followed by silica gel column chromatography and quantification was done by HPLC. The recoveries of imidacloprid were 75-109 per cent. The limits of determination of the methods were 0.005, 0.01 and 0.02 mg kg⁻¹ for rice, straw and soil, respectively.

Fernandez-Alba *et al.* (1996) described a HPLC method with diode array detection (DAD) for determination of imidacloprid residues in vegetables at levels ranging from 0.01-0.60 mg kg⁻¹. Spectral data obtained with DAD allowed the identification of imidacloprid residues. The mean recoveries and standard deviations were 95 per cent and 4.7 per cent, respectively in various crops.

Valverde *et al.* (1996) evaluated the supercritical fluid extraction (SFE) method for imidacloprid along with other organochlorine insecticides from pepper and tomato. Mixture of vegetable samples and anhydrous magnesium sulphate was taken in the ratio of 5:7 to carry out the extraction and analysed

by HPLC-DAD at 210 nm. Under chosen conditions imidacloprid could not be extracted as efficiently as with conventional methods. For the insecticides recoveries were more than 80 per cent from spiked samples.

2.5.2.2.2. Gas liquid chromatography (GLC)

Placke and Weber (1993) reported a GLC method for determination of imidacloprid in crops. In this method the compound was oxidised to 6-chloronicotinic acid which was then silylated with N-methyl trimethyl silyl trifluoroacetamide. Total residues (imidacloprid and its degradation products) were estimated as imidacloprid and analysed on GLC using ECD.

Rouchaud *et al.* (1994, 1996) reported a method for estimation of imidacloprid in soil and plants and its metabolites in soil. The soil and plant samples were extracted in acetonitrile-water (80:20) and cleaned-up by silica gel TLC. The samples were analysed by GLC after trifluoroacylation or methylation with trifluoroacetic anhydride or diazomethane on a glass column (1.80 m x 2 mm, i.d.) with 5 per cent SE 30 on Gas Chrom Q, 80-100 mesh at 225°C oven temperature. The recoveries at fortification levels of 0.1 mg kg⁻¹ for soil and plant samples ranged 83 to 96 per cent. The sensitivity was 0.01 mg kg⁻¹ for dry and fresh sugarbeet leaves.

Navalon *et al.* (1997) reported a method for determination of imidacloprid involving hydrolysis in basic medium followed by gas chromatography mass spectrometry and selected ion monitoring. The hydrolysed product was extracted with chloroform, clean up was not necessary when the selected ion monitoring mode was used. The method was applicable for the determination of imidacloprid in vegetables (tomato, cucumber, capsicum and green beans). Recoveries ranged between 94.3 to 105.8 per cent.

2.5.2.3. Endosulfan

Gas chromatographic methods are most useful for the determination of endosulfan residues in vegetables or other substrates (Mitchell, 1976; Verma 1975; Singh and Kavadia, 1989; Gopal and Mukherjee, 1993). Begliomini *et al.* (1978) used gas chromatographic methods for determination of endosulfan residues in bees. Raju and Gupta (1991, 1992) described a simple spectrophotometric method to determine the endosulfan residues in river water and soil. Galeano *et al.* (1992) reported a new and rapid method for resolution and analytical determination of mixtures of alpha- and beta- endosulfan by reverse phase high performance chromatography. A simple, rapid and reproducible method for determination of endosulfan and some other pesticides residues in water was developed using micro liquid-liquid extraction and gas chromatography-mass spectrometry with selected ion monitoring (GC/MS-SIM).

2.5.2.4. Chlorothalonil

Suzuki and Oda (1977) described a simultaneous determination of chlorothalonil, captan and diazinon in apples. Electron capture detection was used for chlorothalonil with 2 ppb limit of detection.

Gas liquid chromatography (GLC) is mostly used for the determination of chlorothalonil in different substrates (Murakami and Tanaka, 1985; Nabarawy and Carey, 1988; Valverde Garcia *et al.*, 1993; Doorn *et al.*, 1995; Vargyas *et al.* 2000). Jongen *et al.* (1991) determined chlorothalonil by HPLC using UV-detector. Lawruk *et al.* (1995) developed a competitive enzyme immunoassay for the quantification of chlorothalonil in water and agricultural products. Yeung and Newsome (1995) developed a sample preparation procedure for the determination of chlorothalonil in food commodities with a commercial immunoassay kit. An indirect competitive enzyme-linked immunosorbent assay

(ELISA) was used to determine photochemically cutin-bound residues of chlorothalonil in enzymatically isolated tomato and apple fruit cuticles (Jahn and Schwack, 2001). In fruit cuticles that were irradiated for 8h by simulated sunlight 0.030 and 0.068 mg/g photo induced cutin-bound residues of wax-free, cuticles (calculated as chlorothalonil) were determined for tomatoes and apples, respectively.

2.5.2.5 Triazophos

Karageorgiev (1981) gave a gas chromatographic method for determining triazophos in tree fruits. Uygun (1997) investigated the determination method of triazophos and some other pesticides using gel permeation chromatography. However, Parrila *et al.* (1997) used a multivariate calibration method for the simultaneous determination of the pesticides folpet, procymidon and triazophos in mixture, by high performance liquid chromatography with photodiode array detection (HPLC-DAD).

2.5.2.6. Beta-cyfluthrin

Beta-cyfluthrin is a synthetic pyrethroid pesticides and it is generally analysed by injecting the samples in capillary gas chromatographic column (Philstrom *et al.*, 1997) using various detector among which the most common is ECD (Mukherjee *et al.*, 2002).

Sinha *et al.* (2002) analysed beta-cyfluthrin using Hewlett-Packard LIC fitted with a glass column (2 m x 2m) packed with 3% SE-30 coated an 80-100 mesh chromosorb-WHP, an electron capture detector (^{63}Ni) and computer operated integrator.

2.5.3. Multiresidues techniques used for extraction and analysis of pesticides from tomato

These methods are of great interest for laboratories being involved in food quality control analysis for consumer protection. The multiresidue extraction methods for different pesticides from tomato are given in Table 1.

Table 1. Multiresidue technique adapted to estimate pesticides in tomato.

Pesticides	Detection technique	References
Methamidophos, chlorpyrifos, endosulfan and procymidon	Supercritical fluid extraction (SFE)	Valverde Garcia A, Fernandez Alba AR (1995)
Pyraclofos	GC-FPD/OV-17, GC-TSD/SE-30 GC/MSD-SIM mode	Valverde <i>et al.</i> (1997)
Carbofuran, carbosulfan, and Furathiocarb	Multiresidue-TLC method	Balinov <i>et al.</i> (1997)
Bifenthrin, fenpropathrin, cyhalothrin, permethrin, cypermethrin, fluvalinate, fenvalarate and deltamethrin	GLC-ECD	Gau Fang <i>et al.</i> (1995)
Chlorothalonil, vinclozolin, diclofluanid, triadimefon, penconazole, chlozolate, captan, procymidone, triadimenol, folpet, hexaconazole, myclobutanil, cyproconazole, nuarimol, captafol, iprodione, fenarimol, bitertanol and tetradifon	Capillary gas chromatography	Sannino <i>et al.</i> (1999)
Methamidofos, chlorpyrifos, captan, chlorothalonil, endosulfan, lambda-cyhalothrin, and copper-oxychloride	GLC	Seguera and Abakerli (1999)
Seventy seven pesticides (12 organohalogen, 45 organonitrogens, 11 organophosphorus and 9 pyrethroids)	GPC followed by GC-ECD and MS	Gelsomino <i>et al.</i> (1997)
Twenty one organophosphorus pesticides	Graphitized carbon powder (GCP) method	Satoh <i>et al.</i> (2001).

MATERIALS AND METHODS

3.1 Materials

3.1.1. Chemicals and reagents

The technical and analytical grade pesticides under study were supplied by different companies as given below in Table 2.

Table 2. Pesticides used in the experiment and their suppliers

Pesticide	Purity (%)	Supplied by
Pendimethalin	99	M/S Cynamid (India) Ltd.
Endosulfan	98	M/S Excel (India) Ltd.
Chlorothalonil	99	M/S Sandoz (India) Ltd.
Imidacloprid	99	M/S Bayer (India) Ltd.
Triazophos	98	M/S AgrEvo (India) Ltd.
β -cyfluthrin	98	M/S Bayer (India) Ltd.

The analytical grade (AR) pesticides were found to be chromatographically (TLC, GLC and HPLC) pure. The structure of the respective compounds were further confirmed by the spectroscopic techniques ($^1\text{H-NMR}$ and FT-IR).

Other reagents and chemicals required for the study were also analytical grade. The solvents used were laboratory grade, purified and distilled before use. The solvents used in HPLC were HPLC grade.

3.2. Field experiment

Studies on interaction and persistence of pesticides in tomato (*Lycopersicon esculentum* Mill.; var. Pusa Ruby) were based on the field experiment conducted in collaboration with the Division of Agronomy in Main

Block (Mid B/B) at the farm of Indian Agricultural Research Institute (IARI), New Delhi during *rabi* season 2001-2002 using split-plot design (SPD).

3.2.1. Nursery raising

Seeds of tomato var. Pusa Ruby was purchased from seed selling counter of seed corporation of India, Pusa Campus, New Delhi-110012. The seeds were sown in the nursery bed on 14.10.02. The seedlings were protected by against rains and other extreme climatic conditions. For the further growth of the plant NPK was added as fertigation @ 20-20-20 at 1.5g/litre.

3.2.2. Method of transplanting

a. Root dip treatment : The seedlings of untreated seeds were uprooted and the roots were washed with tap water and gently dried with filter paper. The roots of these plants were dipped in imidacloprid and chlorothalonil (individual and combinations) solution (1%) for one hour. The plants were taken out and excess solution was allowed to drain out.

b. One month old tomato seedlings were transplanted from the nursery to the plots (2.5 m x 2.5m) in the main field on 14th November 2001. The spacing between plant to plant and row to row was maintained at 50 cm and in each plot 25 seedlings were transplanted. Usual agronomic practices were following the tomato crop watering was done immediate after transplanting.

3.2.3. Layout of the experiment

Crop	:	Tomato (<i>Lycopersicon esculentum</i> Mill).
Variety	:	Pusa Ruby
Main-plot treatments	:	2
Sub-plot treatments	:	23
Replications	:	3
Plot size	:	2.5 m x 2.5 m

Rows in each plot	:	5
Row to row distance	:	50 cm
Plant to plant distance	:	50 cm
No. of plants/plot	:	25
Design	:	Split Plot Design (SPD)
Soil type	:	Sandy loam

3.2.4. Fertilizer application at the time of transplanting

N	:	120 Kg ha ⁻¹ (as urea)
P ₂ O ₅	:	40 Kg ha ⁻¹ (as SSP)
K ₂ O	:	40 Kg ha ⁻¹ (as MOP)

3.2.5. Irrigation

About half an inch of irrigation every fortnight, total 15 irrigations.

3.2.6. Method of pesticide application

The quantity of different pesticides required for three replications were calculated based on active ingredients (a.i.) present in the commercial products, diluted with water and sprayed uniformly using a Knapsack sprayer. At volume rate equivalent to 500 L ha⁻¹. The rate of application of different pesticides is given in Table 3.

Table 3. Commercial product, type and rate of application of various experimental pesticides

Pesticides	Commercial product	Rate of application (kg a.i. ha ⁻¹)
Pendimethalin	Stomp [®]	1.000
Endosulfan	Thiodan [®]	0.350
Chlorothalonil	Kavach [®]	0.500
Imidacloprid	Confidor [®]	0.020
Triazophos	Hostathion [®]	0.700
β-cyfluthrin	Bull Dock [®]	0.0125

The different treatments of pesticides is given in Table 4. Only the herbicide pendimethalin was applied as pre-plant at a day before transplanting i.e., 13.11.2001. The chlorothalonil and imidacloprid were used for root dipping on the day of transplanting and as spray at the flowering stage (Module-I) (Plate I). Endosulfan, triazophos and β -cyfluthrin were sprayed onto the crop at flowering at higher infestation of fruit borer and white fly (Module II). To infer the result related to pendimethalin, hand weeding and untreated controls (three replicates each) were included in the experiment.

Table 4. Different treatments used for the root dip/spray in the tomato field.

Sl. No.	Treatment no.	Treatments name
1.	T ₁	Foliar spray of imidacloprid
2.	T ₂	Root-dip treatment of imidacloprid
3.	T ₃	Foliar spray of chlorothalonil
4.	T ₄	Root-dip treatment of chlorothalonil
5.	T ₅	Foliar spray of endosulfan
6.	T ₆	Sequential root dip treatment of imidacloprid followed by chlorothalonil
7.	T ₇	Sequential spray of imidacloprid followed by chlorothalonil
8.	T ₈	Root-dip treatment in mixture of imidacloprid + chlorothalonil
9.	T ₉	Foliar spray of mixture of imidacloprid + chlorothalonil
10.	T ₁₀	Sequential spray of imidacloprid, chlorothalonil followed by endosulfan
11.	T ₁₁	Foliar spray of mixture of imidacloprid + chlorothalonil + endosulfan
12.	T ₁₂	Sequential root-dip of imidacloprid - chlorothalonil (transplanting) and sequential spray imidacloprid – chlorothalonil – endosulfan (flowering)
13.	T ₁₃	Root dip in mixture of imidacloprid + chlorothalonil (at transplanting) and foliar spray of mixture of imidacloprid + chlorothalonil + endosulfan at flowering
14.	T ₁₄	Foliar spray of triazophos
15.	T ₁₅	Foliar spray of β -cyfluthrin
16.	T ₁₆	Foliar spray of mixture of β -cyfluthrin + endosulfan
17.	T ₁₇	Foliar spray of mixture of endosulfan + triazophos
18.	T ₁₈	Foliar spray of mixture of β -cyfluthrin + triazophos
19.	T ₁₉	Sequential spray of triazophos-endosulfan- β -cyfluthrin
20.	T ₂₀	Foliar spray of mixture of endosulfan + triazophos + β -cyfluthrin
21.	T ₂₁	One hand weeding
22.	T ₂₂	Weed free
23.	T ₂₃	Control



Plate 1. Pesticides applied in tomato field

Table 5. Physico-chemical properties of IARI farm soil

Properties	Values
Mechanical analysis	
Sand (%)	65.3
Silt (%)	17.5
Clay (%)	17.2
Textural class	Sandy loam
Bulk density (g cm^{-3})	1.47
Field capacity (%)	16.0
Chemical composition	
Organic carbon (%)	0.34
Available nitrogen (kg ha^{-1})	104.72
Available phosphorus (kg ha^{-1})	7.86
Available Potassium (kg ha^{-1})	177.32
Physical parameters	
pH (Soil : Water = 1:2.5)	7.2
Electrical conductivity (dSm^{-1})	0.32
Cation exchange capacity [$\text{Cmol (p}^+) \text{ kg}^{-1}$]	7.30

3.2.7. Field operations

Manual weeding was done regularly in weed free plots with the help of *khurpi* in the hand weeding plots. First weeding was done 20 days after transplanting followed by another weeding after 60 days.

Fertilizers (nitrogen, phosphate and potash) were applied in the form of urea, single super phosphate and muriate of potash at the rate of 60, 40 and 40 kg ha^{-1} , respectively at the time of transplanting. Remaining 60 kg N ha^{-1} was applied at 4 weeks after transplanting after the first irrigation.

A total of 15 irrigations were given during the entire growth period of the crop. The first irrigation was given on 30.11.02 followed by on 15.12.02, and remaining irrigations were given at a 15 days intervals.

3.2.8. Climate and weather conditions

Delhi is situated at 28°40'N latitude and 77°12'E longitude at an altitude of 228.16 meters above mean sea level. The climate of Delhi is sub-tropical semi arid. The important weather parameters during cropping period are presented in Figure 1. The weather data on weekly interval are summarized in Annexure I.

3.2.9. Field observations recorded

3.2.9.1. Crop-plant population

The number of plants in each plot were counted 15 days after transplanting. There should be 25 plants per every 2.5 x 2.5 m plots. The gap was filled up with healthy seedling.

3.2.9.2. Insect-pests

The insect pests infecting the tomato plants were counted by tagging 5 plants randomly chosen from each plot and average number of infestation per plant was calculated. For counting of the aphids and white flies 30 cm twigs from the top of plants were considered. The infestation of the fruit borer (*Helicoverpa armigera*) was counted as whole plant basis.

3.2.9.3. Diseases

The disease scoring was done in the same way as insect pest infestation by tagging the plants. The mean value was taken for further calculations. The disease scoring was started one month after the transplanting when the infestation was observed.

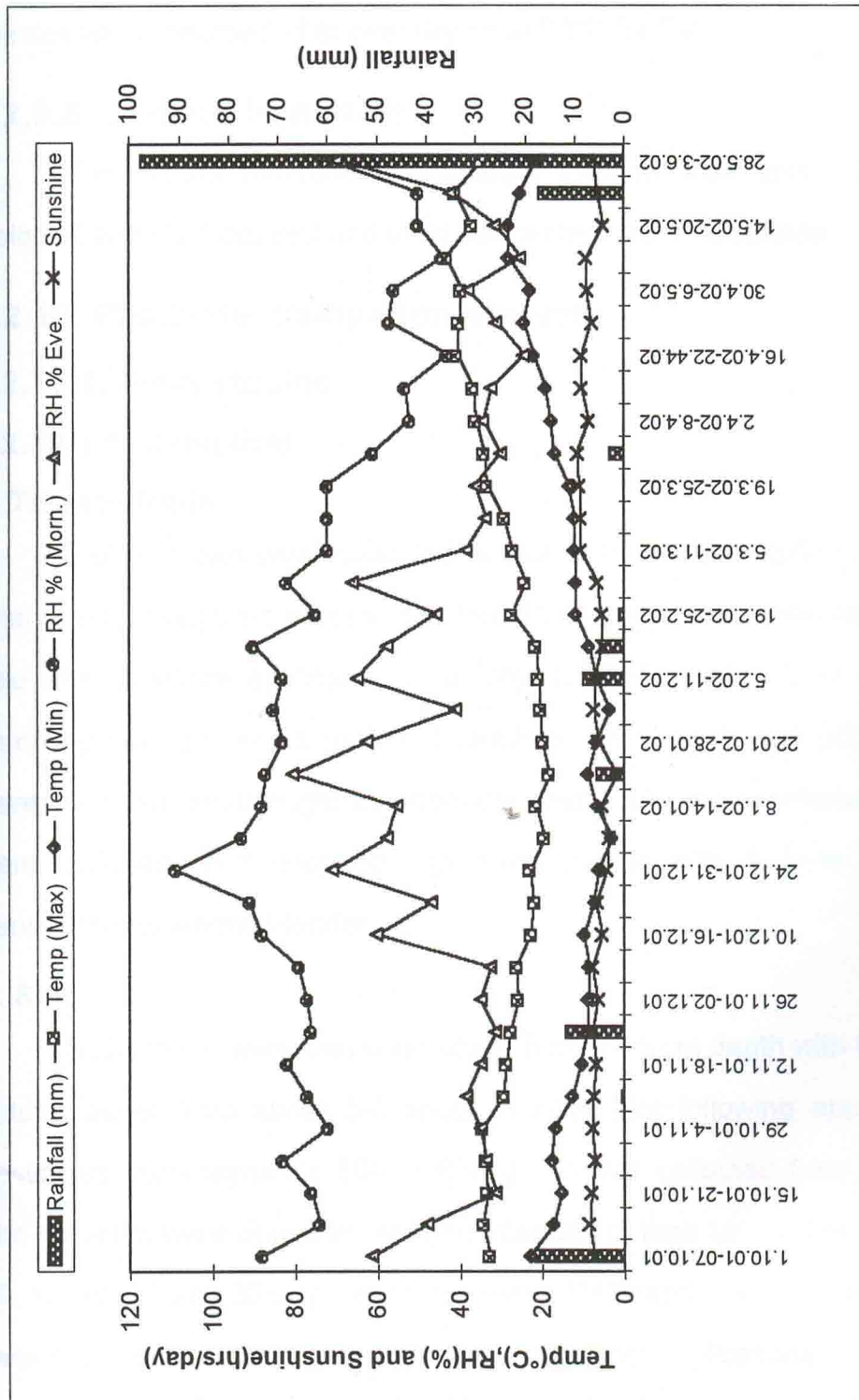


Fig. 1. Meteorological observation of the period during field experiment

3.2.9.4. Weeds

Weed counting was done at 30, 60 days after transplanting and at harvest (90 days) with the help of quardrets. Dry weights of different weed species were recorded after over drying at 60°C for 24h.

3.2.9.5. Tomato fruit yield

The tomato harvested in different pickings from each plots were weighed and yield per plot and yield per hectare were calculated.

3.2.10. Pesticide dissipation studies

3.2.10.1. Field studies

3.2.10.1.1. Sampling

a. Tomato fruits

Fruit samples were collected randomly from each replications and a total of six pickings were done. The tomato samples were collected from the field after pesticide application of 0 (2h), 1, 3, 5, 7, 10, 15, 20 days. The sampling was continued further if required. From each plot 50g samples were collected and brought to laboratory where 50g representative samples were weighed, and chopped into small pieces with a knife and then transferred to waring blender.

b. Soil

Soil sample were drawn randomly from 0-15 cm depth with the help of a tube auger from about 5-6 spots in each plot following application of pesticides. Approximately 500 to 600 g soil was collected from each plot. The samples were drawn at different intervals of time i.e., 0 (2h), 1, 3, 5, 7, 10, 15, 20, 30 and 60 days after treatment (DAT) and at harvest (90 DAT) in case of pendimethalin application from all the three replications.

Each of the collected samples was thoroughly mixed, air dried, ground in pestle and mortar and was passed through 2 mm sieve. Finally, a 50 g representative sample was taken for quantitative analysis.

3.3. Laboratory studies

3.3.1. Soil dissipation studies

Soil samples collected from the control plots were used for the soil dissipation studies in the laboratory. Six sets (6x2) of conical flasks 500mL were taken and 250g of untreated soil (control soil) was kept into it. Four individual pesticides viz., imidacloprid, endosulfan, chlorothalonil and pendimethalin and one mixture of all of these were treated with soil at 10 ppm level in duplicate and incubated at the room temperature. A control in duplicate was also maintained under identical condition in the laboratory. The field capacity of the soil was maintained by adding water time to time and through mixing it with a glass rod. A control flask was also taken with same amount of soil and same quantity of acetone was added into it instead of pesticide solution. Ten grams of soil sample was taken out from each of the flask with the interval of 0 (2h), 1, 3, 5, 7, 10, 15, 20 and 30 days and extracted with suitable solvent as mentioned in the section.

3.3.2. Adsorption studies

In order to study the adsorption of different pesticides and their mixtures in soil, 1g of finely ground soil sample was taken in 25 mL stoppered test tube and treated with 10 mL of (0.01 N CaCl_2) aqueous solution of pesticides of varying concentration (0, 1, 5, 10, 15 and 25 $\mu\text{g mL}^{-1}$) in duplicate. Blank samples (without soil) containing 10 mL of 0.01 N CaCl_2 (aqueous) solution of pesticides at above mentioned concentration were also used for the study. The contents were equilibrated for 24 h by shaking on horizontal shaker. They were then centrifuged at 2000 rpm for 30 minutes.

The supernatants were decanted off and 2 mL aliquots were drawn from each sample for analysis of remaining pesticides in the solution. Three pesticides viz., pendimethalin, imidacloprid and chlorothalonil were studied for their adsorption in soil.

The pattern of adsorption of these pesticides in soil was studied using Freundlich adsorption isotherm.

Freundlich relationship for calculating adsorption isotherm is as follows :

$$x/m = KC^{1/n}$$

Where ,

x/m = weight of substance adsorbed per unit of adsorbent ($\mu\text{g g}^{-1}$)

C = equilibrium concentration

K and n = the empirical constant depending upon the nature of adsorbent, adsorbate and temperature.

The parameters ' K ' and ' $1/n$ ' are thought to provide rough estimates of adsorbent capacity and the intensity of adsorption, respectively (Adamson, 1967).

The amount of herbicides adsorbed was calculated by the formula.

$$A = (C_0 - C) v/m$$

Where,

A = Amount of pesticide adsorbed per unit of adsorbent ($\mu\text{g g}^{-1}$)

C_0 and C = Equilibrium concentration of the pesticides after equilibration with no adsorbent and with adsorbent, respectively ($\mu\text{g mL}^{-1}$)

V = volume of solution

M = weight of adsorbent

3.3.3. Soil degradation studies

3.3.3.1. Rate kinetics in sterile and non-sterile soils

To study the degradation rate of pesticides in sterile and non-sterile soils, two sets of duplicate 20g air-dried soil samples (IARI farm soil, alluvial) were placed in 100 mL Erlenmeyer flask and moistened to 80 per cent of field capacity. One set of Erlenmeyer flasks were plugged tightly with cotton and sterilized by autoclaving at 110°C and 15 psi pressure for 30 minutes in an autoclave. The flasks were removed and sterilized again after 72 hours by autoclaving. The process was repeated once more. The contents were cooled in the laminar flow chamber and soil in each flask was treated with 2 mL acetone solution of pesticides ($10 \mu\text{g mL}^{-1}$) and incubated at $28 \pm 1^\circ\text{C}$ in the incubator. The soil in each of the flask of the second set was also treated with 2 mL acetone solution of pesticides ($100 \mu\text{g mL}^{-1}$) and incubated in BOD incubator at $28 \pm 1^\circ\text{C}$. The samples were drawn at 0, 5, 10, 10, 20 and 30 days intervals for the analysis of pesticide residues as per the procedure described in section 3.4.2. Blank sterile and non-sterile soil samples were also incubated under identical conditions.

3.3.3.2. Rate kinetics of pesticides in nutrient broth

Czapek Dox medium containing 2.5 g of NaNO_3 , 0.5g KCl, 1.0g K_2HPO_4 , 0.5g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 30.0g sucrose and 0.01 of FeCl_3 in 1 litre of distilled water (pH 7.0) was used as fungal broth culture. Erlenmeyer flasks (500 mL) containing medium (250 mL) were autoclaved separately at 110°C and 15 psi for 0.5 hours. The autoclaved liquid medium (10 mL) was transferred to a series of pre-autoclaved Erlenmeyer flasks (50 mL). The content of each flask was amended with pesticides ($50 \mu\text{g}$) inoculated with 1 mL of soil inoculum and incubated in BOD at $28 \pm 1^\circ\text{C}$. The untreated inoculated (UI) and pesticide treated uninoculated (TUI) control were also

incubated in the experiment. Duplicate flasks from each control and treatment were withdrawn initially and then at intervals of 0 (2 h), 5, 10, 15, 20 and 30 days and degradation ceased by adding organic solvent. The contents were transferred to separatory funnel and partitioned with a mixture of dichloromethane and ethyl acetate (1:1, 40 mL). The organic phase was separated and extraction of aqueous phase was repeated twice more with the same solvent mixture (2 x 20 mL). The pooled solvent extracts were dried by passing through anhydrous sodium sulphate and evaporated to dryness using rotary evaporator (40°C).

3.3.4. Effect of pesticides and their mixture on soil microorganism

In order to study the effect of pesticides on soil microorganisms soil was collected from each plot from at least 5 spots from two depths 0-7.5 cm and 7.5-15 cm at 0, 3, 10, 20 and 30 days following pesticides and their mixture application. Soil from each plot was mixed thoroughly and air-dried. A 10g air-dried soil was taken for microbial count. Nutrient agar and Martin-Rose Bengal agar (Table 6) were used to estimate soil bacteria and fungi-counts, respectively.

Table 6. Composition of different media used for microbial population counts

	Media	Composition	Amount
i)	Nutrient agar	Beef extract	3.0g
		Peptone	5.0g
		Agar	15.0g
		Distilled water	1000 mL
ii)	Rose-Bengal agar	Glucose	10.00g
		Peptone	5.00g
		KH ₂ PO ₄	1.00g
		MgSO ₄ , 7H ₂ O	0.50g
		Streptomycin	30.00mg
		Agar	15.00
		Rose-Bengal	0.035g
		Distilled water	1000 mL

Nematode counts was done by taking the soil on double layer of tissue paper supported on wire gauze for screening.

3.3.5. Synthesis of possible degradation products of pesticides

3.3.5.1. Pendimethalin

I. N-(1-ethylpropyl)-3,4-dimethyl-2-nitrobenzene-1,6-diamine (I)

A solution of pendimethalin (I, 3.5g) in absolute ethyl alcohol (120 mL) was treated dropwise with 35 mL of 20% aqueous ammonium sulphide solution with constant stirring at room temperature. The mixture was further stirred overnight and then partitioned between aqueous sodium chloride and methylene chloride. The residue was dissolved in warm hexane upon cooling compounds was obtained as deep red crystals, m.p. 79-81°C, yield 82%, Rt 2.615 min, IR 3478, 3385 (NH₂), 1535, 1350 (NO₂) cm⁻¹, MS : 251(M⁺); 222 (M⁺ - C₂H₅); ¹H-NMR (CDCl₃) δ : 0.85 (f, 6H, 2x CH₂CH₃); 1.30 (q, 4H, 2x CH₂CH₃); 2.10 (d, 6H, 2x CH₃); 3.0 (m, 1H-CH); 3.75 (bs, 2H, exchangeable) with D₂O, NH₂); 6.60 (s, 1H, Ar-H).

Elemental analysis found ; C, 63.12; H, 8.14%, calculated for C₁₃H₂₁N₃O₂; C, 62.15; H, 8.36%.

Gas chromatography of the mother liquor revealed presence of another compound (though in small quantity) in addition to compound I. This additional compound, however, could not be isolated by column chromatography. The GC-MS of the mixture gave two peaks on GC at Rt 3.60 and 4.58 min showing following mass spectra.

MS : m/e 251 (M⁺, 68%), m/e 222 (M⁺-C₂H₅, 100%), Rt 4.58 min.

Identical mass spectra of the two compounds suggested latter compounds to be the isomeric diamine (N-(1-ethylpropyl)-3,4-dimethyl-6-nitrobenzene-1, 2-diamine (a)). The fact that the compound I is produced in

higher yield than compound (a) supports the structural assignment of compounds II and (a) as resulting from the reduction of less and more sterically hindered groups, respectively.

II. 3,4-dimethyl-2,6-dinitroaniline (II)

To a solution of pendimethalin (I, 0.5g) in ethyl alcohol (25 mL) was added 10 mL of aqueous potassium hydroxide solution (20%). The mixture was heated on a boiling water bath for 8 hrs. Then the reaction mixture was cooled, poured over crushed ice and neutralized with dilute hydrochloric acid. It was extracted with ethyl acetate, dried over sodium sulphate and distilled. The residue thus obtained, was purified by column chromatography over silica gel using benzene as eluent. Compound II was obtained as orange red needles when crystallized from benzene.

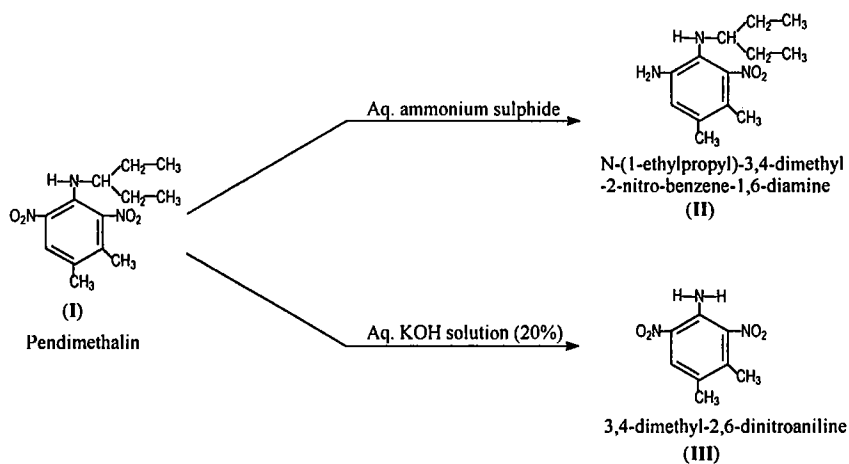
m.p. 142-143°C

Yield, 66%

IR : 3450, 1530, 1348 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3) δ : 2.30 and 2.36 (each 3, each 3H, $2\times\text{CH}_3$) 8.10 (s, 1H, Ar-H); 10.68 (bs, 2H, exchangeable with D_2O , NH_2).

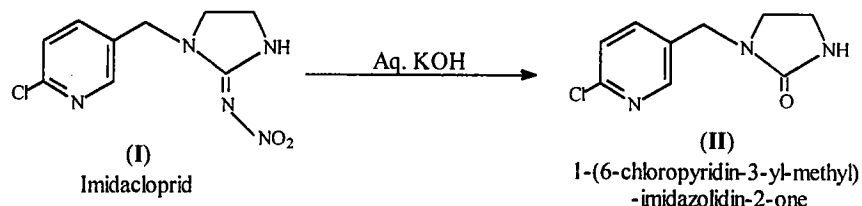
Elemental analysis found : C, 45.68 H, 4.13% calculated for $\text{C}_8\text{H}_9\text{N}_3\text{O}_4$; C, 45.52, H, 4.26%.



3.3.5.2. Imidacloprid

I. 1-(6-chloropyridin-3-yl-methyl) imidazolidin-2-one

Imidacloprid (1g) was refluxed with aqueous potassium hydroxide (100 g L^{-1} ; 60 mL) for 2 h. The reaction mixture was then cooled, neutralized with hydrochloric acid, and extracted with dichloromethane. Concentration and recrystallization with hexane + acetone yielded colourless crystals, mp 172°C , R_f (benzene + acetone, 90+10 ; 0.23). IR $\nu_{\text{max}} \text{ cm}^{-1}$ 3314 (NH), 1680 (CO); PMR (CDCl_3) δ 3.30 (m 4H N-CH₂-CH₂-N), 4.42 (s, 2H CH₂), 5.42 (bs 1H, NH), 7.33-8.20 (m Ar-H)



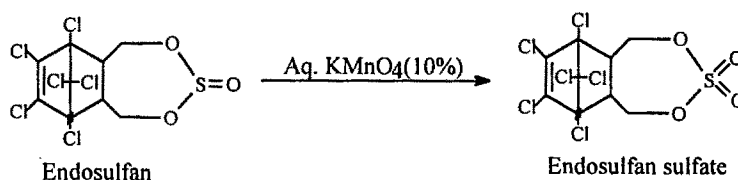
3.3.5.3. Endosulfan

3.3.5.3.1. Separation of α - and β -endosulfan from technical endosulfan

Technical endosulfan consists of 70% α - and 30% β -isomers. The two isomers can be easily separated by column chromatography by taking different fractions. A slurry of silica gel was prepared in hexane. Silica gel was preheated at 120°C for 30 min for activation. A glass column (45 cm $\times$ 3 cm i.d.) was taken and plugged with cotton boll and the silica gel slurry was poured into the column. Two gram of technical endosulfan was taken and dissolved in hexane and then added onto the column. The column was eluted with hexane and fraction of 25 mL each were collected. The fraction were concentrated immediately and subjected to TLC. This fractions eluted α -endosulfan. For β -endosulfan the eluting solvent was 25% benzene in hexane.

3.3.5.3.2. Preparation of endosulfan sulphate

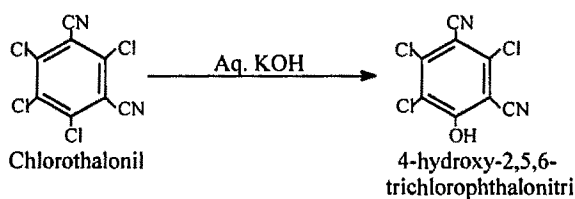
Endosulfan sulphate is an oxidation product of endosulfan. For the preparation of sulphate 1g α -endosulfan was taken and dissolved in small quantity of acetone and 10% aqueous KMnO_4 (10 mL) was added into it. The solution was stirred on magnetic stirrer for 2h at room temperature. The solution was then filtered and washed with sodium bisulphite solution. Finally the endosulfan sulphate was extracted from the solution by the extraction with ethyl acetate.



3.3.5.3. Chlorothalonil

a. Alkaline hydrolysis

Alkaline hydrolysis of chlorothalonil was done using 1% methanolic KOH at room temperature. For that 1 g of chlorothalonil was taken and dissolved in small quantity of acetone and methanolic KOH (1%, 5 mL) was added into it. The content were kept on magnetic stirrer for 2 h. The reaction mixture was diluted with water and acidified with dilute HCl to make it neutral and then extracted with ethyl acetate. The organic layer was collected and evaporated under rotary evaporator under reduced pressure and the solid obtained was recrystallized from ethanol + water. R_f (benzen + acetone 95+5; 0.5).



3.4. Quantitative analysis of pesticides and mixtures

Three most important steps involved in pesticide residue analysis are extraction, clean-up and estimation.

3.4.1. Tomato fruit

3.4.1.1. Extraction

Fruit samples (50g) from treated and control plots were chopped into small pieces and blended in waring blender using suitable solvent/solvent mixture (100 mL) for particular pesticide or their mixture (Section 3.4.4.1). The contents were then filtered through filter paper (Whatman 42). The extraction procedure was repeated twice more (2 x 50 mL) with solvent and filtered. The solvent was then reduced by evaporating in a rotary vacuum evaporator. The combined concentrated filtrate was then diluted with 10% NaCl solution and partitioned with dichloromethane/ethyl acetate. The organic phase then concentrated on the rotary evaporator and transferred into a test tube and volume was made prior injection into GC or HPLC.

3.4.1.2. Cleanup

Except endosulfan treated samples, other samples were thoroughly cleaned up by column chromatography using different adsorbents (Table 7).

Table 7. Adsorbents used for the clean up of pesticides

Pesticides	Adsorbent
Imidacloprid	Neutral alumina
Chlorothalonil	Silica gel
Pendimethalin	Silica gel
Mixture	Florisil/Silica gel

Five gram of adsorbent was packed sandwiched between anhydrous sodium sulphate (2g) on both sides in a glass column (45cm x 1.5 cm i.d.). The concentrated fruit extract was mixed-up with small amount of adsorbent

to make it a free flowing mixture and transferred carefully to the top of the column. The column was then eluted first with hexane (200 mL) followed by 2 per cent acetone in hexane (200 mL). Fractions (50 mL each) were collected separately and concentrated to about 5 mL using rotary evaporator. In case of imidachloprid the eluting solvent was hexane + acetone (9:1) and the adsorbent was neutral alumina.

3.4.2. Soil

3.4.2.1. Extraction

Soil samples collected from the field after treatment were air-dried and sieved through 2.0 mm sieve. To the soil sample (50 g) in an Erlenmeyer flask 100 mL of solvent (as required for different pesticides mentioned in Table 10.) was added. The flask was stoppered and shaken on horizontal shaker for 30 minutes. Replicates were taken and one of the flask was taken from the control soil. For the extraction of chlorothalonil and imidacloprid acidified acetone was used where as for pendimethalin simply acetone was used. After shaking the contents of the flasks were filtered through buchner funnel with filter paper Whatman 42 using a pump. The extraction was done twice more (50 mL, each time) with the same solvent and filtered in the same way. The combined filtrate concentrated and diluted with 10% NaCl solution and then partition with dichloromethane. The solvent was evaporated on rotary vacuum evaporator to dryness. The residue was dissolved in suitable solvent, transferred into a test tube and volume made prior injection into GC or HPLC.

3.4.2.2. Clean up

Extracts of soil samples required no additional cleanup.

3.4.3. Water

3.4.3.1. Extraction

Aqueous sample (100 mL) was transferred to a 250 mL separatory funnel. To it 50 mL of saturated sodium chloride solution was added. The aqueous solution was partitioned with 50 mL of dichloromethane. The organic phase containing pesticide was passed through anhydrous sodium sulphate. The aqueous phase was partitioned twice more with the solvent and passed through anhydrous sodium sulphate. The extracts were combined and solvent evaporated to dryness using a rotary evaporator at 50°C.

3.4.3.2. Clean up

Extracts of aqueous samples required no additional clean up.

3.4.4. Extraction of multiresidues

3.4.4.1. Tomato fruits

The mixture of endosulfan, imidacloprid and chlorothalonil (module I) and endosulfan, triazophos and β -cyfluthrin (module II) were extracted from tomato fruits and cleaned using the following procedure.

a. Extraction

The fruits samples (50g) were chopped into small pieces and blended in Waring blender using a acetone as solvent and the remaining procedure was same as mentioned in case of soil (section 3.4.2.1.). The concentrated extract (10 mL) was used for clean up.

b. Clean up

i) For Pendimethalin, Imidacloprid, chlorothalonil, and endosulfan mixture.

A glass column (60 cm x 22 mm i.d.) was packed with 5g mixture of Florisil (60-100 μ m) and activated charcoal (5:1, w/w) in between two layers

of sodium sulphate. The column was prewashed with hexane (50 ml), loaded with the concentrated extract and eluted with 125 mL mixture of ethyl acetate : hexane (3:7, v/v). The eluate was concentrated to almost dryness and final volume was made upto 2 mL with ethyl acetate : n-hexane (3:7, v/v) prior injection in GC.

ii) For endosulfan, triazophos and β -cyfluthrin mixture.

The column is loaded with half (5ml) of the concentrated extract and eluted with a mixture of ethyl acetate : hexane (3:7 v/v). The second half (5mL) of the extract was passed through a column packed with 5g adsorbent mixture of silica gel (60-230) and activated charcoal (5:1, w/w) and eluted with 125 mL mixture of acetone : hexane (3:7 v/v). The eluate was dried over anhydrous sodium sulphate, concentrated and final volume was adjusted to 2 mL with acetone : n-hexane (3:7, v/v) prior injection in GC/HPLC.

3.4.4.2. Soil

The mixture of imidacloprid, chlorothalonil and pendimethalin in soil was extracted and cleaned up using following methods.

a. Extraction

Soil sample were collected from the field and was air-dried and sieved through 2 mm sieve. A 50g sample was taken in an Erlenmeyer flask and was extracted with 100 mL acetone by mechanical shaking for 1h. There were three replicates for treated and one for control soil. The content was filtered through filter paper (Whatman No. 42). The extraction was repeated twice more (50 mL in each time) with acetone and filtered in the same way. The combined filtrate was then concentrated by evaporating the solvent on a rotary vacuum evaporator and transferred into separatory funnel diluted with 10% NaCl and extracted with ethyl acetate followed by dichloromethane (50, 25, 25 mL). The individual organic phase was concentrated and

combined under reduced pressure and finally volume analysis was made up for GC/HPLC.

b. Clean up

Extracts of soil samples required no additional clean up.

3.5. Calibration of pesticides

3.5.1. Individual pesticides

Fifty mg of individually pesticides taken in 50 mL volumetric flask was dissolved in suitable solvent and made upto the mark to obtain a stock solution. A containing $1000\mu\text{g a.i. mL}^{-1}$. A 10 mL aliquot from the stock solution A was transferred to a 100 mL volumetric flask and was diluted to the mark with the solvent. This gave stock solution B containing $100\mu\text{g a.i. mL}^{-1}$. A 10 mL aliquot from stock solution B was transferred to 100 mL volumetric flask and the volume was made to the mark with acetone. This gave stock solution C containing, $10\mu\text{g a.i. mL}^{-1}$. From stock solution C, 0.1, 0.2, 0.5 and 1.0 mL aliquots were transferred to separate 10 mL volumetric flasks and the volume was made to the mark to give 0.1, 0.2, 0.5 and $1.0\mu\text{g a.i. mL}^{-1}$ solutions, respectively.

A $3\mu\text{L}$ aliquot of each of the solutions containing 0.1, 0.2, 0.5 and $1.0\mu\text{g a.i. mL}^{-1}$ pesticide were injected into GLC. Each run was duplicated and detector response was measured in terms of peak areas. Calibration curves for different pesticides was prepared by plotting concentrations of pesticides in $\mu\text{g mL}^{-1}$ on X-axis against peak area on Y-axis.

For imidacloprid, the working concentrations of 1, 5, 10, 25, $50\mu\text{g mL}^{-1}$ were injected in HPLC and calibration curve was prepared in the same manner, described earlier.

Different solvents used for the preparation of standard solutions are given in Table 8.

Table 8. Solvent used for the preparation of standard solutions of different pesticides

Pesticides	Solvent used for preparation of stock solution
Pendimethalin	n-hexane
Imidacloprid	Methanol
Endosulfan	n-hexane
Chlorothalonil	Acetone
Triazophos	Acetone
β -cyfluthrin	Acetone
Pendimethalin+Imidacloprid+ chlorothalonil + endosulfan [PICE mixture]	n-hexane
Endosulfan + triazophos + β -cyfluthrin [ET β mixture]	n-hexane

3.5.2. Pesticide mixture

Standard solution of individual pesticide of 5 and 10 ppm were made in 10 mL volumetric flask as mentioned in section 3.4.1.1. From this individual solution 1 mL each of solution was taken in a 25 mL volumetric flask and the volume was made up to the mark with distilled hexane. This gave concentration of 0.4 μg of each pesticide in the standard mixture. Two replicates were taken with two other concentration (0.1 and 0.2 $\mu\text{g mL}^{-1}$) in the same manner. A 3 μL aliquot of each of the solutions 0.1, 0.2 and 0.4 $\mu\text{g mL}^{-1}$ pesticide were injected in GLC. Each run was duplicated and detector response was measured in terms of peak areas. Calibration curves of individual pesticides in the mixture was prepared by plotting the concentrations of pesticide in $\mu\text{g mL}^{-1}$ on x-axis against peak area on Y-axis.

For the estimation of Imidacloprid higher concentration (5 ppm) was taken so that it can be injected to HPLC and calibration curves was made in the same manner mentioned above.

3.6. Recovery studies

3.6.1. Individual pesticide

Laboratory experiments were carried out to see the efficacy of extraction, clean up and determinative steps followed in the analysis using fortified substrates viz., tomato fruit, soil or aqueous media. The control (untreated) samples were spiked with known amounts of pesticides. Various solvents or their mixtures were tried for extraction and different adsorbents were taken for clean up. The instrumental conditions were also optimized to get accurate and précised results. Overall the best combinations were adopted for residue analysis.

3.6.1.1. Preparation of stock solution

The analytical pesticides were weighed and dissolved in suitable solvent in a volumetric flask (50 mL) and diluted upto mark with the solvent to get $2000 \mu\text{g L}^{-1}$ (2 mg L^{-1}) solutions. From this solution serial dilutions were made to obtain the desired concentrations of 1000, 100 and 50 mg L^{-1} (ppm).

3.6.1.2. Fortification substrates

a. Tomato fruit

Tomato fruits were chopped into small pieces and representative sample (50g) were taken into Erlenmeyer flasks and spiked at different levels (Table 9). The samples were then incubated at room temperature for $\frac{1}{2}$ h for uniform distribution of pesticides prior analysis.

b. Soil

Replicate air dry soil samples (50g) collected from top 15cm soil layer were sieved to pass through a 2 mm sieve and spiked at the levels mentioned in Table 9. The fortification were done in Erlenmeyer flasks with thorough mixing. After 1h, 3-4 drops of aqueous ammonia were added to each flask and stirred with a glass rod. The flasks were kept undisturbed until the smell of ammonia was disappeared. The control samples were fortified with same amount of acetone. Three replicates were taken for each treatment.

c. Czapek-Dox media

Required amount of media fortified with different levels (Table 9) are shaken slowly for uniform distribution of pesticides.

Table 9. Level of pesticide fortification in substrates for recovery study

Pesticides	Substrates	Concentration ($\mu\text{g g}^{-1}$)
Pendimethalin	Soil	0.1, 0.2, 0.4, 0.5, 0.7, 1.0,
	Tomato fruit	0.1, 0.5, 1.0
	Minimal slat medium	0.2, 1.0, 2.0
	Czapek Dox medium	0.2, 1.0, 2.0
Imidacloprid	Soil	5, 10, 25, 50
	Tomato fruit	5, 10, 25, 50
	Czapek Dox medium	5, 10, 25, 50
Endosulfan	Soil	0.1, 0.2, 0.4, 0.5, 0.7, 1.0,
	Tomato fruit	0.1, 0.5, 1.0
	Czapek Dox medium	0.2, 1.0, 2.0
Chlorothalonil	Soil	0.1, 0.2, 0.4, 0.5, 0.7, 1.0,
	Tomato fruit	0.1, 0.5, 1.0
	Czapek Dox medium	0.2, 1.0, 2.0
Triazophos	Tomato fruit	0.1, 0.5, 1.0
β -cyfluthrin	Tomato fruit	0.1, 0.5, 1.0

3.6.1.3. Extraction and clean up of fortified samples

The extraction and clean up techniques for different pesticides for fortified samples are given below (Table 10).

Table 11. Extraction and clean-up techniques of different pesticides

Pesticide	Substrate	Solvent/solvent mixture	Extraction method	Clean-up
Pendimethalin	Soil	Acetone	Shaking, partitioning with dichloromethane	Silica gel column clean-up
	Media	Acetone	Partitioning with dichloromethane	Silica gel
Imidacloprid	Soil	Acetone	Shaking, partitioning with dichloromethane	Neutral alumina column clean-up
	Tomato fruit	Acetone	Shaking, partitioning with dichloromethane	Neutral alumina column clean-up
	Media	Acetone	Partitioning with dichloromethane	Neutral alumina column clean-up
Endosulfan	Soil	Hexane-isopropanol (2:1, v/v)	Washing with 2% brine solutions followed by discarded of isopropanol aqueous layer	Cleaned-up with Darco G 60 in n-hexane-acetone (9:1)
	Tomato	Hexane-isopropanol (2:1, v/v)	Washing with 2% brine solutions followed by discarded of isopropanol aqueous layer	Cleaned-up with Darco G 60 in n-hexane-acetone (9:1)
	Media	Hexane-isopropanol (2:1, v/v)	Washing with 2% brine solutions followed by discarded of isopropanol aqueous layer	Cleaned-up with Darco G 60 in n-hexane-acetone (9:1)
Chlorothalonil	Soil	Acetone	Shaking, partitioning with dichloromethane	Neutral alumina column clean-up with 1% acetone in hexane
	Tomato	Acetone	Shaking, partitioning with dichloromethane	Neutral alumina column clean-up with 1% acetone in hexane
	Media	Acetone	Shaking, partitioning with dichloromethane	Neutral alumina column clean-up with 1% acetone in hexane
Triazophos	Tomato	Acetone	Shaking, partitioning with dichloromethane	Neutral alumina column clean-up with 1% acetone in hexane
β -cyfluthrin	Tomato	Acetone	Shaking, partitioning with dichloromethane	Neutral alumina column clean-up with 1% acetone in hexane

3.6.2. Recovery of multiresidues

Recoveries of multipesticides were carried out by processing the spiked samples (soil, tomato fruits, culture media) with the mixture of

pesticide containing imidacloprid, pendimethalin, chlorothalonil and endosulfan and β -cyfluthrin, endosulfan and triazophos. The chopped and macerated 25 g samples (25g) of tomato was spiked with 1 mL of above.

For soil, tomato and the aqueous extracts the level of fortification were 0.1, 0.5, 1.0 and 2.0 for all the pesticides except Imidacloprid which was spiked at the level of 5, 10, 25 and 50 $\mu\text{g g}^{-1}$.

The method of extraction and clean up of the multiresidues has been mentioned in section 3.4.4.2.

3.7. Estimation

3.7.1. Gas liquid chromatography (GLC)

Gas liquid chromatographic technique was used for the quantitative estimation of pesticides (pendimethalin, endosulfan, chlorothalonil, triazophos, and β -cyfluthrin) and their metabolic products. Gas chromatograph model Shimadzu GC-17A was used for the analysis.

3.7.1.2. Chromatographic conditions

The stationary phase consisted 3 per cent of OV 17 coated on 530 μ mesh silica megabore column (30m x 0.53 mm i.d.). The mobile phase was Nitrogen gas maintained at a flow rate of 30 mL min^{-1} . The detector was electron capture detector (ECD) for all the pesticides except triazophos which was detected by FTD (flame thermoionic detector). The column, injector port and detector temperature for various pesticide are described in Table below (Table 11). A 3 μL of sample solution was injected each time and chromatogram were recorded on computer printer recorder. The instrument had a microprocessor controlled data system that allowed automatic calculation of detector response in terms of peak area. One unit area was equal to 10 μV second of analogue input.

Table 11. GLC condition used for different pesticides

Pesticide	Temperature (°C)			Detector Used
	Column	Injector	Detector	
1. Pendimethalin	210	230	300	ECD
2. Endosulfan	210	230	300	ECD
3. Chlorothalonil	190	220	300	ECD
4. Triazophos	240	260	300	FTD
5. β -cyfluthrin	260	280	300	ECD

3.7.1.3. High performance liquid chromatography (HPLC)

A high performance liquid chromatographic technique was used for the quantitative estimation of imidacloprid and its metabolic products.

a. Instrument

A Hewlett-Packard HPLC instrument (Series 1100) equipped with a degasser, quaternary pump, diode-array detector connected with, loop injection system and a computer (Model Vectra) was used for the analysis of imidacloprid.

b. Chromatographic condition

The stationary phase consisted of Lichrosphere 1000 RP-C₈ packed stainless column (250 mm x 4 mm i.d.). The mobile phase was acetonitrile water (70:30, v/v) maintained at a flow rate of 1.0 mL min⁻¹. The diode-array detector was operated at a wavelength of 270 nm ($\lambda_{\max}$). A 20 μ L volume of sample was injected each time and chromatogram recorded in Windows' 95 NT based HP Chemstation programme.

3.7.1.4. Estimation of multiresidues by GLC/HPLC

a. Gas liquid chromatography (GLC)

The samples were analysed by Gas Chromatograph, Model Shimadzu GC 17A, equipped with electron capture detector (ECD) and nitrogen

phosphorus detector (NPD). Endosulfan, chlorothalonil, and β -cyfluthrin, pendimethalin, were estimated by GLC equipped with ECD fitted with megabore column (30m x 0.53 mm i.d.). Nitrogen was used as carrier gas at linear gas velocity of 30 mL min⁻¹. Though column and make up gas 60 mL min⁻¹. The injector port was maintained at and the oven temperature was 190°C for 6 min, then @ 10 °C min⁻¹ to 270°C for 5 min. All other gas chromatographic conditions remaining same except column oven temperature. Triazophos was detected on NPD using the same column. The flow rate was also the same. The injector port was maintained at 240°C. The oven and detector temperature was 270°C and 280 °C, respectively.

For the pesticide estimation first individual reference standards and then standard cumulative solutions were injected.

b. High performance liquid chromatography (HPLC)

Following the GLC analysis, the same sample was used for the HPLC analysis. The solvent was removed from the sample to dryness and then proper dilution was done with acetonitrile. A 20 μ L aliquot of the sample was injected through the Rheodyne injector in HPLC. The mobile phase used for the estimation of Imidacloprid was acetonitrile : water (70:30 v/v) at a flow rate of 1.00 mL min⁻¹. The photodiode array UV detector with a RP-C₈ column gave the λ_{max} at 270 nm for Imidacloprid.

3.8. Compatibility

Interaction of pesticides when applied sequentially or simultaneously were evaluated by mixing and incubating at room temperature in the analytical/technical compounds in organic solvent, formulation product and/or formulation products diluted with water.

Pesticides are applied in tomato crop depending upon the insect pests, disease and weeds infestation during the early growth period or at

the time of flowering. The pesticides were, therefore, categorized into two group as follows :

1. Pendimethalin, imidacloprid, chlorothalonil and endosulfan (PICE)
2. Endosulfan, triazophos and β -cyfluthrin (ET β)

In order to study the compatibility of two batches of pesticides which were taken for the application on the tomato crop were selected. One batch was applied at the early stage of the crop growth (as preemergent, or root dip application or sprayed the other batch was applied as spray after flowering stage (or the fruiting stage) of the crop.

3.8.1. Compatibility of pesticides mixture in standard solution

Standard solution of 10 ppm was prepared from the each individual pesticides. From this standard 1 ml of each of solution was taken into a 25 mL volumetric flask and the volume was made upto 25 mL mark with hexane except for Imidacloprid (CH_3OH). Likewise three different concentrations were prepared [(0.2, 0.4, 0.4 and 5 ppm for pendimethalin, chlorothalonil, endosulfan and imidacloprid respectively (for module I) and 0.4, 0.5 and 0.4 ppm for endosulfan, triazophos and β -cyfluthrin (for module II)] 25 mL volumetric flask. A 3 μ l of standard was injected to GC (in HPLC injection volume, 20 μ l) and observed the changes peak area in the standard solution when there was no interaction no extra peak was found other than the individual peaks. The operational parameters of GLC and HPLC is mentioned in Table 16.

3.8.2. Compatibility of pesticides formulation mixture

3.8.2.1. PICE

Two sets of 250 mL of volumetric flask were taken and to each flask 0.3mg Kavach[®] 75 WP (Chlorothalonil), 0.7 mg Thiodan[®] 35EC, 1.4 mg

Confidor® (Imidacloprid) and 0.8 mg of Stomp® (pendimethalin) formulation was added. The volume was then made up to 250 mL mark with water. The formulations were then shaken proper by holding it on hand and kept in observations.

- i) There was no or very little frothing on it
- ii) There was very little sedimentation which may be due to the additive in WP formulations of chlorothalonil
- iii) There was uniformity in colour

Likewise different combinations of formulations were made and kept for the observation. The different combinations are given below:

Pendimethalin + Imidacloprid
 Imidacloprid + Chlorothalonil
 Chlorothalonil + Endosulfan
 Endosulfan + Pendimethalin
 Pendimethalin + Chlorothalonil
 Imidacloprid + Endosulfan
 Pendimethalin + Imidacloprid + Chlorothalonil
 Imidacloprid + Chlorothalonil + Endosulfan
 Chlorothalonil + Endosulfan + Pendimethalin
 Endosulfan + Pendimethalin + Imidacloprid
 Pendimethalin + Endosulfan + Chlorothalonil + Imidacloprid

It was observed that the formulation are stable in the formulation upto 30 days of study.

3.8.2.2. ET β mixture

Two replicates of 250 mL volumetric flask was taken and three formulation was taken in to make the following combination. A 0.286 ml of Thiodan® was mixed with 5 mL of Bulldock® (β -cyfluthrin) and 0.25 mL of Hostathion® which was diluted to 250 mL mark by water to obtain the 10ppm

equivalent concentration mixture. This was further diluted for the lower concentrations. The combination was first subjected to compatibility study and then study for chemical interaction.

Endosulfan + Triazophos
 Triazophos + β -cyfluthrin
 β -cyfluthrin + Endosulfan
 Endosulfan + Triazophos + β -cyfluthrin

The through observation on the mixture revealed that the formulation was miscible in the mixture. There was no frothing, no sedimentation or no break-up the formulations. For the indepth study of compatibility chemical interaction study was done.

3.8.2.3. Chemical interactions of pesticide formulation

To study the chemical interaction of the pesticide formulations, 2 mL of each mixture formulation was taken at different intervals (0 (2h), 1, 3, 7 and 10 days) and diluted with distilled water. The diluted formulation was extracted with dichloromethane. The dichloromethane layer was passed through the 3-5 cm layer of anhydrous Na_2SO_4 . The solvent was then evaporated in a rotary vacuum evaporator. The dried sample was then diluted with suitable solvent for analysis in GLC or HPLC.

3.9. Analysis of active ingredients in the commercial formulations

Based on the a.i. content in the formulation, solutions were prepared in acetone to contain 1 mg mL^{-1} concentration. The solutions were injected in GLC. A methanol solution of imidacloprid (1 mg mL^{-1}) formulation was injected in HPLC. The quantity of pesticide (a.i.) present in the commercial product were calculated using the following formula :

$$Y = \frac{A \times C}{B} \times \frac{V}{W} \times 100$$

Where,

Y = Active ingredient in the commercial formulation (%)

A = Peak area of sample solution

B = Peak area of standard solution

C = Concentration of standard solution ($\mu\text{g m L}^{-1}$)

V = Volume of the sample extracts (mL)

W = Weight of the sample (g)

3.10. Sample analysis

Concentrated sample (soil, plant or water) extracts were dissolved in hexane (or hexane : acetone, 4:1) separately. A 3 μL aliquot of standard solution or sample extract was injected in GLC whereas, a 20 μL methanol aliquot was injected in HPLC. The retention time, peak areas of the standards and sample extracts were recorded.

The quantity of residues in the sample were calculated using following equation:

$$Y = \frac{A \times C}{B} \times \frac{V}{W}$$

Where,

Y = Concentration of pesticide residue in the substrate ($\mu\text{g g}^{-1}$)

A = Peak area of sample solution

B = Peak area of standard solution

C = Concentration of standard solution ($\mu\text{g m L}^{-1}$)

V = Volume of the sample extracts (mL)

W = Weight of the sample (g)

3.11. Interpretation of residue data

3.11.1. Regression equation

Theoretically, the pesticide residues or deposits decrease logarithmically, since the amount lost per unit time is proportional to the total amount present at a particular time, provided the entire quantity of residues is equally exposed to the different agencies of dissipation such as weathering, metabolism and degradation reactions etc. (Hoskins, 1961).

Therefore, the regression analysis between the time elapsed and quantity of residues of pesticides can be described by first order under model as:

$$Y = a + bX$$

$$\text{or } \log C_t = \log C_o + kt$$

Where,

Y = log of residues concentration (C_t) at anytime (t or x) in days after application of pesticide

k or b = Dissipation rate constant or slope of regression equation

C_o = Initial deposit concentration at zero time

a = $\log C_o$ i.e., log of initial deposit.

Alternatively, the pesticide disappearance can be written as the first-order exponential model

$$C_t = C_o e^{kt}$$

$$C_t = C_1 e^{-k_1 t} + C_2 e^{-k_2 t}$$

Where,

C_1 = Initial deposit obtained for fast dissipation phase

C_2 = Initial deposit obtained by extrapolating the line to zero time for slow dissipation phase.

k_1 and k_2 = Dissipation rate constant for fast and slow phase

The residue data of the individual pesticides were subjected to regression analysis and the regression equation were calculated.

3.11.2. Half life (RL_{50} or $T_{1/2}$)

For calculating $T_{1/2}$ for biphasic dissipation, the rate constant (k) will have two values one value for first phase of dissipation and the other for second phase of dissipation.

$$T_{1/2} = \log 2/k = 0.301/k$$

3.11.3. Safety interval (T_{MRL})

T_{MRL} is minimum time (in days) required for pesticide residues to reach maximum residue limit (Hoskins, 1961).

$$T_{MRL} = \frac{\log C_0 - \log (MRL)}{k}$$

3.11.4. Maximum possible intake (MPI)

The MPI value is defined as the maximum permissible intake of insecticide and can be calculated as per the expression given below:

$$MPI = ADI \times \text{Average body weight}$$

Where ADI = Acceptable daily intake.

3.11.5 Theoretical Maximum Residue Concentration (TMRC)

TMRC values were calculated by the following formula.

$$\begin{aligned} TMRC &= \text{Daily consumption of food commodity} \times \text{Residue level (mg kg}^{-1}\text{)} \\ &= \text{Food factor} \times \text{Daily food intake} \times \text{Residue level (mg kg}^{-1}\text{)} \end{aligned}$$

$$\text{Where, Food factor} = \frac{\text{Contaminated food consumed}}{\text{Total daily food intake}}$$

3.12. Qualitative analysis of pesticides by the thin layer chromatography (TLC)

Slica gel pates (20 x 5 cm) were prepared by coating glass plates with silica gel (E. Merck, Type 60) containing 13% binder using TLC applicator.

The thickness of silica gel layer was 250 μ . The plates were activated by placing them in oven at 110°C for 1h.

All the sample extracts were pooled and solvent was evaporated to approximately 0.5 mL by passing cool-air from hair-dryer. The sample solution along with standard solution of individual pesticides and its metabolites were simultaneously spotted on the activated plates at 2 cm from the bottom of the plate, with the help of micro pipettes. The plates were developed by placing in a glass chamber containing acetone : hexane (1:1, v/v), hexane : benzene (1:1 v/v) and/or benzene. When the solvent front moved upward to about 12 cm from the bottom the plates were removed, air-dried and visualized by exposing them to iodine in the chamber.

3.13. Structure characterization and confirmation of pesticides

The structure assessment and confirmation of all the pesticides was done by (i) Nuclear Magnetic Resonance (NMR) and (ii) Gas-Chromatographic-Mass Spectroscopy (GC-MS).

3.13.1. Nuclear Magnetic Resonance Spectroscopy (NMR)

¹H-NMR spectra of different pesticides were obtained in deuterated solvents in a Varian Model EM-360L, 60 MHz instrument in the Division of Agricultural Chemicals, using TMS as internal standard.

3.13.2. Gas Chromatography-Mass Spectroscopy (GC-MS)

A HRGC-MEGA-2 series gas chromatograph coupled to a FISON TRIO, 1000 ion trap mass spectrometer connected with a Panasonic KX-P 1150 multi-mode printer was used. The ionization potential was 70 eV.

RESULTS AND DISCUSSION

4.1 Standardization of analytical methods

The two most important steps involved in the standardization of the methodology for residue determination of pesticides include :

- a. Development and/or standardization of analytical technique and
- b. Recovery experiments with the substrates

4.1.1. Standardization of GLC technique for residue analysis

4.1.1.1. Analytical technique

Efforts were directed for the development of a GLC technique for estimation of pesticides. The possibility of detection examined by different detectors is given in the section of materials and methods (Table 12).

The analysis using GLC fitted with electron capture detector (ECD) was highly satisfactory for the quantitative analysis of pendimethalin, endosulfan, chlorothalonil and β -cyfluthrin. However, Triazophos gave response to flame thermoionic detector (FTD) only rather than ECD. The essential pre-requisites for the development of GLC technique are choice of suitable column and selection of a suitable oven temperature for good resolution of the compounds for detection by ECD and FTD.

4.1.1.2. Selection of suitable column

Megabore column (30 m x 0.53 mm i.d.) containing 3 per cent OV-17 as coating material most satisfactory for the analysis of pesticides.

4.1.1.3. Choice of suitable column temperatures

Column temperatures were varied for different compounds mentioned in Table 12 for the separation of the compounds from their co-extractives with distinct peaks (Figure 2a to 2e).

Table 12. Column temperatures suitable for different pesticides and their respective retention time (Rt) values

Sl. No.	Compounds	Column Temperature	Detector	Rt
1.	Pendimethalin	210°	ECD	2.772
2.	Endosulfan	210°	ECD	4.465(α) 5.839(β)
3.	Chlorothalonil	190°	ECD	3.001
4.	β -cyfluthrin	260°	ECD	3.630
5.	Triazophos	240°	FTD	2.359

4.1.1.4. Preparation of standard curve

The peak areas corresponding to various concentrations ranging from 0.1 to 2.0 $\mu\text{g mL}^{-1}$ solutions of different pesticides are presented in the Table 13a to 13f.

The calibration graphs of concentrations versus peak areas for these pesticides have been shown in Figure 3a to 3e. The curves show linearity over the range from 0.1 to 2.0 $\mu\text{g mL}^{-1}$ concentration of pesticides. The sensitivity of the GLC method for these pesticides are presented in Table 15.

Table 13a. Calibration of pendimethalin by GLC technique

Concentration ($\mu\text{g mL}^{-1}$)	Replicate	Peak area		Average peak area
		R ₁	R ₂	
0.1	2	68184	68327	68255.5
0.2	2	112521	113578	113049.5
0.5	2	352351	349711	351031.0
1.0	2	519526	538421	528973.5
1.5	2	690235	692020	691127.5
2.0	2	869725	883344	876534.5

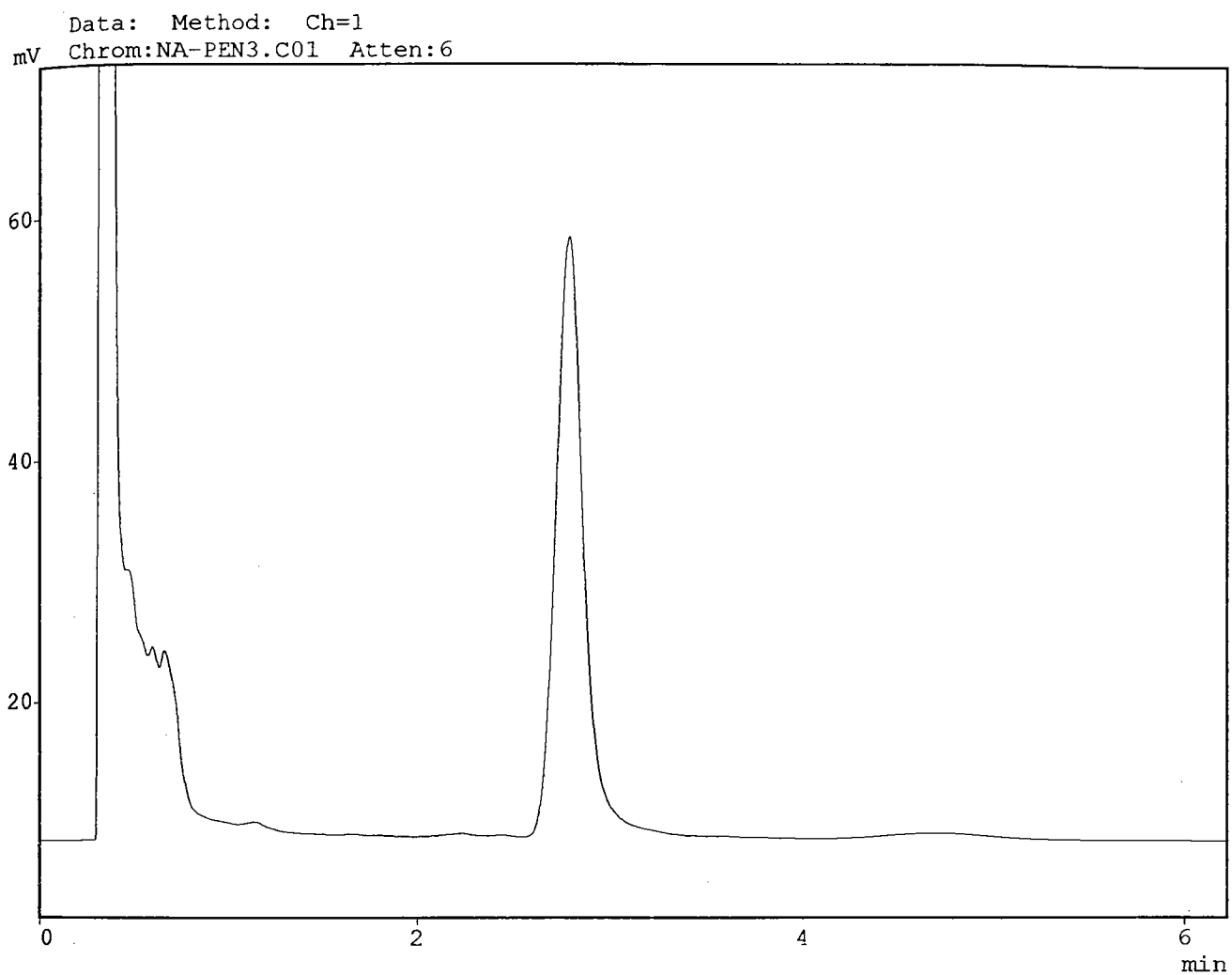


Fig. 2a. GLC chromatogram of pendimethalin

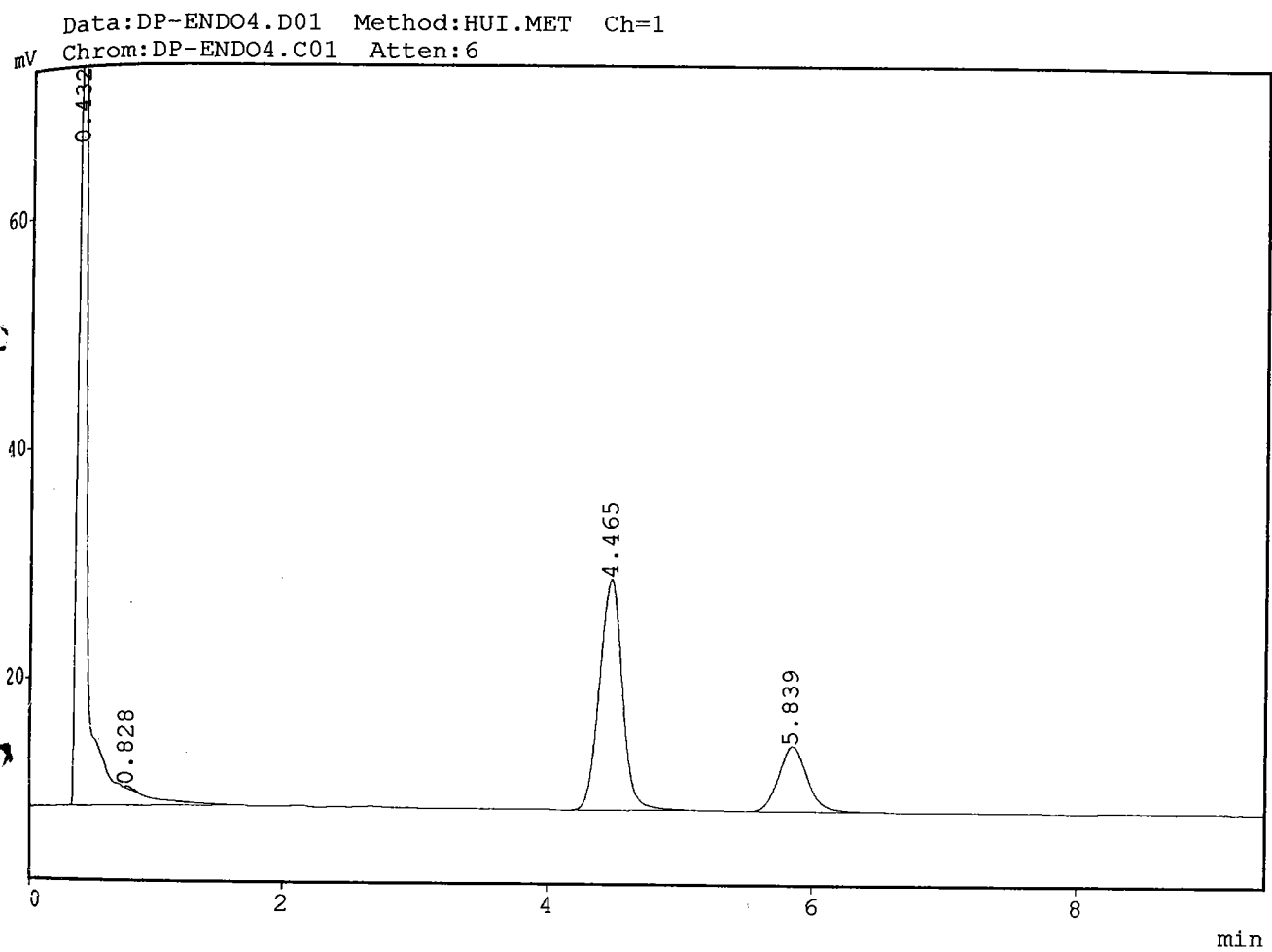


Fig. 2b. GLC chromatogram of endosulfan

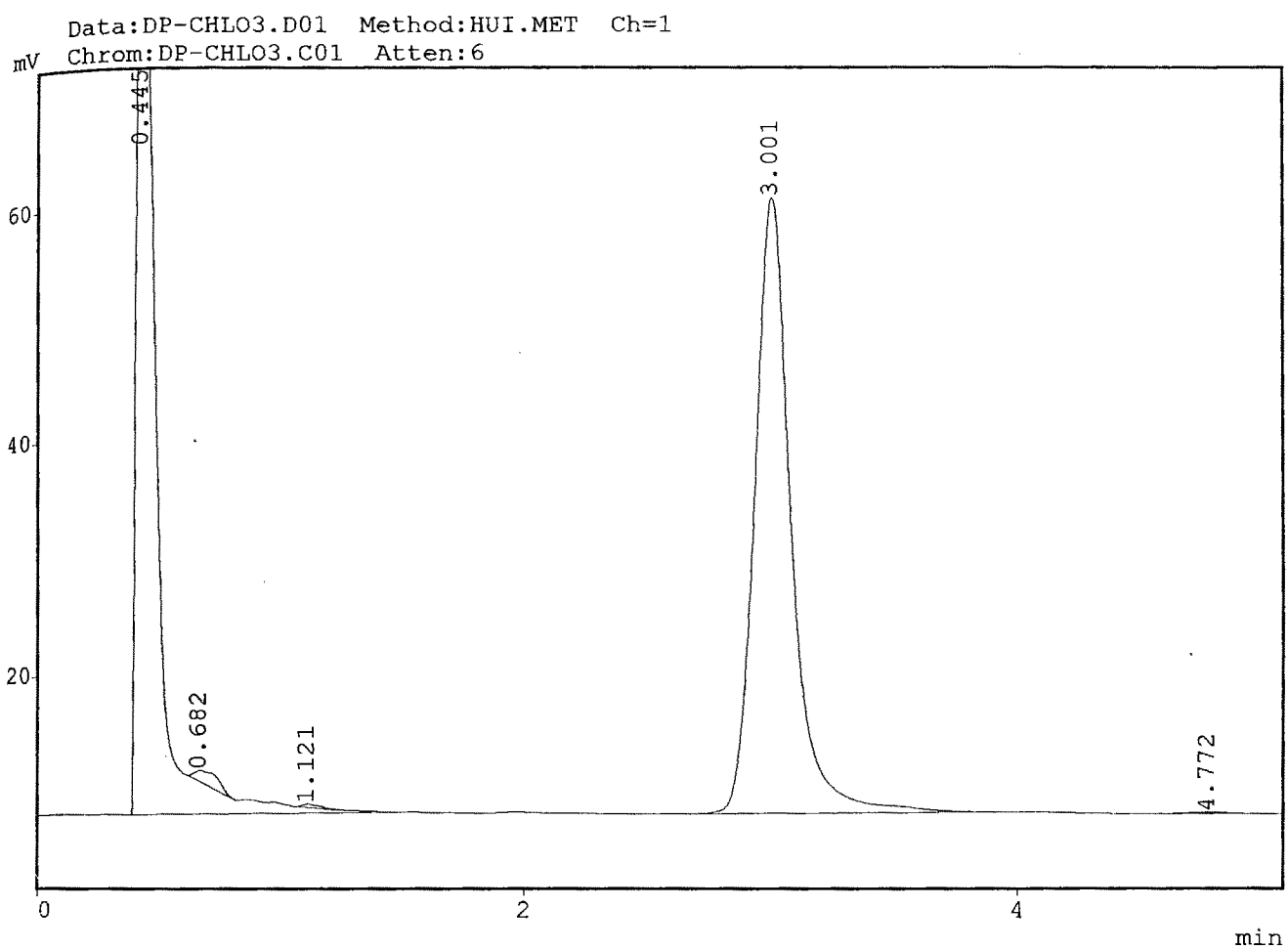


Fig. 2c. GLC chromatogram of chlorothalonil

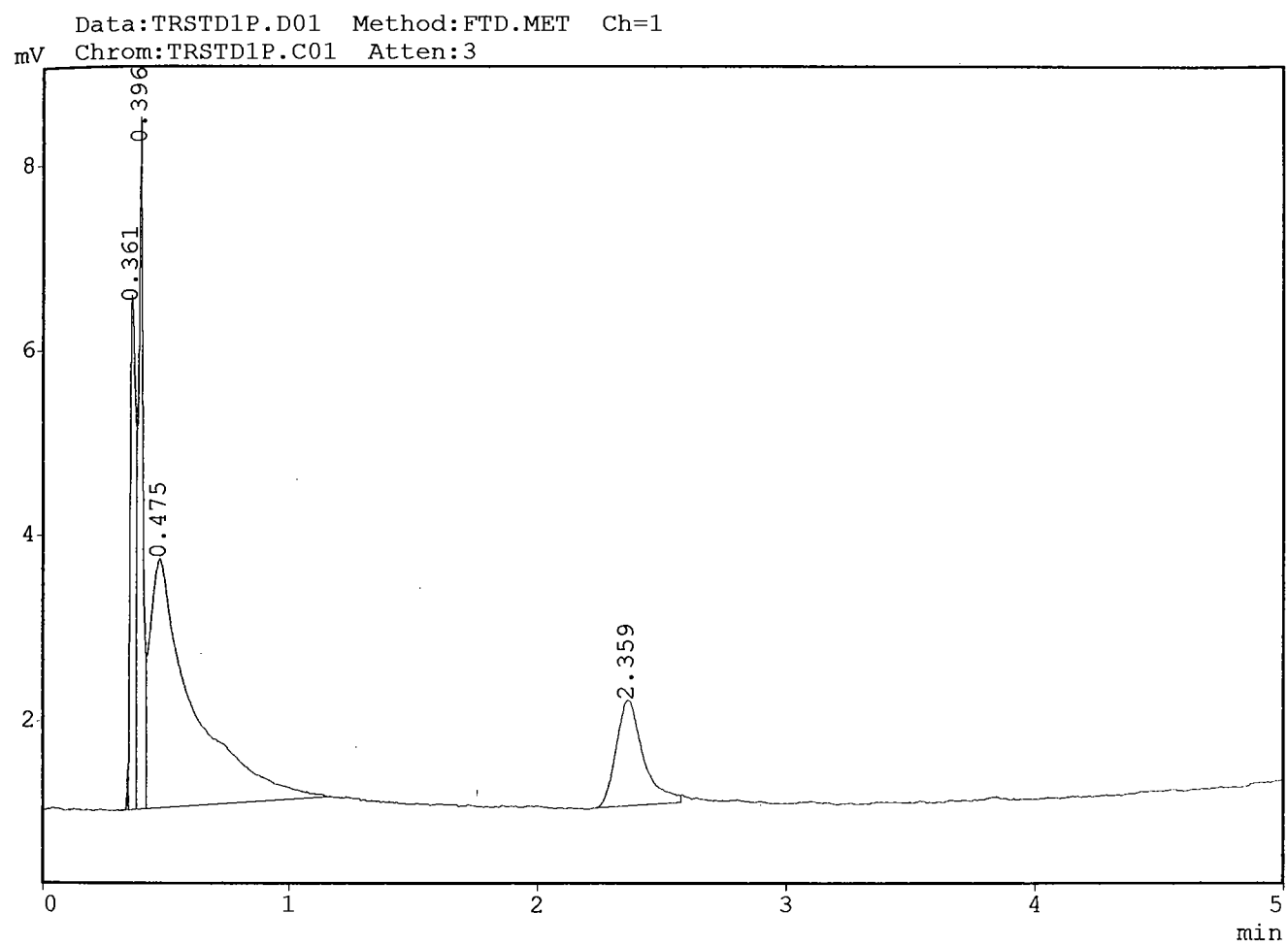


Fig. 2e. GLC chromatogram of triazophos

Table 13b. Calibration of endosulfan by GLC technique

Concentration ($\mu\text{g mL}^{-1}$)	Replicate	Peak area				Average peak area	
		R ₁	R ₂	R ₁	R ₂		
		α	β	α	β	α	β
0.1	2	23266	99638	2737055	101595	234860.5	100616.5
0.2	2	452231	193810	461372	197728	456801.5	195769.0
0.5	2	1033890	443165	1136760	487183	1085325	465174.0
1.0	2	2165798	928015	2104572	901958	2155185	914986.5
1.5	2	3027392	1297454	3172736	1359745	3100064.0	1328599.5
2.0	2	2989152	1708532	3832666	1642572	3910909	1675552.0

Table 13c. Calibration of chlorothalonil by GLC technique

Concentration ($\mu\text{g mL}^{-1}$)	Replicate	Peak area		Average peak area
		R ₁	R ₂	
0.1	2	102608	98720	100664.0
0.2	2	199623	198276	198949.5
0.5	2	540423	558376	549404.5
1.0	2	1176188	1176947	1176567.5
1.5	2	1787995	1768783	1778389.0
2.0	2	2227199	2387130	2307164.5

Table 13d. Calibration of triazophos by GLC technique

Concentration ($\mu\text{g mL}^{-1}$)	Replicate	Peak area		Average peak area
		R ₁	R ₂	
0.1	2	1789	1986	1887.5
0.2	2	3527	3422	3474.5
0.5	2	8524	8826	8675.0
1.0	2	18687	18325	18506.0
1.5	2	27469	28258	27863.5
2.0	2	36253	38292	37272.5

Fig. 3a Calibration of pendimethalin by GLC

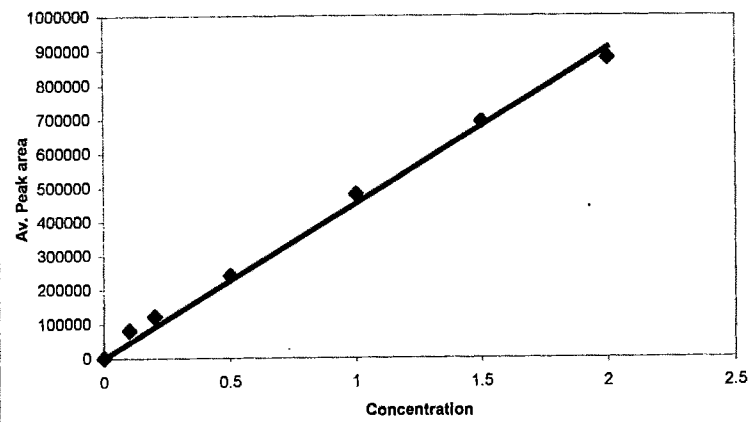


Fig.3b Calibration of endosulphan by GLC

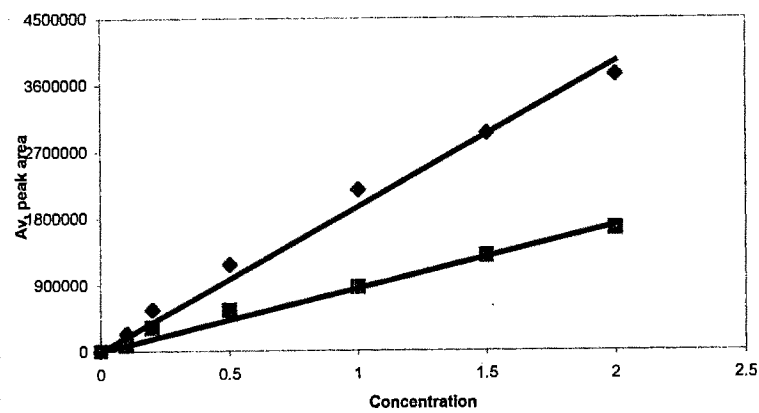


Fig.3c Calibration of chlorothalonil by GLC

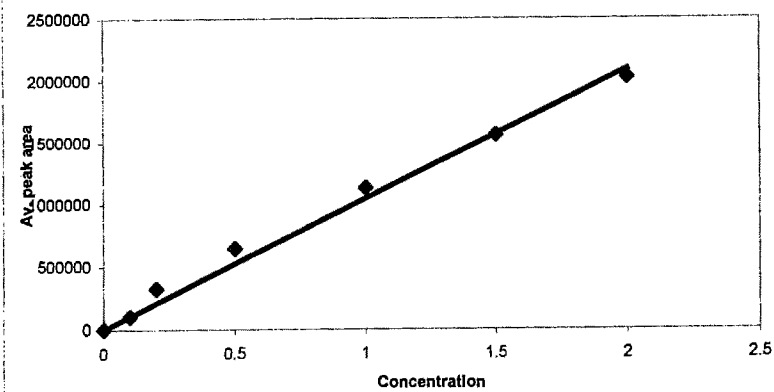


Table 13e. Calibration of β -cyfluthrin by GLC technique

Concentration ($\mu\text{g mL}^{-1}$)	Replicate	Peak area		Average peak area
		R ₁	R ₂	
0.1	2	144963	143725	144344.0
0.2	2	292136	293602	292869.0
0.5	2	804362	754108	779235.0
1.0	2	1607240	1533840	1570540.0
1.5	2	2393417	2399174	2396295.5
2.0	2	2947728	2930722	2939225.0

4.1.2. Standardization of HPLC technique for estimation of Imidacloprid

Efforts were also directed for the development of a HPLC technique for the estimation of the Imidacloprid and its transformation products. Imidacloprid gave no response to GLC. Therefore, a sensitive and simple HPLC technique was standardized to detect this compound.

4.1.2.1. Analytical technique

The essential pre-requisites for the development of a HPLC technique are choice of suitable column and a suitable solvent system for good resolution of the compound at its λ_{max} for detection.

4.1.2.2. Determination of λ_{max} of the compound

The absorbance of imidacloprid and its transformation products ($25 \mu\text{g mL}^{-1}$) were scanned in the wavelength range of 190 to 400 nm using a photodiode array detector. Absorbance of imidacloprid and its hydrolysed product showed a maxima at 270 nm (Fig. 4a). Therefore, λ_{max} of 270 nm was selected for all the estimations of these compounds in HPLC.

4.1.2.3. Selection of suitable column

Most satisfactory results were obtained with Lichrosphere 1000 Reverse Phase C₈ paired stainless steel column (250 mm x 4 mm i.d.) and thus selected for all estimations.

Current Chromatogram(s)

DAD1 A, Sig=270,24 Ref=400,100 (27DC0203.D)

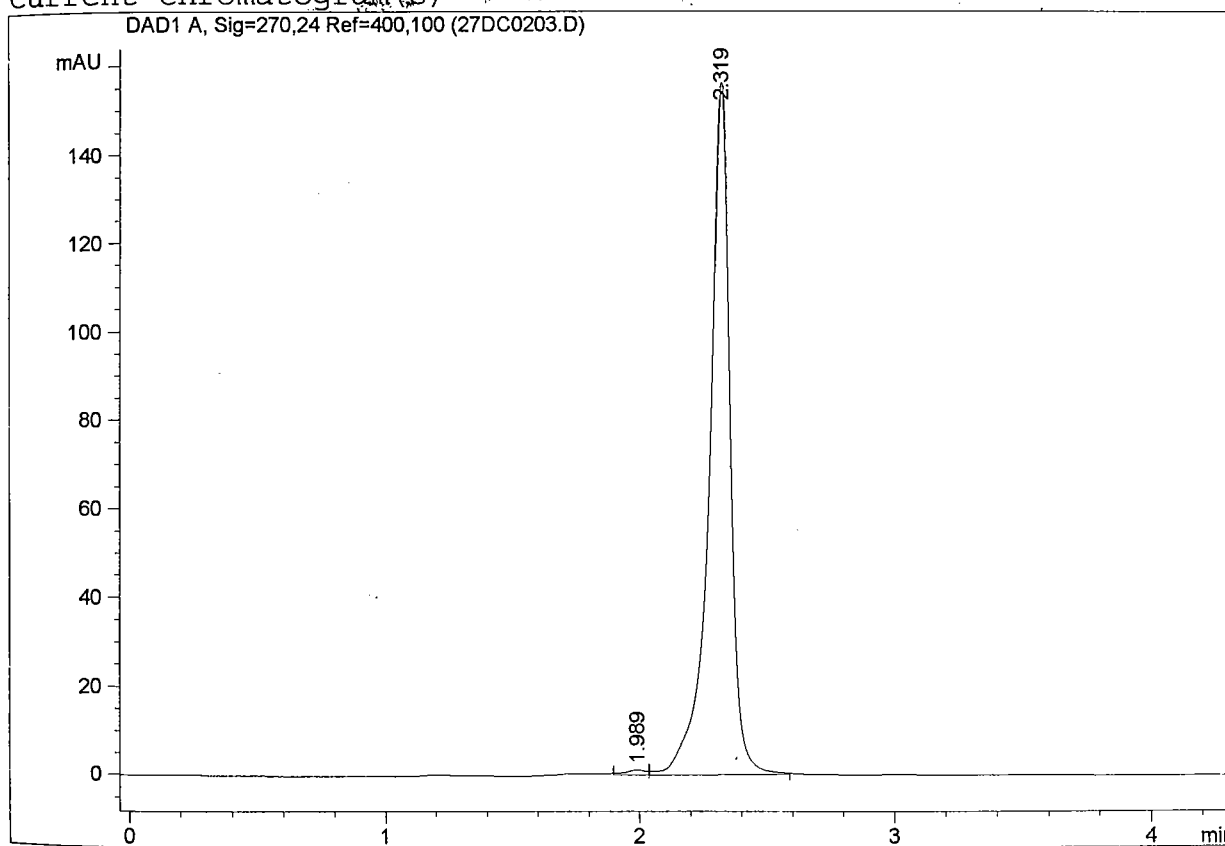
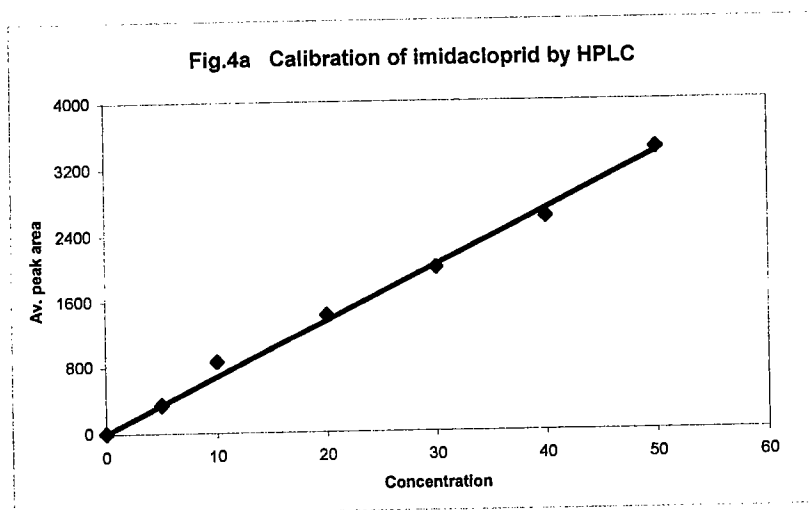
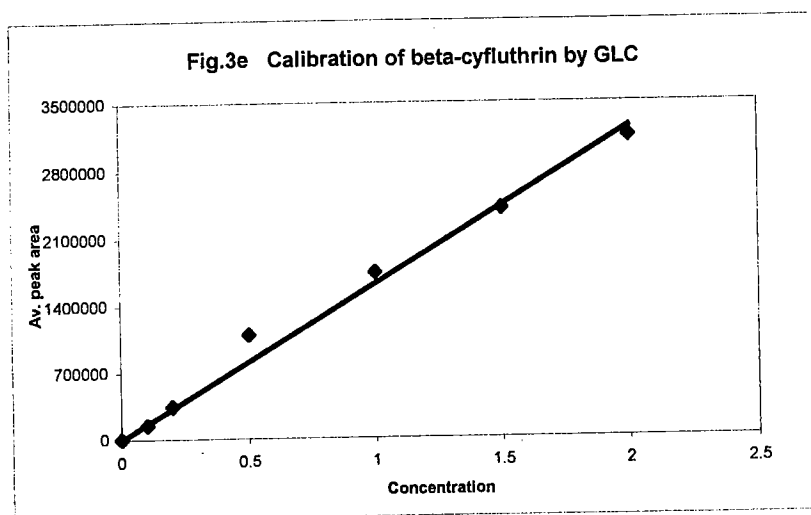
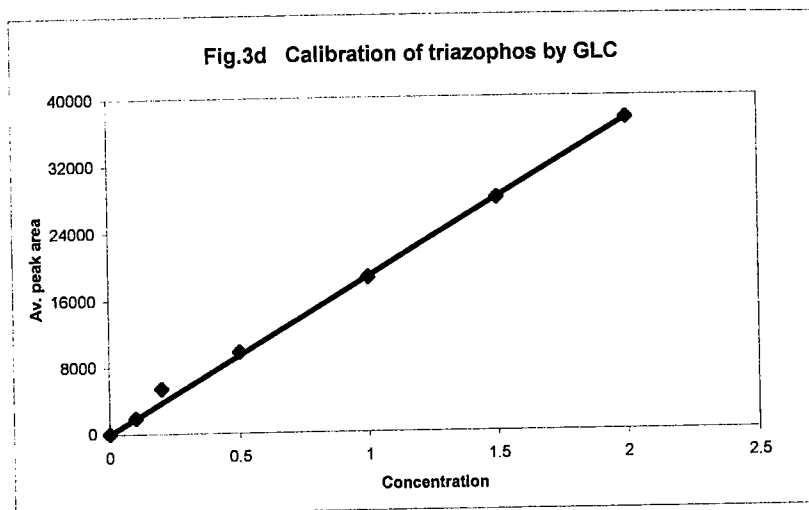


Fig. 4b. HPLC chromatogram of imidacloprid



4.1.2.4. Choice of solvent system

Mobile phase consisting of acetonitrile and water in various proportions were examined with respect to their suitability as eluting solvent using RP-C₈ column. The mixture of acetonitrile and water in 70:30 proportion gave the best resolution of both the compounds with distinct peaks (Fig. 4a). Imidacloprid showed the *R_t* at 2.319.

4.1.2.5. Preparation of standard curve

The peak areas corresponding to various concentrations ranging from 5 to 50 µg mL⁻¹ solution of imidacloprid are presented in Table 14.

The calibration graph of concentrations versus peak areas has been shown in Fig. 4b. The curve shows linearity over the range from 5 to 50 µg mL⁻¹. The sensitivity of the method for imidacloprid was 2 µg mL⁻¹.

Table 14. Calibration of imidacloprid by HPLC technique

Concentration (µg mL ⁻¹)	Replicate	Peak area		Average peak area
		R ₁	R ₂	
5	2	337.06	340.82	338.94
10	2	675.13	663.17	852.5
20	2	1274.26	1356.43	1413.5
30	2	1933.22	2072.65	1983.5
40	2	2539.93	2657.25	2598.59
50	2	3465.36	3363.24	3414.30

Table 15. Sensitivity of GLC/HPLC method for different pesticides

Sl. No.	Pesticides	Detected in	Sensitivity of the method
1.	Pendimethalin	GLC	0.1 ng
2.	Endosulfan	GLC	1.0 ng
3.	Chlorothalonil	GLC	0.01 ng
4.	β-cyfluthrin	GLC	0.1 ng
5.	Triazophos	GLC	0.2 ng
6.	Imidacloprid	HPLC	0.01 µg

4.1.3. Standardization of the multiresidues GLC/HPLC technique for analysis of mixture

4.1.3.1. Analytical technique

In order to know the extent of residual contamination and the magnitude of exposure to human being, efforts were directed to develop a multiresidue analytical technique, which should be efficient, precise, sensitive and capable of detecting and estimating low concentration of pesticides residues. While adopting for a multiresidue technique for two different modules (pesticides taken in two part of the crop growth, the initial pesticides pendimethalin, imidacloprid, chlorothalonil and endosulfan are in module I, where as at the later stage of crop growth three more insecticides endosulfan, triazophos and β -cyfluthrin was taken to control whitefly and fruit borer mainly, are in module II) of pesticides, difficulties were faced in getting quantitative recoveries, especially in case of module I, where four heterogenous pesticides viz., pendimethalin, imidacloprid, endosulfan and chlorothalonil were taken. Three of the molecule viz., pendimethalin, endosulfan and chlorothalonil are relatively non-polar in nature and contain -NH and Cl substituents. Moreover, all these molecule remains stable at medium range temperature (upto 300°C). Therefore, possibility of their separation through GLC using electron capture detector was high. The fourth member, imidacloprid was analysed by HPLC after the analysis of other three in GLC and the method of standardization is already described in section 4.1.2.

In case of module II, three pesticides viz., Endosulfan, β -cyfluthrin and triazophos were taken and while all the individual pesticides were analysed by GLC for the above said reason, the multipesticides was also standardized through GLC. The only modification here was that the first

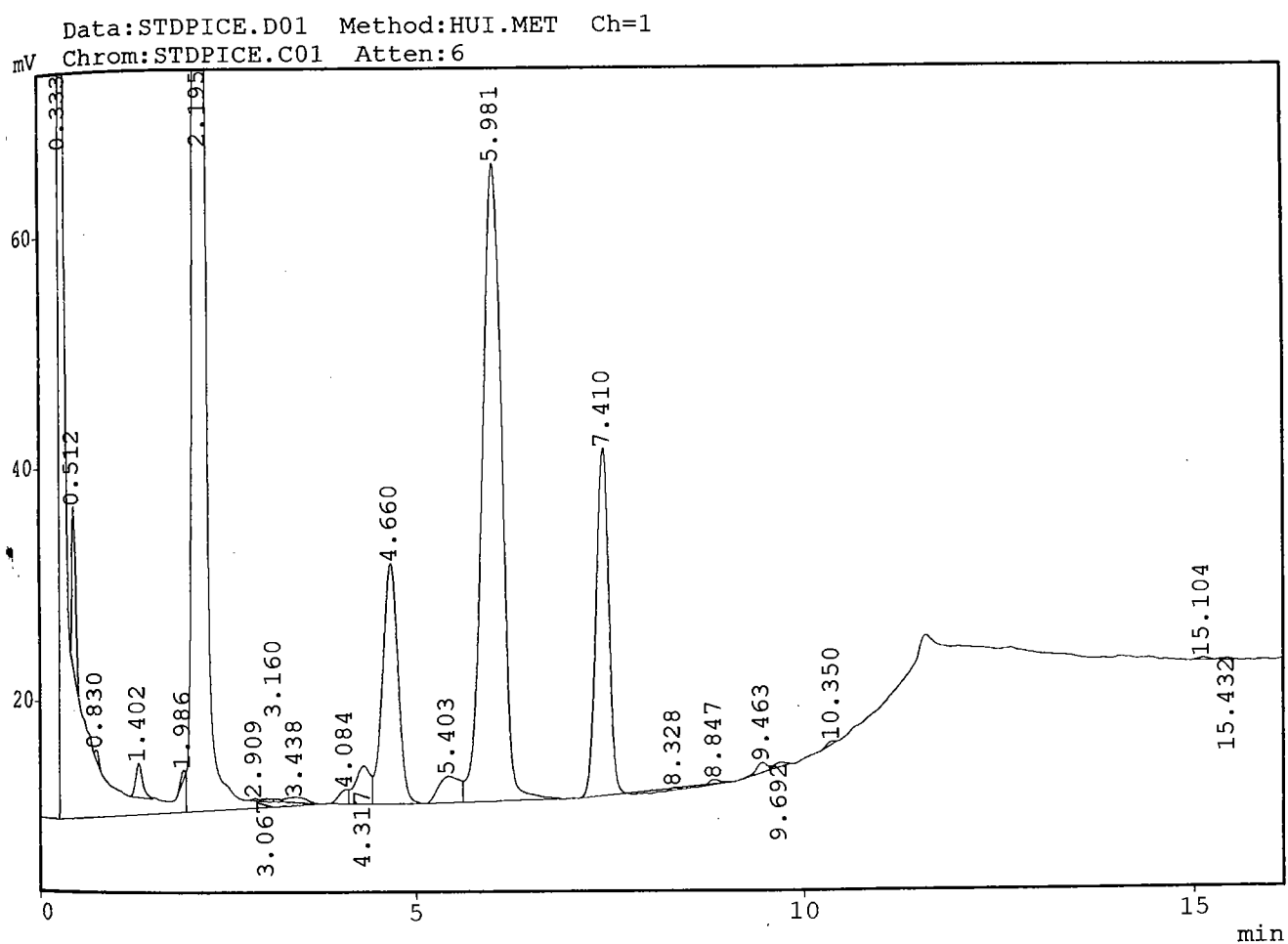


Fig 5a. GLC Chromatogram of multiresidue in module-I

two (endosulfan and β -cyfluthrin) were analysed using ECD while the last one (triazophos) was analysed by FTD after the completion of analysis through ECD.

The analysis using GLC fitted with electron capture detector was highly satisfactory and the method could be used for quantitative analysis of multipesticides and their possible metabolite residues. The HPLC method was also posses satisfactory results for the estimation of imidacloprid. The essential pre-requisite for the development of GLC/HPLC technique were choice of suitable column and selection of suitable oven temperature for good resolution of the compounds.

4.1.3.2. Selection of suitable column

Megabore column (30 m x 0.53 i.d.) containing 3% OV-17 as coating material was found most satisfactory for analysis of pesticides.

4.1.3.3. Choice of suitable column temperature in GLC

The column temperature were programmed for the estimation of multiresidue in GLC in both the module and illustrated in Table 16.

Table 16. GLC temperature programming suitable for multiresidue analysis of pesticides

Sl. No.	Module	Compounds	Retention time (Rt)	Temperature programming
1.	Module I	Chlorothalonil	2.195	
		Pendimethalin	4.660	190°C (6 min) ... @ 10° min ⁻¹
		Endosulfan (α)	5.981	270°C (5 min)
		Endosulfan (β)	7.410	
2.	Module II	Endosulfan (α)	3.633	
		Endosulfan (β)	4.638	220°C (6 min) ... @ 10° min ⁻¹ ..
		β -cyfluthrin	10.187	270 (5 min)
		Triazophos	2.322	[240°C....270°C... 280°C (FTD)]

4.1.3.4. Validation of the GLC technique

The GLC technique in both the modules were validated by injecting same standard mixture solution three times. The curves showed the peaks of standard pesticides resolved to a desirable extent (Fig. 5a).

4.1.4. Standardization of HPLC technique for analysis of imidacloprid as mixture component.

Only the insecticide imidacloprid was analysed in HPLC, after the analysis of other mixture component in GLC. The injection in HPLC was done later due to reason it needs more injection volume (20 μ L) than GLC (2-3 μ L). Since, only component was analysed in HPLC, the standardization technique was same as mentioned in case of single compound (section 4.1.2.).

4.1.5. Recovery experiments

4.1.5.1. Individual pesticides

4.1.5.1.1. Tomato fruits

The efficiency of extraction method was assessed by fortifying the known amount of plant samples (50g) with known quantities of pesticide(s) and extracting the fortified sample with method described the solvent used for extraction with per cent recoveries of the compound are presented in Table 17 and section 3.4.2. The recoveries of pesticides from plant samples fortified at 0.1, 0.5 and 1.0 $\mu\text{g g}^{-1}$ level are presented in Table 18a to 18f. The recoveries of pendimethalin ranged from 84 to 88 per cent. The recovery range of chlorothalonil was 82 to 89 per cent. Endosulfan was recorded in the range of 82 to 85 per cent whereas the highest recovery of imidacloprid, triazophos and β -cyfluthrin were 83, 93 and 95 per cent, respectively.

4.1.5.1.2. Soil

The efficiency of extraction method was assessed by fortifying the known amount of soil samples (50g) with known quantities of pesticides (0.1

to 1.0 ppm) and extracting the fortified samples with different solvents. The solvents used for extraction with per cent recoveries of the compounds are presented in Table 17.

Different solvents or solvent-system were examined for getting an acceptable recovery from soil. There were three pesticides applied or treated in soil. The recovery of the three pesticides from soil has been shown in Table 17. Among the different solvents, acetone gave the highest recovery. Whereas for imidacloprid, dichloromethane was the best solvent.

Only acetone proved to be best solvent for chlorothalonil for its higher recovery from the soil. The recoveries of all the pesticides fortified at 0.1, 0.2, 0.4, 0.5, 0.7 and 1.0 $\mu\text{g g}^{-1}$ of soil using different solvents are presented in Table 18a to 18f.

Table 17. Solvents used for extraction of pesticides and their mixture from tomato and soil

Pesticides	Solvents	Average Recovery range (per cent)	
		Tomato	Soil
Pendimethalin	Acidified acetone	84-88	85-98
Imidacloprid	Acetone	79-83	82-86
Endosulfan	Hexane:isopropanol (2:1)	83-88	82-90
Chlorothalonil	Acetone	82-89	91-97
β -cyfluthrin	Acetone	92-95	-
Triazophos	Acetone	89-93	-
PICE mixture	Acetone	74-83	77-90
ET β mixture	Acetone	75-85	-

4.1.5.1.3. Culture media

The efficiency of extraction method was assessed by fortifying the known amount of liquid media (Czapek Dox) sample (100 mL) with known quantities of pesticide(s) and extracting the fortified sample with suitable solvent mentioned in Table 17. The recoveries of pesticides from liquid culture media sample fortified at 0.2, 1.0 and 2.0 $\mu\text{g mL}^{-1}$ are presented in Table 18a to 18f.

Table 18a. Recoveries of pendimethalin from various substrates

Amount added ($\mu\text{g g}^{-1}$)	Amount recovered ($\mu\text{g g}^{-1}$)		Average recovery ($\mu\text{g g}^{-1}$) $\pm$ SD	Pendimethalin recoveries (%)
	R ₁	R ₂		
A. Soil				
0.1	0.082	0.087	0.085 $\pm$ 0.004	85.00
0.2	0.189	0.194	0.192 $\pm$ 0.004	96.00
0.5	0.462	0.461	0.462 $\pm$ 0.001	92.40
1.0	0.925	0.952	0.939 $\pm$ 0.020	93.90
B. Tomato fruit				
0.1	0.085	0.082	0.084 $\pm$ 0.002	84.00
0.5	0.432	0.451	0.442 $\pm$ 0.013	88.40
1.0	0.842	0.845	0.844 $\pm$ 0.002	84.40
C. Minimal salt medium				
0.2	0.192	0.190	0.191 $\pm$ 0.001	95.50
1.0	0.921	0.920	0.921 $\pm$ 0.001	92.10
2.0	1.872	1.873	1.873 $\pm$ 0.001	93.65
D. Czapek-Dox medium				
0.2	0.179	0.182	0.181 $\pm$ 0.002	90.50
1.0	0.872	0.828	0.850 $\pm$ 0.031	85.00
2.0	1.851	1.873	1.862 $\pm$ 0.016	93.10

Table 18b. Recoveries of chlorothalonil from various substrates

Amount added ($\mu\text{g g}^{-1}$)	Amount recovered ($\mu\text{g g}^{-1}$)		Average recovery ($\mu\text{g g}^{-1}$) $\pm$ SD	Chlorothalonil recoveries (%)
	R ₁	R ₂		
A. Soil				
0.1	0.089	0.093	0.091 $\pm$ 0.003	91.00
0.2	0.197	0.188	0.193 $\pm$ 0.006	96.50
0.5	0.475	0.496	0.486 $\pm$ 0.015	97.20
1.0	0.938	0.934	0.936 $\pm$ 0.03	93.60
B. Tomato fruit				
0.1	0.079	0.087	0.083 $\pm$ 0.006	83.00
0.5	0.443	0.448	0.446 $\pm$ 0.004	89.10
1.0	0.832	0.810	0.821 $\pm$ 0.016	82.10
C. Czapek-Dox medium				
0.2	0.185	0.188	0.187 $\pm$ 0.002	93.50
1.0	0.876	0.870	0.873 $\pm$ 0.004	87.50
2.0	1.835	1.875	1.855 $\pm$ 0.028	92.75

Table 18c. Recoveries of endosulfan from various substrates

Amount added ($\mu\text{g g}^{-1}$)	Amount recovered ($\mu\text{g g}^{-1}$)		Average recovery ($\mu\text{g g}^{-1}$) $\pm$ SD	Endosulfan recoveries (%)
	R ₁	R ₂		
A. Soil				
0.1	0.079	0.084	0.082 $\pm$ 0.004	82.00
0.2	0.173	0.172	0.173 $\pm$ 0.001	86.50
0.5	0.445	0.448	0.447 $\pm$ 0.002	89.40
1.0	0.893	0.897	0.895 $\pm$ 0.003	89.50
B. Tomato fruit				
0.1	0.082	0.079	0.083 $\pm$ 0.002	83.00
0.5	0.425	0.431	0.428 $\pm$ 0.004	85.60
1.0	0.880	0.886	0.883 $\pm$ 0.004	88.30
C. Czapek-Dox medium				
0.2	0.179	0.182	0.181 $\pm$ 0.002	90.50
1.0	0.905	0.911	0.908 $\pm$ 0.004	90.80
2.0	1.82	1.80	1.81 $\pm$ 0.014	90.50

Table 18d. Recoveries of Triazophos from tomato fruits

Amount added ($\mu\text{g g}^{-1}$)	Amount recovered ($\mu\text{g g}^{-1}$)		Average recovery ($\mu\text{g g}^{-1}$) $\pm$ SD	Triazophos recoveries (%)
	R ₁	R ₂		
A. Tomato fruits				
0.1	0.094	0.091	0.093 $\pm$ 0.002	93.00
0.2	0.182	0.176	0.179 $\pm$ 0.004	89.50
0.5	0.453	0.462	0.458 $\pm$ 0.006	91.60
1.0	0.912	0.932	0.922 $\pm$ 0.014	92.20

Table 18e. Recoveries of β -cyfluthrin from tomato fruits

Amount added ($\mu\text{g g}^{-1}$)	Amount recovered ($\mu\text{g g}^{-1}$)		Average recovery ($\mu\text{g g}^{-1}$) $\pm$ SD	β -cyfluthrin recoveries (%)
	R ₁	R ₂		
A. Tomato fruits				
0.1	0.096	0.094	0.095 $\pm$ 0.001	95.00
0.2	0.192	0.187	0.190 $\pm$ 0.004	95.00
0.5	0.457	0.462	0.460 $\pm$ 0.004	92.00
1.0	0.932	0.941	0.937 $\pm$ 0.006	93.70

Table 18f. Recoveries of imidacloprid from various substrates

Amount added ($\mu\text{g g}^{-1}$)	Amount recovered ($\mu\text{g g}^{-1}$)		Average recovered ($\mu\text{g g}^{-1}$) $\pm$ SD	Imidacloprid recovery (%)
	R ₁	R ₂		
A. Soil				
20	16.275	16.320	16.298 $\pm$ 0.045	81.49
50	39.450	42.932	41.191 $\pm$ 2.462	82.38
100	86.273	84.199	85.236 $\pm$ 1.467	85.24
B. Tomato fruit				
20	15.392	16.242	15.817 $\pm$ 0.601	79.09
40	32.273	33.125	32.699 $\pm$ 0.602	81.75
50	42.133	39.156	40.645 $\pm$ 2.105	81.29
C. Czapek-Dox medium				
20	17.225	18.175	17.700 $\pm$ 0.672	88.50
40	36.629	33.932	35.281 $\pm$ 1.907	88.20
50	44.720	45.829	42.272 $\pm$ 0.780	84.54

4.1.5.2. Recoveries of pesticides mixtures from various matrices

4.1.5.2.a Module I

The efficiency of extraction method of the multiresidue was assessed by fortifying the known amount of pesticides and extracting the fortified samples with 100 mL acetone by mechanical shaking for 1 hour. The extract was filtered through 3-4 cm bed of anhydrous sodium sulphate and transferred into separatory funnel diluted with 10% sodium chloride solution and extracted with ethyl acetate followed by dichloromethane (50, 25, 25 mL). The organic phase concentrated and combined to about 5 mL under reduced pressure and cleaned up as follows.

The total extract was divided into 2 equal parts and cleaned up by two different procedures. Firstly, A glass column (60 cm x 20 mm) was packed with 5g mixture of florisil (60-100 mesh) and charcoal (5:1, w/w) in between two layers of anhydrous sodium sulphate. The column was prewashed with 50 mL hexane, loaded with first part of concentrated extract and eluted with 125 mL mixture of ethyl acetate : hexane (3:7 v/v). The eluate was concentrated and final volume was made up to 2 mL with ethyl acetate : n-hexane (3:7 v/v) and analysed in GLC followed by HPLC after solvent evaporation and volume making in acetonitrile.

Secondly, a chromatographic column was prepared by packing 5g adsorbent mixture of silica gel (60-230 mesh) and activated charcoal (5:1 w/w). Column was pre-washed and loaded with the second part of the concentrated extract and eluted with 125 mL of mixture of acetone : n-hexane (3:7 v/v). Eluate was concentrated and final volume was adjusted to 2 mL with acetones : n-hexane (3:7 v/v) and analysed in GLC. The solvent was evaporated to dryness and 2 mL acetonitrile added for analysis by HPLC.

The recoveries of each pesticides in the mixture in different substrates (soil, tomato fruits and culture media) are presented in Table 19a to 19c.

4.1.5.2.1. Tomato fruits

In tomato fruits, each component of the pesticide mixture recovered as : pendimethalin 81.65 to 82.90 per cent; imidacloprid 97.00 to 82.64 per cent; chlorothalonil 74.0 to 81.2 per cent and endosulfan 75.9 to 82.6 per cent (Table 19a). The recoveries of each component of the pesticide mixture from tomato fruits were less than soil and aqueous media. This might be due to the clean up of pigments from the fruit sample. The removal of ample amount of pigment leads to the removal of some amount of pesticides at the time of clean-up.

4.1.5.2.2. Soil

In the soil (Table 19b) the recoveries of pendimethalin were 80, 82.4, 89.5 and 86.8 when the fortification level were 0.1, 0.5, 1.0 and 2.0, respectively.

Imidacloprid recovery was lower than pendimethalin and the recoveries were 81.84, 77.38, 82.5 and 79.6. When the level of fortification were 5, 10, 25 and 50, respectively.

The recoveries of chlorothalonil and endosulfan varied from 78.00 to 81.00 and 80 to 86.8 per cent, respectively when the level of fortification were 0.1, 0.5, 1.0 and 2.0, respectively.

4.1.5.2.3. Aqueous extract / culture media

In aqueous extracts, the recovery of pendimethalin varied from 88.7 to 91.0 per cent, while the recovery of chlorothalonil was 85.4 to 94.9 per cent when the fortification level was same as in the case of soil. The recoveries for endosulfan ranged from 85.85 to 90.00 per cent in this case. The insecticide imidacloprid recoveries varied from 83.7 to 92.48 per cent (Table 19c).

Table 19a. Recovery of pesticides from tomato fruits fortified with pesticide mixture

Amount added ($\mu\text{g g}^{-1}$)	Amount recovered ($\mu\text{g g}^{-1}$)		Average amount recovered ($\mu\text{g g}^{-1}$) $\pm$ SD		Per cent recovery
	R ₁	R ₂			
Pendimethalin					
0.1	0.079	0.083	0.081	0.003	81.00
0.5	0.423	0.383	0.403	0.028	80.60
1.0	0.823	0.835	0.829	0.008	82.90
2.0	1.583	1.682	1.6333	0.070	81.65
Imidacloprid					
5	3.927	3.772	3.850	0.110	77.00
10	7.562	8.211	7.887	0.459	78.87
25	21.275	20.145	20.710	0.800	82.84
50	39.212	39.929	39.571	0.507	79.14
Chlorothalonil					
0.1	0.075	0.073	0.074	0.001	74.00
0.5	0.395	0.417	0.406	0.016	81.20
1.0	0.738	0.745	0.742	0.005	74.20
2.0	1.473	1.589	1.531	0.082	76.55
Endosulfan					
0.1	0.078	0.082	0.080	0.003	80.00
0.5	0.412	0.413	0.413	0.001	82.60
1.0	0.842	0.765	0.804	0.054	80.40
2.0	1.499	1.587	1.518	0.056	75.90

Table 19b. Recovery of pesticides from soil fortified with pesticide mixture

Amount added ($\mu\text{g g}^{-1}$)	Amount recovered ($\mu\text{g g}^{-1}$)		Average amount recovered ($\mu\text{g g}^{-1}$) $\pm$ SD		Individual recovery (%)
	R ₁	R ₂			
Pendimethalin					
0.1	0.076	0.084	0.080	0.006	80.00
0.5	0.425	0.398	0.412	0.019	82.40
1.0	0.878	0.912	0.895	0.024	89.50
2.0	1.725	1.801	1.763	0.054	86.80
Imidacloprid					
5	4.221	3.962	4.092	0.183	81.84
10	7.272	8.203	7.738	0.658	77.38
25	21.202	20.045	20.624	0.818	82.50
50	38.375	41.221	39.798	2.012	79.60
Chlorothalonil					
0.1	0.077	0.079	0.078	0.001	78.00
0.5	0.392	0.420	0.406	0.020	81.20
1.0	0.825	0.795	0.810	0.021	81.00
2.0	1.527	1.625	1.576	0.069	78.80
Endosulfan					
0.1	0.075	0.084	0.080	0.006	80.00
0.5	0.423	0.415	0.419	0.006	83.80
1.0	0.915	0.821	0.868	0.066	86.80
2.0	1.732	1.650	1.691	0.058	84.55

Table 19c. Recovery of pesticides from culture media fortified with pesticide mixture

Amount added ($\mu\text{g g}^{-1}$)	Amount recovered($\mu\text{g g}^{-1}$)		Average recovery recovered ($\mu\text{g g}^{-1}$) $\pm$ SD		Individual recovery
	R ₁	R ₂			
Pendimethalin					
0.1	0.092	0.089	0.091	0.002	91.00
0.5	0.447	0.452	0.450	0.003	90.00
1.0	0.913	0.935	0.924	0.016	92.40
2.0	1.762	1.785	1.774	0.016	88.70
Imidacloprid					
5	4.250	4.125	4.188	0.088	83.76
10	9.263	8.128	8.670	0.803	86.70
25	22.450	23.789	23.120	0.947	92.48
50	44.029	45.560	44.795	1.083	89.59
Chlorothalonil					
0.1	0.093	0.087	0.090	0.004	90.00
0.5	0.421	0.432	0.427	0.008	85.40
1.0	0.951	0.947	0.949	0.003	94.90
2.0	1.762	1.825	1.794	0.045	89.70
Endosulfan					
0.1	0.087	0.083	0.085	0.003	85.00
0.5	0.423	0.476	0.450	0.037	90.00
1.0	0.882	0.875	0.879	0.005	87.90
2.0	1.745	1.698	1.717	0.033	85.85

4.1.5.2.b. Recoveries of pesticides mixture from fortified tomato fruits in module II:

The efficiency of extraction method of the pesticide mixture (Module II) was assessed by fortifying the known amount of tomato fruit samples (25g) at the level of 0.1, 0.5, 1.0 and 2.0 $\mu\text{g g}^{-1}$ and extracting the fortified sample with the method described in Section 3.4.4. The recoveries of pesticides from tomato fruit extract are presented in Table 20.

The recoveries of the pesticides in mixture used in method II are also presented in Table 20.

Table 20. Recoveries of pesticides from tomato fruit fortified with pesticide mixture (Endosulfan + Triazophos+ β -cyfluthrin)

Amount added ($\mu\text{g g}^{-1}$)	Amount recovered($\mu\text{g g}^{-1}$)		Average recovery recovered ($\mu\text{g g}^{-1}$) $\pm$ SD		Per cent recovery
	R ₁	R ₂			
Endosulfan					
0.1	0.072	0.077	0.075	0.003	75.00
0.5	0.413	0.405	0.409	0.005	81.80
1.0	0.775	0.782	0.779	0.004	77.90
2.0	1.685	1.720	1.7025	0.024	85.13
Triazophos					
0.1	0.083	0.087	0.085	0.002	85.00
0.5	0.413	0.418	0.423	0.003	83.10
1.0	0.812	0.823	0.818	0.007	81.80
2.0	1.625	1.586	1.600	0.028	80.00
β -cyfluthrin					
0.1	0.824	0.835	0.830	0.007	83.00
0.5	0.422	0.416	0.419	0.004	83.80
1.0	0.845	0.852	0.845	0.005	84.50
2.0	1.676	1.723	0.170	0.033	85.00

4.2 Persistence of pesticides

Investigation on persistence of pesticides in tomato fruit and soil under field conditions were conducted by spraying of these pesticides at the rate

given in Table 3 (Materials and Methods). The pesticide contents in the tomato fruits and soil (0-15 cm) depth were monitored at regular intervals by GLC/HPLC method.

4.2.1. Persistence in/on tomato fruits

Persistence of pesticides in fruit following its application in tomato crop was determined by estimating pesticide residues at regular intervals.

4.2.1.1. Individual pesticides

4.2.1.1.a. Imidacloprid

The initial concentration of imidacloprid residues from triplicate samples and their per cent dissipation in tomato fruits treated at the level of 20 g a.i. ha⁻¹ in 500 litres of water are presented in Table 21a.

Table 21a. Residues and per cent dissipation of imidacloprid in tomato fruits

Interval (days)	Imidacloprid residues ($\mu\text{g g}^{-1}$)			Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Dissipation (%)
	R ₁	R ₂	R ₃		
0	1.56	1.72	1.63	1.64 $\pm$ 0.080	-
1	1.27	1.22	1.38	1.29 $\pm$ 0.082	21.34
3	1.06	0.98	1.12	1.05 $\pm$ 0.070	35.98
5	0.88	0.82	0.95	0.88 $\pm$ 0.065	46.34
7	0.38	0.33	0.41	0.37 $\pm$ 0.040	77.44
10	0.26	0.21	0.18	0.22 $\pm$ 0.040	86.59
15	0.05	0.03	0.08	0.05 $\pm$ 0.025	96.95
20	BDL	BDL	BDL	-	-
SEm $\pm$				0.03	
CD at 5%				0.09	
Y = -0.0778X+3.2116					
R ² = 0.9787					
T _{1/2} = 3.9 days					

The initial concentration of imidacloprid in tomato fruit was $1.64 \mu\text{g g}^{-1}$ which dissipated in plants to 1.29, 1.05, 0.88, 0.37, 0.22, $0.05 \mu\text{g g}^{-1}$ and below detectable limit after 1, 3, 5, 7, 10, 15 and 20 days of application, respectively (Fig 6a).

The residues of imidacloprid in tomato plants dissipated with time (Fig. 6b). It could be seen from dissipation curve that the rate of disappearance in plants was very rapid during initial period which could be due to hydrolysis and the dilution effect due to rapid growth of the tomato crop. During later period degradation could be attributed to hydrolysis within plants leading to almost complete dissipation of the imidacloprid residues in plants at harvest of the fruits (75 days).

The logarithmic plots of insecticide residues versus time obtained by fitting the regression equation ($Y = 3.2116 - 0.0778X$) have been presented in Figure 6c. The plot indicated the disappearance of parent compound fitted a first order kinetics decay curve well ($r = -0.989$). The slope of the curve, correlation coefficient, rate constant and half-life of the herbicide as obtained from the regression equation are summarized in Table 21a. The half-life of imidacloprid in tomato fruit under field condition estimated by best fit was found to be 3.9 days. Kumar (1999) also reported a half-life of 4.0 days for imidacloprid in mustard plants. Mukherjee and Gopal (2000) observed the half-life of imidacloprid in brinjal, cabbage leaves and curd as 3.0, 3.7 and 3.4 days, respectively. Our result was close conformity with that. The ADI (acceptable daily intake) of imidacloprid is 0.057 mg kg^{-1} body weight (Tomlin, 1997). Multiplying the ADI with the weight of average Indian person (55 kg), the maximum, the maximum permissible intake (MPI) was obtained as 3.135 mg^{-1} per person per day. Assuming 80g of tomato out of 1.5 kg

total diet, and multiplying the maximum amount of residues detected (not the average value of the replicate) on tomato by the amount of vegetables consumed per day per person the Theoretical Maximum Residue Contribution (TMRC) was obtained. The TMRC, thus calculated from the residue data were found to be $0.1968 \text{ mg kg}^{-1}$ in tomato. This is lower than the MPI ($3.135 \text{ mg kg}^{-1} \text{ day}^{-1}$) calculated from toxicological data. Therefore, the application of imidacloprid could be taken to be safe from the crop protection and environmental contamination aspect.

Some of the metabolites of imidacloprid are biologically active and act in concert with remaining levels of the parent insecticide and thus provide effective and long-lasting control in crops. In the present study, the urea compound and chloronicotinic acid were detected by TLC of the treated tomato sample on day 10, but the olefinic metabolite was not detected. The identities of the metabolic products were confirmed by comparison with authentic samples prepared in the laboratory.

4.2.1.1.b. Endosulfan

The data pertaining to initial concentration of endosulfan residues from triplicate samples and their dissipation in tomato fruits treated at the level of $350 \text{ g a.i. ha}^{-1}$ (0.05% aqueous solution) are presented in Table 21b.

The total residues of endosulfan ($\alpha + \beta + \text{sulfate}$) after 5, 10, 15d were found to be 1.99, 0.66 and $0.16 \text{ } \mu\text{g g}^{-1}$, respectively. The corresponding loss of residue was 51.34, 83.86 and 96.09% when endosulfan was applied at recommended dose. Endosulfan sulfate, a toxic metabolite of endosulfan

Table 21b. Residues and per cent dissipation of endosulfan in the tomato fruits

Days	Endosulfan residues						Average residues ($\mu\text{ g}^{-1}\pm\text{SD}$)						Dissipation (%)			
	α			β			Sulphate			α	β	Sulphate	$\alpha+\beta$ sulphate	α	β	$\alpha+\beta$ sulphate
	R ₁	R ₂	R ₃	R ₁	R ₂	R ₃	R ₁	R ₂	R ₃							
0	2.82	2.89	2.90	1.23	1.25	1.18	-	-	-	2.87	1.22	-	4.09	-	-	-
1	2.38	2.42	2.46	1.12	1.14	1.13	-	-	-	2.42	1.13	-	3.55	15.67	7.38	13.20
3	1.60	1.63	1.63	1.03	1.07	1.08	-	-	-	1.62	1.06	-	2.68	56.45	13.11	34.47
5	1.05	1.10	1.06	0.87	0.93	0.96	0.23	0.26	0.26	1.07	0.92	0.25	1.99	62.72	24.59	51.34
7	0.68	0.69	0.79	0.70	0.65	0.63	0.44	0.40	0.54	0.72	0.66	0.46	1.38	74.91	45.90	66.26
10	0.10	0.14	0.15	0.52	0.53	0.54	0.56	0.53	0.56	0.13	0.53	0.55	0.66	95.47	56.58	83.86
15	-	-	-	0.03	0.05	0.04	0.18	0.14	0.16	-	0.04	0.12	0.16	BDL	90.16	96.09

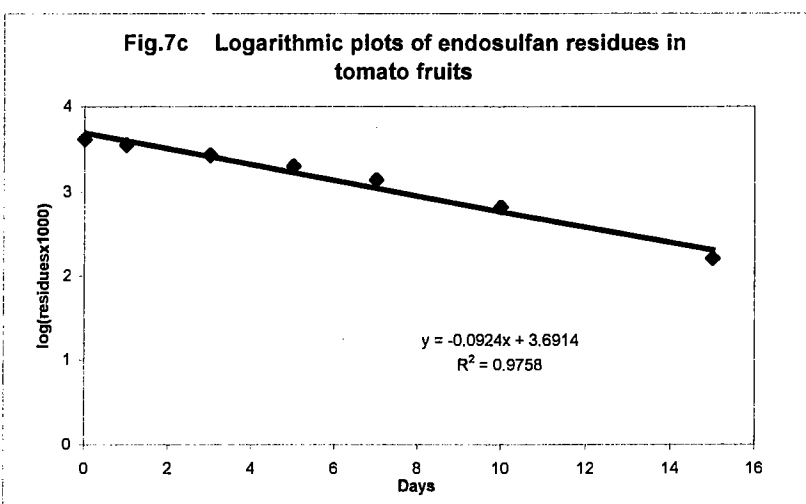
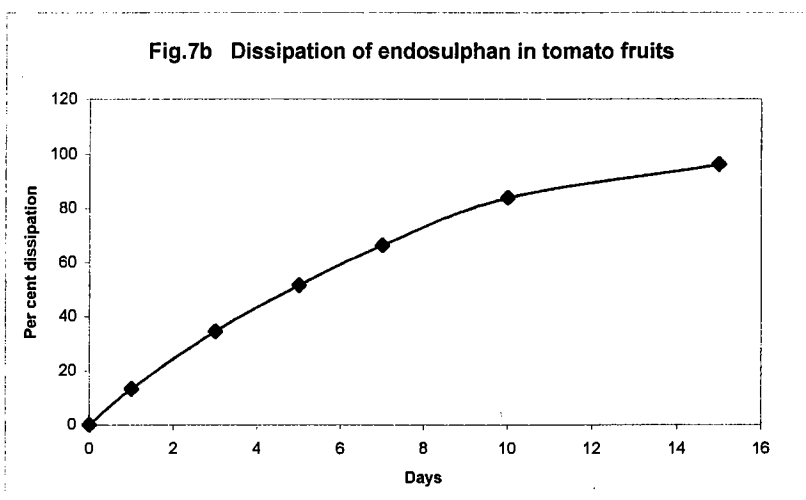
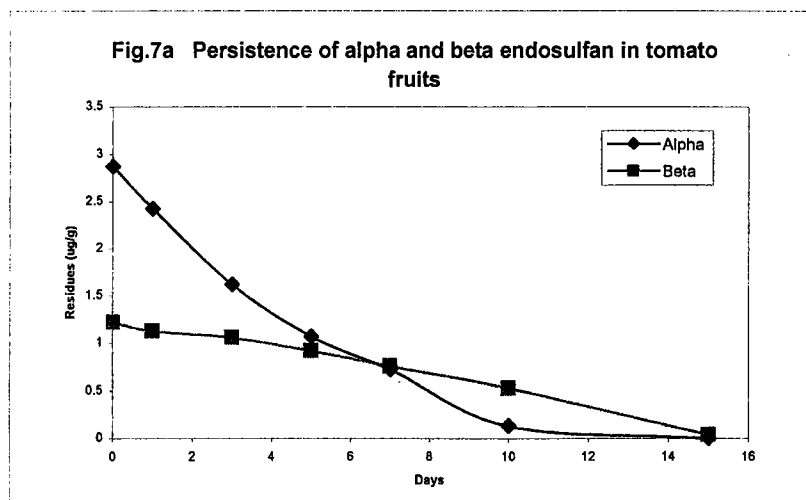
$Y = -0.0924x+3.6914$
 $R^2 = 0.9758$
 $T_{1/2} = 3.3\text{ days}$

appeared on ($0.25\mu\text{g g}^{-1}$) fruits after 5d of spraying increased to $0.55\mu\text{g g}^{-1}$ on the 10th day and then reduced to $0.12\mu\text{g g}^{-1}$ in the 15 days.

The persistence study of α and β isomers of endosulfan revealed that the α -isomer decreased to 62.72% of initial deposits (Fig. 7a) after 5d and the corresponding value for β -isomer was 24.59 per cent. After 10 days α -isomers and β -isomer declined by 95.47 and 56.58%, respectively. Endosulfan sulphate was first detected on 5th day after spraying and it gradually increased reaching its maxima by 10th day, thereafter, it dissipated slowly by 15th day (Fig. 7b).

The sharp decline in residues of endosulfan initially could be attributed to the weathering factors. The alpha isomer degraded faster than β -isomer during the first 7 days, but, later on the degradation of the two isomers was nearly the same. Thus in the fruit samples of tomato, the degradation pattern of different isomers of endosulfan and its metabolite was $\alpha > \beta > \text{sulfate}$. Similar trend of dissipation was observed on tomato (Antonious *et al.*, 1998) sunflower and cowpea (Kumari *et al.*, 1996), soybean (Pal and Handa, 1994), chickpea (Sukul and Handa, 1984) pigeon pea (Tanwar and Handa, 1998) leaves (Harrison *et al.*, 1967) plant surface (Maier Bode H, 1966) and grape vine (Singh and Chawla, 1978).

The dissipation pattern of endosulfan on tomato fruits followed the first order kinetics (Fig. 7c). The half life values of endosulfan (Table 21b) on tomato was 3.3 days, when applied @ 350g a.i. ha^{-1} . Antonious *et al.* (1998) reported the half-life of α and β isomers as 1.5 and 3.3 days, respectively.



Our result revealed the similar observation. The maximum residue limit (MRL) of endosulfan on vegetables as assigned by Codex Alimentarius Commission (2000) is 2 ppm. The waiting period, therefore, for the recommended dose of endosulfan on tomato was found as one day.

4.2.1.1.c Chlorothalonil

The per cent dissipation of chlorothalonil and their residues in the tomato fruits are presented in the Table 21c.

Table 21c. Residues and per cent dissipation of chlorothalonil in tomato fruits

Interval (days)	Chlorothalonil residues ($\mu\text{g g}^{-1}$)			Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Dissipation
	R ₁	R ₂	R ₃		
0	0.562	0.528	0.506	0.532 $\pm$ 0.028	-
1	0.397	0.401	0.405	0.401 $\pm$ 0.004	24.62
3	0.286	0.282	0.299	0.289 $\pm$ 0.008	45.68
5	0.252	0.254	0.250	0.252 $\pm$ 0.002	52.63
7	0.204	0.201	0.201	0.202 $\pm$ 0.001	62.03
10	0.162	0.158	0.169	0.163 $\pm$ 0.005	69.36
15	0.014	0.020	0.017	0.017 $\pm$ 0.003	96.80
20	BDL	BDL	BDL	-	-
SEm $\pm$			0.01		
CD at 5%			0.03		
Y = -0.0868X+2.7852					
R ² = 0.8721					
T _{1/2} = 3.5 days					

The fruit samples of tomato were collected an hour after application when the spray solution on the plant had completely dried. The residue data revealed that the initial deposit of chlorothalonil (Kavach[®], 75WP) was 0.532 ppm when applied @ 0.5 kg ha⁻¹. The residues gradually declined with time and became non-detectable (< 0.001 ppm) on 20th day (Fig. 8a). The

Fig.8a Persistence of chlorothalonil in tomato fruits

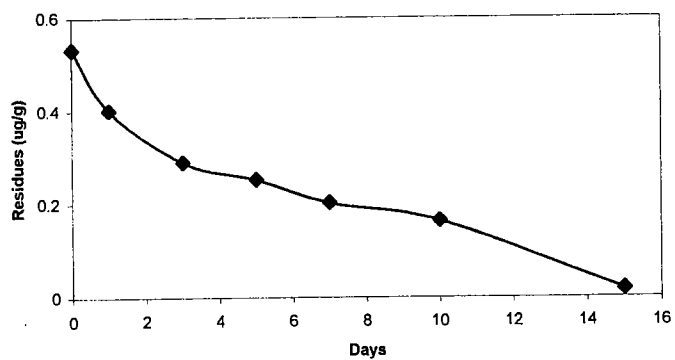


Fig.8b Dissipation of chlorothalonil in tomato fruits

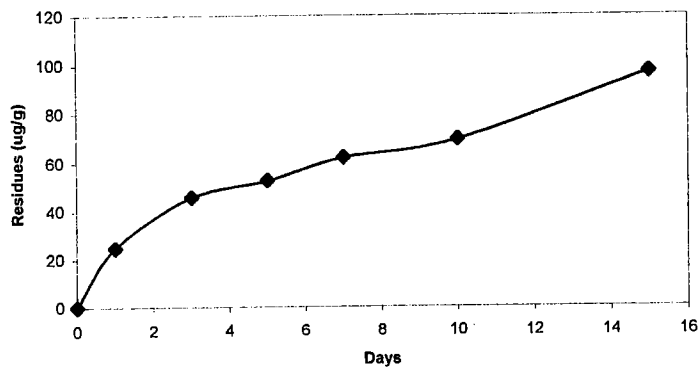
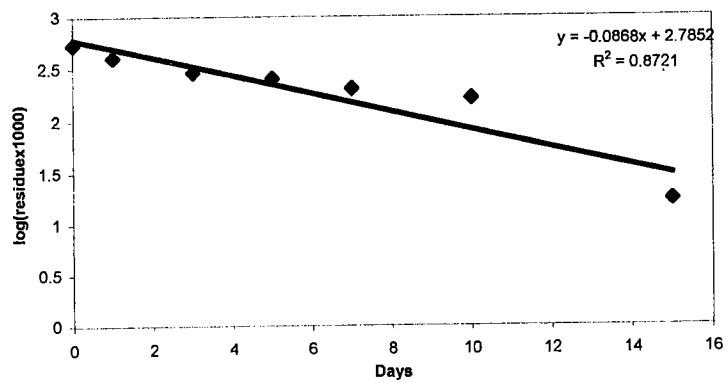


Fig.8c Logarithmic plots of chlorothalonil residues in tomato fruits



dissipation of chlorothalonil after first day of application was 24.62% which was further dissipated to 45.68% within 3rd day (Fig. 8b). On the 10th and 15th day there was 0.163 and 0.017 ppm of residues in fruits which was equivalent to about 70 and 97 per cent of dissipation. The results connots that the rate of loss was faster during 10-20 days than in the initial period of 0-10 days. The observation was very much similar to the observation of Agnihotri *et al.* (1995) in potato and tomato crop.

The loss of chlorothalonil followed a first order kinetics with a dissipation model $Y = -0.0868X + 2.7852$ and the half life was 3.48 days when applied @ 0.5 kg ha⁻¹ (Table21c). The correlation co-efficient was $r = -0.934$ (Fig. 8c). Since the maximum residue limit of chlorothalonil on tomatoes is 5 mg/kg (Codex Alimentarius Commission, 2000), a safe waiting period of one day can be safely recommended for use of chlorothalonil 75 WP on tomato crop applied @ 0.5 kg a.i. kg⁻¹. No residue of metabolic product was found in the same upto 15 days sample.

4.2.1.2. Persistence of pesticides (Module -I) in/on tomato fruits when applied sequentially

The sequential application of imidacloprid followed by chlorothalonil and endosulfan at the normal dose on the tomato crop was studied in the tomato field and the data on average individual residues from triplicate samples and their per cent dissipation from fruits is presented in Table 22. The results revealed that the quantity of pesticides recovered from tomato fruits at different intervals and their corresponding per cent losses.

Table 22. Residues and per cent dissipation of pesticides in/on tomato fruit when applied in sequence of imidacloprid-chlorothalonil-endosulfan

Interval (Days)	Average residues recovered ($\mu\text{g g}^{-1}$)				Dissipation (%)			
	Imida	Chloro	Endo		Imida	Chloro	Endo	
			α	β			α	β
0	1.523	0.652	2.751	1.176	-	-	-	-
1	1.425	0.601	2.412	1.121	6.43	7.82	12.32	4.68
3	1.024	0.562	1.243	1.053	32.76	13.80	54.82	10.46
5	0.856	0.500	0.952	0.853	43.80	23.31	65.39	27.47
7	0.684	0.421	0.524	0.566	55.09	35.43	80.95	51.87
10	0.478	0.325	0.096	0.429	68.61	50.15	96.51	63.52
15	0.321	0.056	0.01	0.121	78.92	91.41	99.63	89.71

The initial concentration of imidacloprid, chlorothalonil, α and β endosulfan in/on the tomato fruits were 1.523, 0.652, 2.751 and 1.176 ppm, respectively (Fig. 9).

The indepth analysis of data revealed the presence of high concentration of imidacloprid initial stage of the plants. The high concentration might be due to application technique which provided a high proximity between the insecticide and young plant. The other vital reason being the high systemic activity of imidacloprid. The initial concentration of 1.523 ppm declined with time elapse and reached to 0.478 ppm after 10 days of treatment. The concentration was found sufficient to control aphid, whitefly and fruit borer population. Thus observation is in agreement with findings of Moriya *et al.* (1992) who reported that as low as 0.3 mg kg⁻¹ (ppm) residues of imidacloprid were enough to check the insect population. The residues declined further and were found to be 0.321 ppm after 15 days of application. The half life of imidacloprid on tomato fruits worked out as 6.5 days.

Fig.9 Persistence pattern of pesticides in tomato fruits when applied sequentially

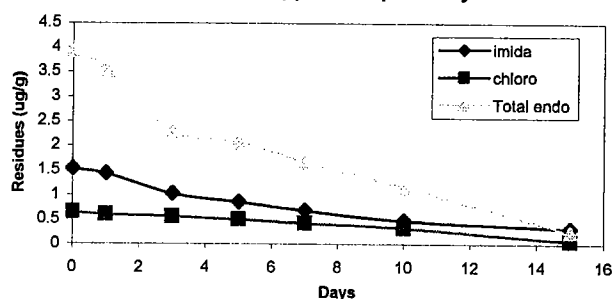


Fig.10a Persistence pattern of pesticides in tomato fruits when applied as mixture

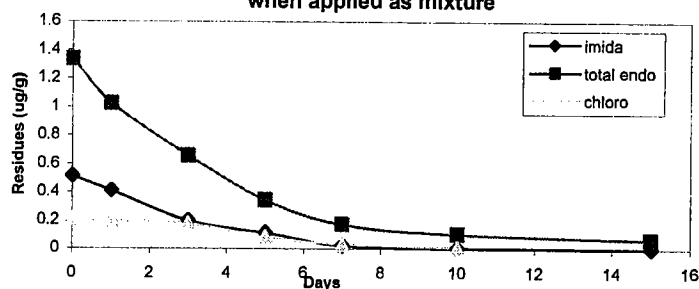


Fig.10b Dissipation of pesticides when applied as mixture

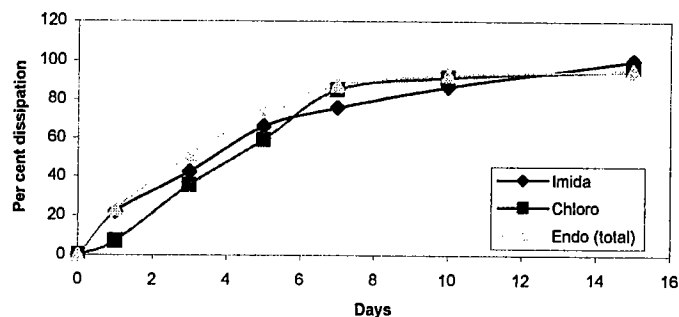
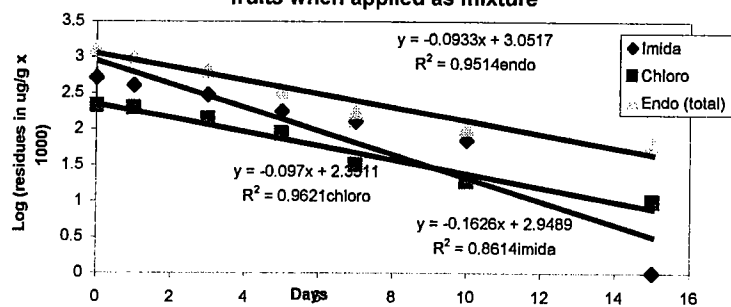


Fig.10c Logarithmic plots of pesticide residues in tomato fruits when applied as mixture



The chlorothalonil concentration declined over time from 0.652 ppm initial concentration to 0.601, 0.562, 0.50, 0.421, 0.325 and 0.056 ppm in 1, 3, 5, 7, 10 and 15 days, respectively. The initial residue was far below than the MRL (5 ppm). There was over 90 per cent dissipation of chlorothalonil from the tomato fruit after 15 days. The waiting period observed was 7 days.

Endosulfan concentration was highest among all the pesticides in the fruits. The initial concentration of 2.751 ppm (alpha) declined to 2.412, 0.952, 0.096 and 0.01 ppm in 1, 5, 10 and 15 days, respectively. At the 15th day the dissipation of endosulfan was 99.63 per cent. Beta endosulfan dissipated at much lower rate from tomato fruits. The initial concentration of 1.176 ppm beta isomer dissipated at 1.121, 0.853, 0.429 and 0.121 ppm in 1, 5, 10 and 15 days, respectively. At the 15th day the dissipation of beta endosulfan was 89.71 per cent from the tomato fruits.

The sequential application of imidacloprid, chlorothalonil and endosulfan on tomato caused the dissipation of pesticides which followed the first order Kinetics. The half-lives of imidacloprid, chlorothalonil and endosulfan were 6.5, 4.7 and 4.1 days, respectively when applied sequentially (Table 24). Therefore, sequential application of these three pesticides increased the persistence slightly than the individual application. This may be due to longer retention (slower metabolism) of the pesticides in the plant system due to continuous chemical interference.

4.2.1.3. Persistence of pesticides (Module I) in /on tomato fruits when applied as mixture

The application of the tank mixture of imidacloprid + chlorothalonil + endosulfan at the rate of one-third of their normal dose to the tomato crop was studied in the tomato fruits and data on average individual residues from triplicate samples and their per cent dissipation is presented in Table 23.

Table 23. Residues and per cent dissipation of pesticide when applied as mixture (Imidacloprid + Endosulfan + chlorothalonil at one third rate of application) in tomato fruits

Pesticide Component	Interval (days)	Residues ($\mu\text{g g}^{-1}$)			Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Dissipation (%)
		R ₁	R ₂	R ₃		
Imidacloprid	0	0.480	0.520	0.540	0.513 $\pm$ 0.031	-
	1	0.397	0.403	0.403	0.401 $\pm$ 0.003	21.83
	3	0.288	0.301	0.302	0.297 $\pm$ 0.007	42.11
	5	0.173	0.180	0.172	0.175 $\pm$ 0.004	65.87
	7	0.120	0.125	0.133	0.126 $\pm$ 0.006	75.44
	10	0.07	0.072	0.068	0.070 $\pm$ 0.002	86.35
	15	BDL	BDL	BDL	-	-
Chlorothalonil	0	0.215	0.218	0.203	0.212 $\pm$ 0.008	-
	1	0.185	0.202	0.204	0.197 $\pm$ 0.010	7.08
	3	0.132	0.138	0.141	0.137 $\pm$ 0.004	35.38
	5	0.136	0.130	0.130	0.087 $\pm$ 0.003	58.96
	7	0.070	0.063	0.062	0.032 $\pm$ 0.004	84.91
	10	0.019	0.016	0.022	0.019 $\pm$ 0.003	91.04
	15	0.009	0.011	0.01	0.010 $\pm$ 0.001	95.28
Endosulfan (total)	0	1.321	1.359	1.322	1.334 $\pm$ 0.022	-
	1	1.065	1.072	0.929	1.022 $\pm$ 0.081	23.39
	3	0.652	0.653	0.666	0.657 $\pm$ 0.007	50.75
	5	0.338	0.345	0.343	0.342 $\pm$ 0.004	74.36
	7	0.170	0.168	0.178	0.172 $\pm$ 0.005	87.11
	10	0.105	0.098	0.103	0.102 $\pm$ 0.003	92.35
	15	0.062	0.058	0.072	0.064 $\pm$ 0.007	95.20

The data on the persistence of pesticide mixtures in tomato fruits revealed that the initial deposits of imidacloprid, chlorothalonil and

endosulfan were 0.513, 0.212 and 1.334 ppm, respectively (Fig. 10a) The initial residues of imidacloprid declined from 0.513 to 0.401 ppm in one day, 0.297 ppm in 3 days, 0.126 in 7 days, 0.070 ppm in 10 days and became non-detectable in 15 days.

The initial deposit of chlorothalonil residue was 0.212 ppm which was further dissipated to 0.197, 0.137, 0.087, 0.032, 0.019 ppm and 0.010 ppm in 1, 3, 5, 7, 10 and 15 days, respectively. There was 95.28 per cent dissipation of chlorothalonil at 15th day after spray.

Endosulfan dissipated much faster in tomato fruits. The initial deposit 1.334 ppm declined to 1.022, 0.657, 0.342, 0.172, 0.102 and 0.064 ppm in 1, 3, 5, 7, 10 and 15 days, respectively. The corresponding dissipation were 23.39, 50.75, 74.36, 87.11, 92.35 and 95.20 per cent, respectively.

The residues of pesticides on/in tomato fruits exhibited a declining pattern as a function of time (Fig.10a). It could be seen from the dissipation curve that the rate of disappearance in/on tomato fruits were rapid during initial period which could be due to hydrolysis and dilution effect due to rapid growth of the plants. During later period degradation could be attributed to hydrolysis within plants leading to almost complete dissipation of pesticides in fruits.

The logarithmic plots of pesticide residue versus time (in sequential mixture application) obtained by fitting the regression equations has been presented in Fig. 10c. The plot indicated that the disappearance of parent compound fitted a first-order kinetics decay curve (Table 24). The slope of the curve, correlation co-efficient, rate constant and half-life of the pesticides as obtained from the regression equations are summarized in Table 24.

Table 24. Regression equations, rate constant, correlation co-efficients and half-lives of pesticides in sequential and mixture application on tomato

Condition	Pesticide	Dissipation model	Rate constant- K (days ⁻¹)	Correlation coefficient (r)	Half-life (days)
Sequential application	Imidacloprid	Y=-0.0463X+3.1712	0.1066	-0.995	6.5
	Chlorothalonil	Y=-0.0637X+2.9342	0.1467	-0.907	4.7
	Endosulfan	Y=-0.0736X+3.649	0.1695	-0.967	4.1
Mixture application	Imidacloprid	Y=-0.0864X+2.7037	0.1989	-0.998	3.5
	Chlorothalonil	Y=-0.097X+2.3511	0.2234	-0.980	3.1
	Endosulfan	Y=-0.0933X+3.0517	0.2149	-0.975	3.2

Application of the mixture caused slightly faster dissipation as compared to sequential application. However, the half-lives were very much closer to the half-lives of pesticides in individual application. Thus, application of the mixture did not exceed the persistence which was our concern for the safety aspects. Therefore, the pesticide mixture can safely be used for the vegetables like tomato.

4.2.1.4. Persistence of pesticides (Module-II) in/on tomato fruits when applied individually

4.2.1.4.a. Triazophos

The per cent dissipation of triazophos and their residues in the tomato fruits treated at the level of 0.7 kg a.i. ha⁻¹ are presented in the Table 25.

Table 25. Residues and per cent dissipation of triazophos in tomato fruits

Interval (days)	Residues (µg g ⁻¹)			Average residues (µg g ⁻¹ ± SD)	Dissipation (%)
	R ₁	R ₂	R ₃		
0	0.080	0.040	0.040	0.053±0.023	-
1	0.036	0.041	0.037	0.038±0.003	28.30
3	0.024	0.028	0.029	0.027±0.002	49.06
5	0.019	0.018	0.017	0.018±0.001	66.04
7	0.016	0.016	0.013	0.015±0.001	71.70
10	0.013	0.010	0.007	0.010±0.003	81.13
15	BDL	BDL	BDL	-	100.00
SEm±			0.01		
CD at 5%			0.02		
Y = -0.0702 + 1.6653X, R ² = 0.9748, T _{1/2} = 4.3 days					

Fig.11a Persistence of Triazophos in tomato

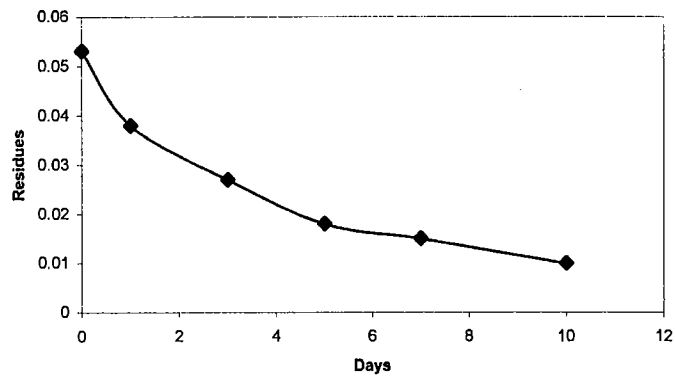


Fig.11b Per cent dissipation of triazophos in tomato fruits

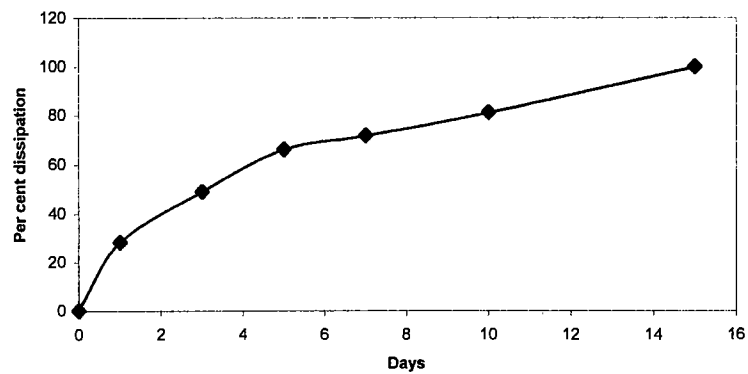
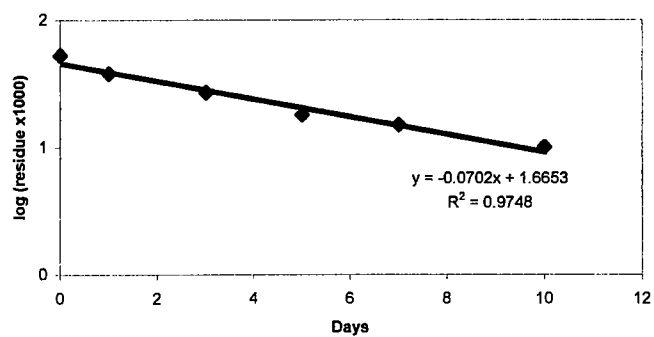


Fig.11c Logarithmic plots of triazophos residues in tomato fruits



The initial deposit of $0.053 \mu\text{g g}^{-1}$ of Triazophos on tomato fruits dissipated to 0.038, 0.019, 0.013, 0.010, 0.005 and below detection level at 1, 3, 5, 7, 10 and 15 days, respectively (Table 25 and Fig. 11a). The corresponding dissipation was 28.30, 64.15, 75.47, 81.13, 90.57 and 100%, respectively (Fig. 11b). The half life ($T_{1/2}$) value was calculated as 4.3 days (Table 25). The residue reached below MRL (0.20mg kg^{-1}) in one day. Hence, it is suggested that tomatoes can be harvested one day after application of triazophos when applied @ $700 \text{ g a.i. ha}^{-1}$. The persistence pattern of triazophos at 0.05% and 0.1% however, spray required a waiting period of 7 to 10 days (IIHR, Annual Report), perhaps because of the sub-tropical weather condition in Bangalore region.

4.2.1.4.b. β -cyfluthrin

The initial concentration of β -cyfluthrin residues from triplicate samples and their dissipation in tomato fruits treated at the level of $12.5\text{g a.i. ha}^{-1}$ are presented in Table 26.

Table 26. Residues and per cent dissipation of β -cyfluthrin in tomato fruits

Interval (days)	Residues (µg g ⁻¹)			Average residues (µg g ⁻¹ ± SD)	Dissipation (%)
	R ₁	R ₂	R ₃		
0	0.081	0.039	0.058	0.059±0.021	-
1	0.052	0.043	0.045	0.047±0.004	20.34
3	0.035	0.033	0.028	0.032±0.003	45.76
5	0.023	0.021	0.016	0.020±0.003	66.10
7	0.008	0.010	0.012	0.010±0.002	83.05
10	0.003	0.004	0.005	0.004±0.001	93.22
15	BDL	BDL	BDL	-	-
SEm±			0.01		
CD at 5%			0.02		

Y = -0.1165X+1.8133

R² = 0.9886

T_{1/2} = 2.6 days

Fig.12a Persistence of Beta-cyfluthrin in tomato fruits

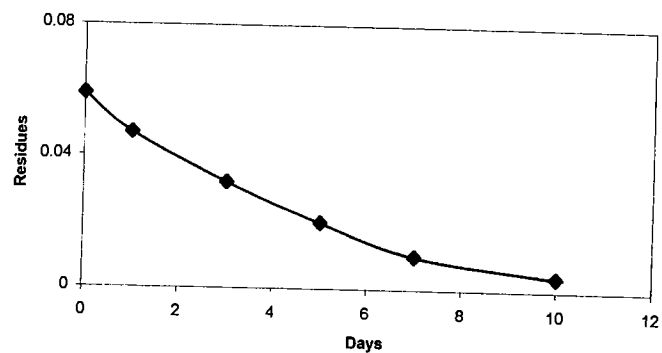


Fig.12b Per cent dissipation of beta-cyfluthrin in tomato fruits

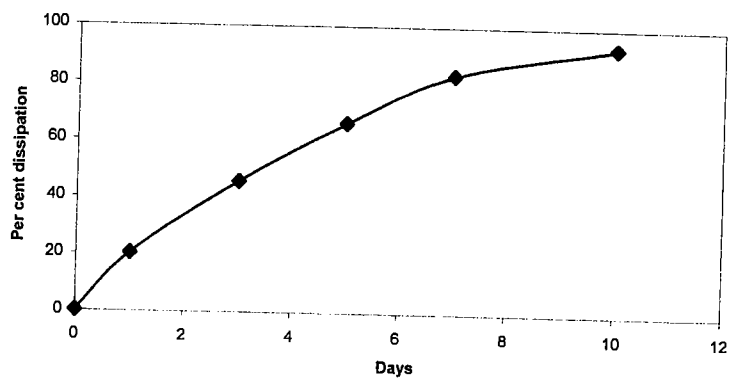
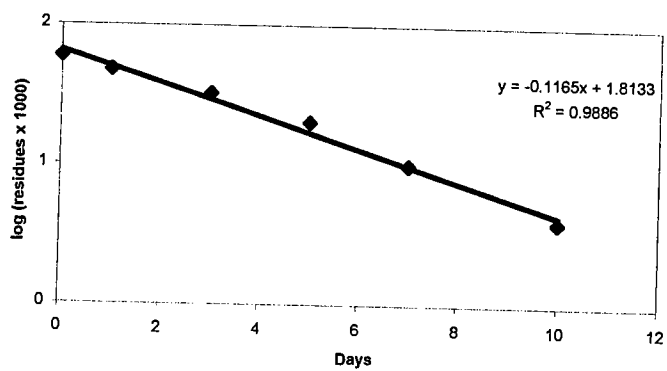


Fig.12c Logarithmic plot of beta-cyfluthrin in tomato fruits



The spray of β -cyfluthrin on tomato crop at the time of flowering resulted in the initial deposit $0.059 \mu\text{g g}^{-1}$. The initial concentration declined further to $0.047 \mu\text{g g}^{-1}$ in 1d, $0.004 \mu\text{g g}^{-1}$ in 3d, $0.003 \mu\text{g g}^{-1}$ in 7 days, 0.002 in 10 days and below detection limit after 15 days (Table 26 and Fig. 12a). There was only 20.34% dissipation of β -cyfluthrin 1 day after application but drastic dissipation of 93.32% was observed within 3 days (Fig. 12b). This may be attributed to the rainfall (28.2cm) 2 days after application. From the 5th day onwards the rate of dissipation was much lower and it was 93.22, 94.92, 96.61 on 5th, 7th and 10th day, respectively. No residue could be detected 15 days after spray.

The relation between the residue present and time after application followed 1st order kinetics (Fig 12c). The results indicate that the decline of β -cyfluthrin residue showed a high correlation with time (days) intervals. Moreover, from the regression equation, it was observed that the time taken for residue to dissipate by half was 2.6 days (Table 26). The residue were below MRL (0.5 ppm) on first day. The waiting period of one day is suggested for harvesting of tomato fruits.

4.2.1.5. Persistence of pesticides (Module II) in/on tomato fruits when applied as mixture

The application of the tank-mix of endosulfan +triazophos + beta-cyfluthrin at the rate of one-third of their individual dose to the tomato crop was studied in the tomato fruits and data on average individual residues from triplicate samples and their per cent dissipation is presented in Table 27.

Perusal of data from the table revealed that the initial deposit of endosulfan, triazophos and beta-cyfluthrin were 3.716, 0.059 and 0.043 ppm. The endosulfan residues progressively reduced to 3.152, 2.243, 1.429,

1.476, 0.953 and 0.282 ppm after 1, 3, 5, 7 10 and 15 days, respectively. The 1st day dissipation 15.18 per cent enhanced to 92.41 per cent within 15 days.

Dissipation of triazophos was quite faster. The initial deposit of 0.059 ppm triazophos dissipated quickly to 0.042, 0.031, 0.022, 0.016 and 0.012 ppm in 1, 3, 5, 7, 10 and 15 days, respectively. Residues declined by almost 80 per cent after 15 days of treatment.

The data on the persistence of residues and dissipation of β -cyfluthrin as mixture component in Table 27 revealed that the initial deposit was 0.043 ppm. Which dissipated gradually to 0.025, 0.016, 0.008 and 0.02 ppm in 1, 3, 5, 7, 10 and 15 days, respectively. The corresponding dissipation was 23.26, 41.86, 62.79, 81.40 and 95.35 per cent.

The residues of pesticides in/on tomato fruits exhibited a declining pattern as a function of time. The dissipation of each pesticide component was rapid during initial period of application but declined thereafter. The logarithmic plots of pesticides residues versus time (days) obtained by fitting the regression equations has been presented in Table 28. The plot indicated the disappearance of parent compound fitted a first-order kinetics decay curve. The slope of the curve, correlation co-efficient, rate constant and half-lives pesticides as obtained from regression equation are summarized in Table 28.

Table 28. Regression equations, rate constant, correlation co-efficients and half-lives of pesticides in mixture application on tomato in Module II.

Pesticide	Dissipation model	Rate constant (K, days ⁻¹)	Correlation coefficient (r)	Half-life (days)
Endosulfan	Y=-0.0706X+3.5999	0.1626	-0.986	4.3
Triazophos	Y=-0.068+1.7133	0.1566	-0.988	4.4
Beta-cyfluthrin	Y=-0.1274X+1.7117	0.2934	-0.976	2.4

The half-life of endosulfan was 4.3 which was at par with the sequential application of endosulfan in module-I. Triazophos, and beta-cyfluthrin dissipated with the same half-life as in the case of individual application (4.2 and 2.6, respectively). Therefore, application of mixture did not hampered the normal persistence of the pesticides and thus gave control over the pest species.

Table 27. Persistence of pesticide when applied as mixture (Module-II)

Pesticide component	Interval (days)	Residues ($\mu\text{g g}^{-1}$)			Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Dissipation (%)
		R ₁	R ₂	R ₃		
Endosulfan	0	3.720	3.715	3.713	3.716 $\pm$ 0.003	-
	1	3.150	3.152	3.154	3.152 $\pm$ 0.002	15.18
	3	2.240	2.246	2.243	2.243 $\pm$ 0.003	39.64
	5	1.935	1.928	1.924	1.929 $\pm$ 0.005	48.09
	7	1.468	1.465	1.495	1.476 $\pm$ 0.016	60.28
	10	0.953	0.981	0.925	0.953 $\pm$ 0.028	74.35
	15	0.280	0.276	0.290	0.282 $\pm$ 0.007	92.41
Triazophos	0	0.053	0.060	0.065	0.059 $\pm$ 0.006	-
	1	0.042	0.045	0.039	0.042 $\pm$ 0.003	28.81
	3	0.030	0.028	0.035	0.031 $\pm$ 0.003	47.46
	5	0.024	0.026	0.016	0.022 $\pm$ 0.005	62.71
	7	0.016	0.015	0.017	0.016 $\pm$ 0.001	72.88
	10	0.014	0.012	0.010	0.012 $\pm$ 0.002	79.66
β -cyfluthrin	0	0.045	0.038	0.045	0.043 $\pm$ 0.004	-
	1	0.039	0.026	0.033	0.033 $\pm$ 0.006	23.26
	3	0.025	0.023	0.027	0.025 $\pm$ 0.002	41.86
	5	0.014	0.018	0.016	0.016 $\pm$ 0.002	62.79
	7	0.007	0.005	0.012	0.008 $\pm$ 0.003	81.40
	10	0.001	0.003	0.002	0.002 $\pm$ 0.001	95.35

4.2.2. Persistence in soil

4.2.2.1. Individual pesticides

4.2.1.2.a Pendimethalin

The average of pendimethalin residues from triplicate sample and their per cent dissipation in the field are presented in the Table 29a. The results indicated the quantity of herbicide recovered at different intervals and their corresponding per cent losses.

Table 29a. Residues and per cent dissipation of pendimethalin in the tomato field soil

Interval (days)	Pendimethalin residues ($\mu\text{g g}^{-1}$)			Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Dissipation (%)
	R ₁	R ₂	R ₃		
0	0.667	0.702	0.698	0.689±0.019	-
5	0.546	0.529	0.521	0.532±0.012	22.79
10	0.352	0.347	0.369	0.356±0.011	48.33
20	0.237	0.229	0.233	0.233±0.004	66.18
30	0.196	0.203	0.204	0.201±0.004	70.83
40	0.118	0.116	0.129	0.121±0.007	82.44
90	BDL	BDL	BDL	-	-
SEm±			0.01		
CD at 5%			0.03		
Y = -0.0179X+2.7922					
R ² = 0.966					
T _{1/2} = 16.8 days					

The initial concentration of pendimethalin in field soil was 0.689 ppm which dissipated gradually in soil to 0.532, 0.356, 0.233, 0.201, 0.121 and below detection limit after 5, 10, 20, 30, 40 and 90 days after application, respectively.

The residues of pendimethalin in soil under field conditions exhibited a declining pattern as a function of time (Fig. 13a). It could be seen from the

data that the rate of dissipation was rapid during the initial 5 days when dissipation was of the order of 22.79 per cent. This followed with 48.33 per cent dissipation in next 5 days. During this period maximum temperature was 29°C, relative humidity of 82 per cent and a rainfall of 12.3mm. The high humidity aided with high temperature might have caused rapid disappearance (per cent) of the herbicide in this period. There was overall 70.83 per cent dissipation after 30 days, 82.44 per cent after 40 days, 100 per cent after 90 days (Figure 13b). The alike environment conditions (temperature, humidity, rainfall etc.) prevailing during the tenure upto 40 days after pendimethalin spray must have resulted almost complete disappearance of the herbicide from the field. In the field, losses due to adsorption, microbial degradation, photodecomposition, removal by plants and/or volatilization were also possible. Walker and Bond (1977) also found that the DT_{50} of pendimethalin in sandy loam soil was inversely related to temperature. The $0.121 \mu\text{g g}^{-1}$ residue persisted in the tomato soil after 40 days of herbicide application. At the end of 40 days 17.56 per cent residues remained in soil which dissipated to below detection level after 90 days.

The logarithmic plots of herbicide residues versus time obtained by fitting the regression equation has been presented in Figure 13c. The plot indicated the disappearance of parent compound fitted a first order kinetics decay curve well ($r = -0.983$) suggesting that the rate of dissipation of pendimethalin was proportional to concentration of the herbicide remaining in soil at a given time. The data fitted well the regression equation $Y = -0.0179X + 2.7922$ and had a half-life of 16.8 days. However, Diwan *et al.* reported that half-life of pendimethalin in onion field soil as 29.5 days when applied as 1.00 kg ha^{-1} . The slope of the curve, correlation co-efficient, rate constant and half life of the herbicide as obtained from the regression equation are summarized in Table 29a.

Pendimethalin has strong bonding with soil colloids, thus only little amount is lost to sub soil by leaching. Also strong adsorption on soil particles renders the chemical less prone to degradation by physical as well as biochemical action. Smith *et al.* (1995) reported only 70% degradation applied pendimethalin after 350 d in field soil. The loss of pendimethalin was less rapid from the surface soil (Savage and Jordan, 1978) it was relatively less volatile than trifluralin (Savage and Jordan, 1980). Walker and Bond (1977) have reported the half-life of 98d at 30°C in a sandy loam soil. However, Tripathi *et al.* (1993) reported pendimethalin residue upto 60d in a clay loam soil.

4.2.2.1.b Imidacloprid

The average of imidacloprid residues from triplicate samples and their per cent dissipation in the field are presented in Table 29b. The results indicated the quantity of insecticide recorded at different intervals and their corresponding per cent losses.

Table 29b. Residues and per cent dissipation of imidacloprid in the tomato field soil

Interval (days)	Imidacloprid residues ($\mu\text{g g}^{-1}$)			Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Dissipation
	R ₁	R ₂	R ₃		
0	0.540	0.543	0.543	0.542 $\pm$ 0.001	-
1	0.506	0.510	0.49	0.502 $\pm$ 0.011	7.38
3	0.462	0.463	0.470	0.465 $\pm$ 0.004	14.21
5	0.428	0.427	0.441	0.432 $\pm$ 0.007	20.30
7	0.395	0.403	0.405	0.401 $\pm$ 0.005	26.01
10	0.338	0.348	0.340	0.342 $\pm$ 0.005	36.90
20	0.297	0.311	0.295	0.301 $\pm$ 0.008	44.46
40	0.175	0.186	0.188	0.183 $\pm$ 0.007	66.24
SEm $\pm$			0.01		
CD at 5%			0.03		

$$Y = -0.0112X + 1.5958,$$

$$R^2 = 0.9636,$$

$$T_{1/2} = 26.9 \text{ days}$$

Fig.14a Persistence of imidacloprid under tomato field soil

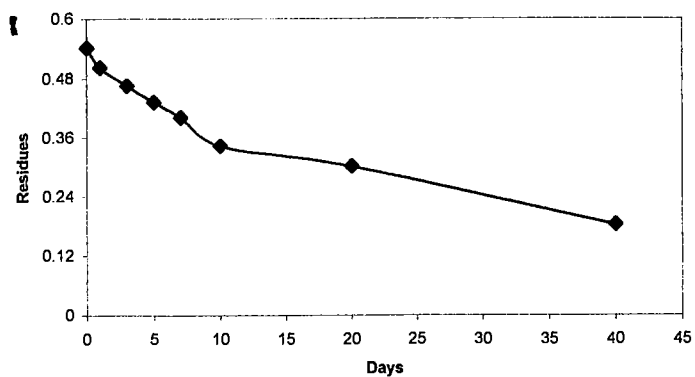


Fig.14b Dissipation of imidacloprid in tomato field soil

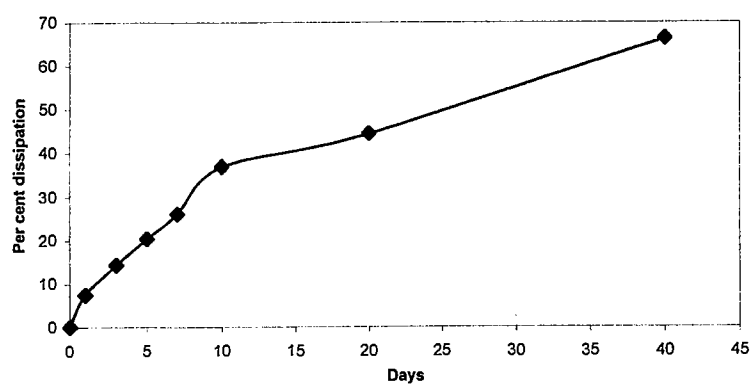
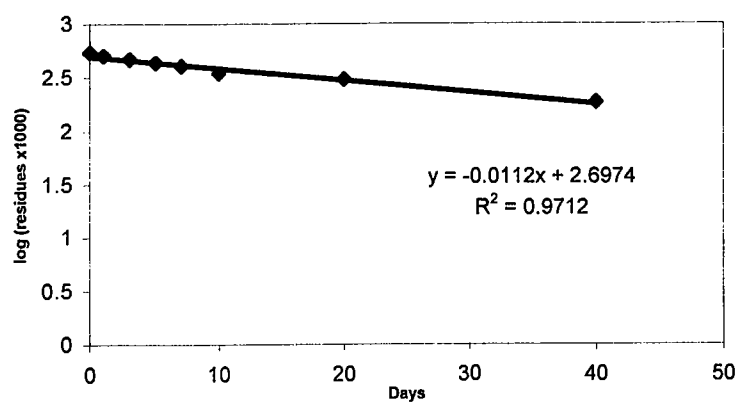


Fig.14c Logarithmic plots of Imidacloprid residues in tomato field soil



The initial concentration of imidacloprid in field soil was 0.542 ppm which was dissipated gradually in soil to 0.502, 0.465, 0.432, 0.401, 0.342, 0.301 and 0.183 $\mu\text{g g}^{-1}$ after 1, 3, 5, 7, 10, 20 and 40 days after application, respectively.

The residues of imidacloprid in soil under field conditions exhibited a declining pattern as a function of time (Fig. 14a). Perusal of data revealed that the rate of dissipation of imidacloprid at the initial stage was very slow especially first five days when nearly 10% (7.38 per cent) of the initial deposit dissipated but further increased to 66.24 per cent at 40 days (Fig. 14b).

The residue data for imidacloprid, when plotted for log residues versus time, fitted to a first order rate kinetics $Y = a + bX$, where 'Y' is the concentration of imidacloprid at any time, 'a' is the log concentration of imidacloprid at time zero, 'b' is the residue decline constant and 'X' is the time in days. The linear plots showing first order rate kinetics are shown in Figure 14c.

The equation yielded dissipation constants of 0.0258 per days. The correlation co-efficient (r) for first order rate equation was 0.9636 (R^2) which indicated significant negative correlation between the concentration of imidacloprid and the time. The dissipation model yielded the half-life of 26.88 days. The result was in accordance with the earlier report (Miles Inc., 1993). The theoretical predicted concentration of residues at zero days i.e., initial concentration was calculated as 0.039 which do not differ substantially than observed values 0.042. Therefore, the mathematical expression can be useful in describing the persistence of imidacloprid and can be used to predict the residues at a particular time.

4.2.2.1.c. Chlorothalonil

The per cent dissipation of chlorothalonil and it's residues in the tomato field soil are presented in the Table 29c.

Table 29c. Residues and per cent dissipation of chlorothalonil (Kavach® 75WP) the tomato field soil

Interval (days)	Chlorothalonil residues ($\mu\text{g g}^{-1}$)			Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Dissipation
	R ₁	R ₂	R ₃		
0	0.033	0.038	0.037	0.036 $\pm$ 0.002	-
3	0.030	0.034	0.032	0.032 $\pm$ 0.002	11.11
5	0.021	0.028	0.029	0.026 $\pm$ 0.004	27.78
7	0.023	0.025	0.015	0.021 $\pm$ 0.005	41.67
10	0.020	0.011	0.011	0.014 $\pm$ 0.005	61.11
20	0.002	0.006	0.007	0.005 $\pm$ 0.002	86.11
40	BDL	BDL	BDL	-	-
SEm $\pm$			0.01		
CD at 5%			0.03		
Y = -0.027X+1.5802					
R ² = 0.9643					
T _{1/2} = 11.2 days					

Results of analysis of samples drawn 2h after application and subsequently at different intervals upto 40 days are presented in Table 29c. The results revealed residues ($\mu\text{g g}^{-1}$) for three replicated field plots, average residues and per cent cumulative dissipation of chlorothalonil.

The initial concentration of chlorothalonil in soil (0-15 cm) was 0.036 $\mu\text{g g}^{-1}$ of soil which dissipated gradually 0.032, 0.026, 0.021, 0.014, 0.005 below detectable limit after 3, 5, 10, 20, 30 and 40 days of application, respectively.

The residues of chlorothalonil in soil exhibited a declining pattern as a function of time (Fig.15a). It can be seen from the dissipation curve (Fig.15b) that the rate of dissipation was rapid during initial period but declined thereafter as the time proceeded. Within 5 days of application 27.78 per cent fungicide dissipated from the field. The dissipation after 20 days and 30 days were 61.11 and 81.11 per cent, respectively which went below detectable level at 40 days. Higher rate of dissipation at initial stage of application might be attributed to the high humidity (72%) aided with high temperature (33.3°C) which influenced soil microflora to enhance microbial degradation in soil.

Fig.15a Persistence of chlorothalonil in tomato field soil

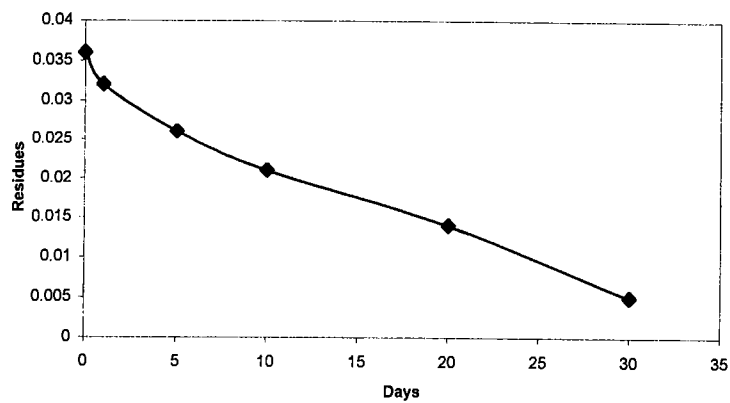


Fig.15b Dissipation of Chlorothalonil under tomato field soil

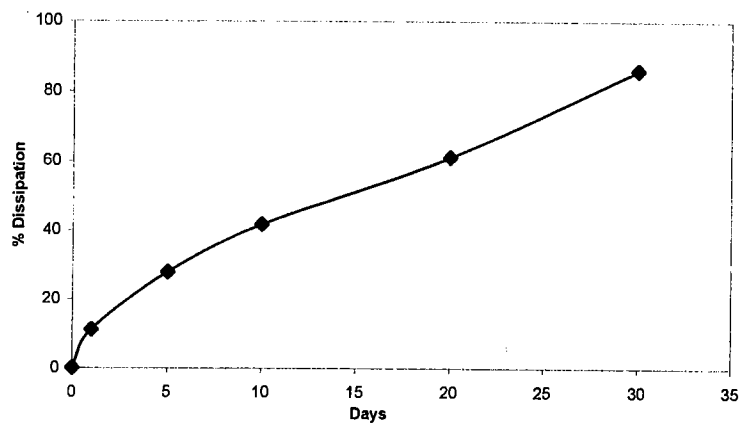
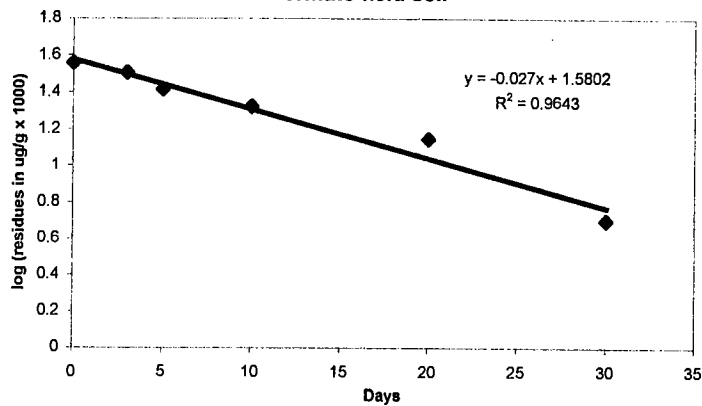


Fig.15c Logarithmic plots of chlorothalonil residues under tomato field soil



The logarithmic plots of fungicide residues versus time obtained by fitting the regression equation has been presented in Figure 15c. The plot indicated the disappearance of chlorothalonil, fitted a first order kinetics decay curve well ($r = -0.982$) suggesting that the rate of dissipation of chlorothalonil was proportional to concentration of the herbicide remaining in soil at a given time. The slope of the curve, correlation coefficient, rate constant and half-life of the compound as obtained from regression equation are summarized in Table 29c.

The initial amount of the chlorothalonil in the top soil layer (0-15 cm) was lower than expected on the basis of dosage minus the interception by the crop. Spray drift can explain a small fraction of the loss from the field (van der Pas *et al.*, 1999).

4.2.2.1.d Endosulfan

The average endosulfan residues from triplicate samples and their per cent dissipation in the field are presented in Table 29d. The results indicated the quantity of insecticide recovered at different intervals and their corresponding per cent losses.

Table 29d. Residues and per cent dissipation of endosulfan in the tomato field soil (0-15 cm) treated @ 350 g a.i. ha⁻¹

Day	Average residues recovered ($\mu\text{g g}^{-1}$)			
	Alpha-endo	Beta-endo	Endo-sulphate	Total endo
0	2.016	0.864	0	2.880
1	1.852	0.828	0	2.680
3	1.520	0.656	0.01	2.186
5	1.330	0.605	0.04	1.975
7	0.940	0.546	0.15	1.636
10	0.630	0.425	0.38	1.435
20	0.100	0.298	0.75	1.148
30	0.025	0.213	0.03	0.268
Y = $-0.0302x + 3.4583$, $R^2 = 0.9207$, $T_{1/2} = 9.9$ days				

Fig.16a Persistence of endosulphan in tomato field soil

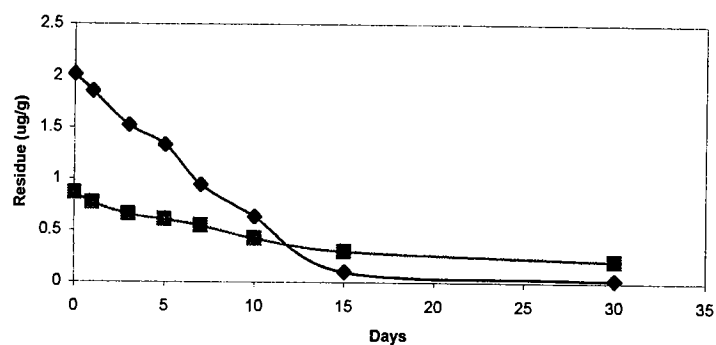


Fig.16b Dissipation of endosulphan in tomato field soil

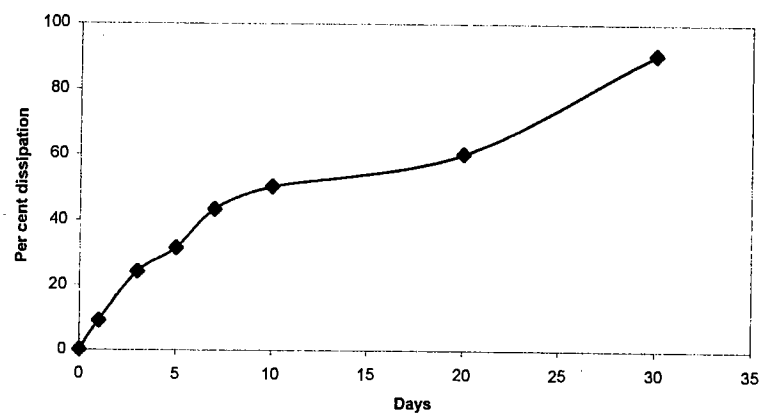
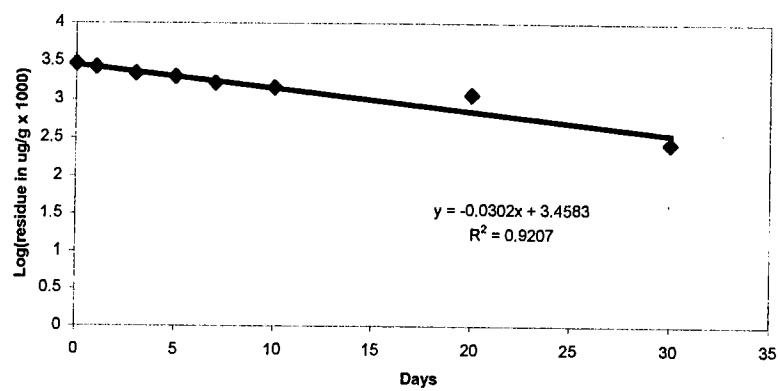


Fig.16c Logarithmic plots of endosulphan residues under tomato field soil



The data pertaining to the initial concentration of endosulfan residues from triplicate samples and its dissipation in tomato field soil treated at the rate of 350 g a.i. ha⁻¹ presented in Table 29d revealed that the initial deposit of endosulfan was 2.880 µg g⁻¹ (Fig. 16a). The residues further dissipated in soil and after 5 days there was 31.42 dissipation of endosulfan was occurred (Fig. 16b). After 10 days, there was 1.435 µg g⁻¹ of endosulfan in the top 0-15 cm soil which reduced to 1.148 µg g⁻¹ after 20 days.

The sharp decline of residue of endosulfan initially could be attributed to the weathering factors. The alpha isomer degraded faster than β-isomers but at the later stage degradation of both the isomers slowed down. The toxic isomer endosulfan sulphate formed in the concentration of sulphate initially increases and then decreased. The degradation pattern of different isomers of endosulfan and metabolite as alpha followed the same trend as tomato fruits ($\alpha > \beta > \text{sulphate}$). Kaur *et al.* (1998) reported the similar observation on endosulfan dissipation from soil.

4.2.2.2. Persistence of pesticides in tomato field soil when applied sequentially

The sequential application of pendimethalin followed by imidacloprid, chlorothalonil and endosulfan (in the same order) at the normal dose to the tomato crop was studied in the tomato field and data on average individual residues from triplicate samples and their per cent dissipation in the field are presented in Table 30. The results revealed the quantity of individual pesticides recovered from the soil at different intervals and their corresponding per cent losses.

Table 30. Residues and per cent dissipation of pesticides in tomato when applied in sequence of pendimethalin-imidacloprid-chlorothalonil and endosulfan

Interval (Days)	Average residues recovered ($\mu\text{g g}^{-1}$)				Dissipation (%)			
	Pendi	Imida	Chloro	Endo	Pendi	Imida	Chloro	Endo
0	0.754	0.153	0.613	2.312	-	-	-	-
1	0.736	0.134	0.594	2.001	2.39	12.41	3.10	6.14
5	0.582	0.114	0.482	1.426	22.81	25.49	21.37	33.11
10	0.513	0.092	0.374	0.530	31.96	39.87	38.99	75.14
15	0.426	0.083	0.310	0.314	43.50	45.75	49.43	86.42
20	0.342	0.054	0.253	0.102	54.64	64.71	58.73	95.88

The initial concentrations of pendimethalin, imidacloprid, chlorothalonil and endosulfan in the tomato were 0.754, 0.153, 0.613 and 2.312 ppm, respectively (Fig. 17). Pendimethalin dissipated gradually to 0.736, 0.582, 0.513, 0.426 and 0.342 ppm after 1, 5, 10, 15 and 20 days, respectively.

Imidacloprid was dissipated to 0.134, 0.114, 0.092, 0.083 and 0.054 after the same period of intervals, respectively.

The initial concentration of chlorothalonil 0.613 ppm in soil dissipated to 0.594, 0.482, 0.374, 0.310 and 0.253 after 1, 5, 10, 15 and 20 days.

Dissipation of total endosulfan was fairly less in sequential application and the residue range was 2.312 to 0.102 $\mu\text{g g}^{-1}$ within 20 days.

Pendimethalin dissipation was very much lower in sequential application as compared to individual application. After 5 days there was 22.79 per cent dissipation of pendimethalin, whereas, after the same intervals, the dissipation at sequential application was 2.39 per cent. After 10 days, the dissipation was 39.87 per cent, which was nearer to the dissipation of individual application (48.333 per cent). At the end of the experimental period (20 days) there was 54.64 per cent dissipation of pendimethalin from tomato field soil.

In case of imidacloprid, the dissipation was at par with individual application. There was no significant difference in the dissipation of imidacloprid upto 10 days, whether applied individually or sequentially with other pesticides (36.90 per cent and 38.99 per cent for individual and sequential) application. After 20 days, the residue of Imidacloprid in the soil was 0.054 ppm which was five time lower than individual application.

Chlorothalonil degraded much slower in the sequential application. The degradation of chlorothalonil after 10 days were 38.99 per cent in sequential application which was much below than the individual dissipation 61.11 per cent. After 20 days, the overall dissipation of chlorothalonil was 58.73 per cent depicted the slow degradation of the fungicide due to presence of different chemical environment.

Dissipation of endosulfan in sequential application was slower (Fig. 17) and than it was at par with the individual dissipation (after 7 days). Within 1 days, there was 6.14 per cent dissipation of endosulfan which further increased to 33.11 per cent on 5th day. At the end of the experiment (20 days) there was 4.41 per cent residue of endosulfan in the soil.

The logarithmic plots of pesticide residues versus time obtained by fitting the regression equations indicated the disappearance of parent compound fitted a first order kinetics decay curve well suggesting that the rate of dissipation of pesticide was proportional to the concentration remaining in soil at a given time. The regression equations and half-lives of all the pesticides presented in Table 32.

4.2.2.3. Persistence of pesticides in tomato field soil when applied as mixture

The analytical data on average individual residues and per cent dissipation of pesticides mixture [pendimethalin (P) + Imidacloprid (I) +

Chlorothalonil (C) + Endosulfan (E) abbreviated PICE] at their reduced dosage to the tomato field soil is presented in Table 31 and Fig. 18. The results revealed the quantity of individual pesticides recovered from the soil at different intervals and their corresponding per cent losses.

Table 31. Residues and per cent dissipation of pesticides in tomato field soil when applied as mixture

Interval (Days)	Average residues recovered ($\mu\text{g g}^{-1}$)				Dissipation			
	Pendi	Imida	Chloro	Endo	Pendi	Imida	Chloro	Endo
0	0.265	0.254	0.124	0.487	-	-	-	-
1	0.232	0.241	0.101	0.356	12.45	5.12	18.55	26.90
5	0.185	0.261	0.084	0.241	30.19	14.96	32.26	50.51
10	0.126	0.198	0.065	0.102	52.45	22.05	47.58	79.06
15	0.102	0.162	0.042	0.042	61.51	34.22	66.13	91.36
20	0.065	0.108	0.031	-	75.47	57.48	75.00	-

The initial concentration of pendimethalin, imidacloprid, chlorothalonil and endosulfan in the tomato field soil was 0.265, 0.254, 0.124 and 0.487 ppm, respectively. The dose of application in case of mixture, however, was taken as one-fourth of the individual applied pesticides.

Dissipation of pendimethalin was studied in the tomato field soil and the residue of pendimethalin remaining in the soil was 0.232, 0.185, 0.126, 0.102 and 0.065 ppm, respectively after 0, 1, 5, 10, 20 and 30 days of application.

After 5 days of mixture application, the dissipation was 12.45 per cent whereas the corresponding dissipation after same interval in individual and sequentially applied pendimethalin were 22.79 and 22.81 per cent. After 10 days, the dissipation for the mixture-component pendimethalin was 52.45 per cent which was higher than individual (48.33 per cent) and sequentially applied pendimethalin (31.96 per cent). At the end (20 days) the pendimethalin left was 24.53 per cent of initial residue in the soil.

Imidacloprid was also degraded faster in the field soil when applied as mixture. The initial concentration 0.254 degraded gradually to 0.241, 0.216, 0.198, 0.162 and 0.108 ppm after 1, 5, 10, 15 and 20 days.

There was 14.96 per cent dissipation of imidacloprid after 5 days of mixture application, whereas, the dissipation was 20.30 and 25.49 per cent in case of individually and sequentially applied imidacloprid, respectively. After 20 days, the dissipation in mixture was much higher (57.48 per cent) than individual (44.46 per cent) and sequential application (45.75 per cent).

Like other compounds, degradation of chlorothalonil was faster in the soil when applied as mixture. The initial residue of 0.124 ppm decreased gradually to 0.101, 0.084, 0.065, 0.042 and 0.031 after 0, 1, 5, 10, 20 and 30 days. The respective per cent dissipation was 18.55, 32.26, 47.58, 66.13 and 75.00 per cent, respectively. Whereas the dissipation of sequentially applied chlorothalonil was 3.10, 21.37, 38.99, 58.73 per cent in 1, 5, 10 and 20 days, respectively.

Dissipation of total endosulfan was also very high from the initial stage. After 5 days, there was 0.241 ppm residue and 50.51 per cent dissipation occurred. However, at the end (20 days) the dissipation was at par (91.36) with sequentially applied endosulfan (91.14 per cent).

The logarithmic plots of the pesticides residues versus time obtained by fitting regression equation indicated the disappearance of pesticides, fitted a first order kinetics decay curve well suggesting the rate of dissipation of pesticide were proportional to the concentration of pesticides remaining in soil at a given time. The slope of the curve,

correlation coefficient rate constant and half-lives of the compounds as obtained from regression equation are summarized in Table 32.

Table 32. Regression equations, rate constant, correlation co-efficients and half-lives of pesticides applied to soil as sequential and as mixture

Condition	Pesticide	Dissipation model	Rate constant- K (days ⁻¹)	Correlation coefficient (r)	Half-life (days)
Sequential application	Pendimethalin	$Y = -0.0172X + 2.8764$	0.0396	-0.989	17.5
	Imidacloprid	$Y = -0.0107X + 2.1159$	0.0246	-0.960	28.1
	Chlorothalonil	$Y = -0.0191X + 2.7748$	0.0440	-0.992	15.8
	Endosulfan	$Y = -0.0316X + 3.4145$	0.1538	-0.994	9.5
Mixture application	Pendimethalin	$Y = -0.0197X + 2.3865$	0.0454	-0.978	15.3
	Imidacloprid	$Y = -0.0118X + 2.4109$	0.0272	-0.989	25.5
	Chlorothalonil	$Y = -0.0198X + 2.0492$	0.0456	-0.988	15.2
	Endosulfan	$Y = -0.0547X + 2.6661$	0.1260	-0.988	5.5

The half-lives of sequentially and mixture applied pesticide did not differ significantly. The half-life of pendimethalin in field soil estimated by best fit as 17.5 and 15.3 days, respectively, while the half-lives of imidacloprid were varied from 25 to 28 days. Chlorothalonil dissipated with a half-life of 15.8 and 15.2 days in sequential and simultaneous application. The dissipation of endosulfan initially was quite faster. In the sequential application the half-life was 4.5 days, while in case of mixture the half-life was 5.5 days only.

Therefore, the sequential or simultaneous (mixture) application of pesticides, exactly followed the first order rate kinetics i.e., the half-lives did not depend on the initial concentration of the pesticides.

4.2.3. Persistence of pesticides in soil under laboratory condition

The average pesticide residues from duplicate soil samples and their per cent degradation in incubated soil treated at $10 \mu\text{g g}^{-1}$ level are presented in Table 33a to 33d. The results indicated the quantity of pesticide recovered at different intervals and their corresponding per cent losses.

The residues of pendimethalin and other three pesticides in laboratory condition exhibited a decreasing pattern as a function of time (Fig. 19a; 20a; 21a and 22a). It was observed from the dissipation curve (Fig. 19b) that the dissipation rate was not so rapid during the initial 5 days marked by 16.18 per cent dissipation of parent compound. This followed with 25.83 per cent dissipation in the next 5 days and 42.24 per cent in subsequent 10 days. At the end of 40 days 30.51 per cent pendimethalin residues persisted in alluvial soil under laboratory conditions which signifies that the compound is fairly persistent in soil.

Table 33a. Residues and per cent dissipation of pendimethalin in soil under laboratory condition treated @ $10 \mu\text{g g}^{-1}$

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Dissipation (%)
	R ₁	R ₂		
0	9.272	9.125	9.199 $\pm$ 0.104	-
5	7.320	8.102	7.711 $\pm$ 0.552	16.18
10	6.270	7.375	6.823 $\pm$ 0.781	25.83
20	5.282	5.343	5.313 $\pm$ 0.043	42.24
30	4.365	4.029	4.197 $\pm$ 0.238	54.38
SEm $\pm$			0.68	
CD at 5%			2.67	
Y = -0.0111X+0.9514				
R ² = 0.996				
T _{1/2} = 27.1 days				

Imidacloprid gave similar dissipation pattern (Fig. 20b) in soil under laboratory condition. The first 5 days dissipation from soil was merely 5.38

Fig.19a Persistence of pendimethalin in soil under laboratory condition

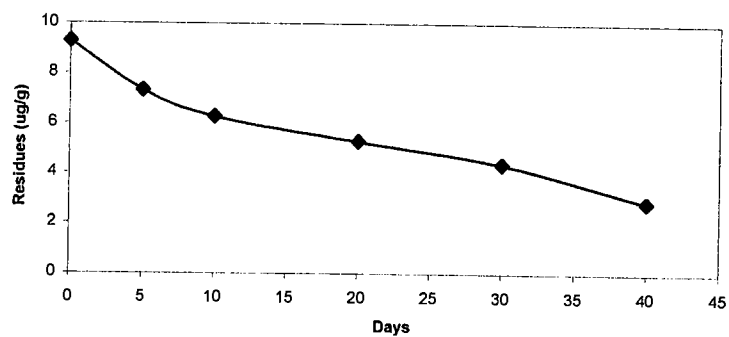


Fig.19b Dissipation of pendimethalin in soil under field and laboratory condition

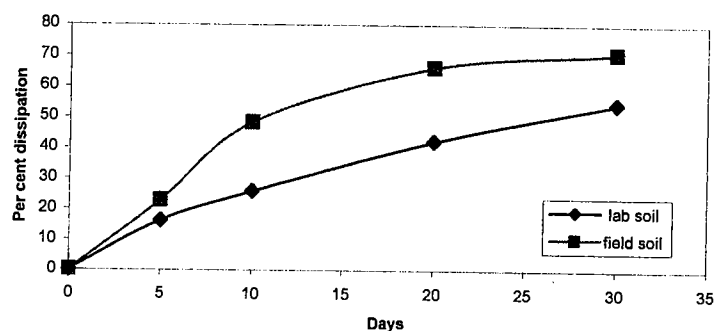
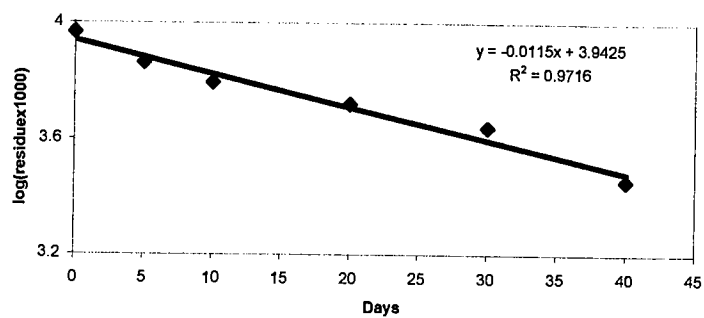


Fig.19c Logarithmic plots of pendimethalin in soil under laboratory condition



per cent. This followed with 22.24 per cent in next 5 days. After 20 days there was only 28.28 per cent dissipation but 20 to 30 days interval gave fairly higher dissipation (42.75 per cent). At the end of the experiment (40 days) there was nearly 60 per cent dissipation and nearly 40 per cent parent compound remained in the soil.

The initial concentration of pendimethalin in soil was 9.199 ppm which dissipated gradually in soil to 7.711, 6.823, 5.313 and 4.197 ppm after 5, 10, 20 and 30 days after application, respectively.

In case of Imidacloprid, the laboratory study gave the initial concentration pesticide in soil as 18.093 ppm when the soil incubated in soil under laboratory condition @ 20 ppm and the initial concentration was 18.093 ppm which dissipated gradually in soil to 17.120, 14.069, 12.977, 10.358 and 7.231 ppm and below detection level after the same period of incubation as other pesticides.

Table 33b. Residues and per cent dissipation of imidacloprid in soil under laboratory condition treated @ 20 $\mu\text{g g}^{-1}$

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Dissipation (%)
	R ₁	R ₂		
0	18.453	17.733	18.093 $\pm$ 0.509	-
5	17.297	16.943	17.120 $\pm$ 0.250	5.38
10	14.135	14.003	14.069 $\pm$ 0.093	22.24
20	12.102	13.852	12.977 $\pm$ 1.237	28.28
30	9.785	11.919	10.358 $\pm$ 1.665	42.75
40	7.015	7.447	7.231 $\pm$ 0.305	60.03
SEm $\pm$			0.59	
CD at 5%			1.68	
Y = -0.0095X + 1.2702,				
R ² = 0.9637,				
T _{1/2} = 31.7 days				

Fig.20a Persistence of imidacloprid in soil under laboratory condition

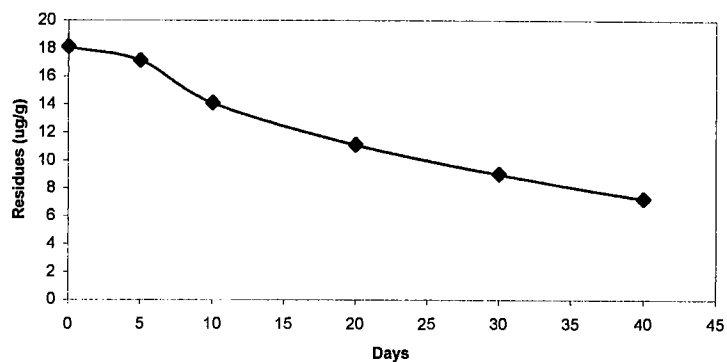


Fig.20b Dissipation of imidacloprid in soil under field and laboratory condition

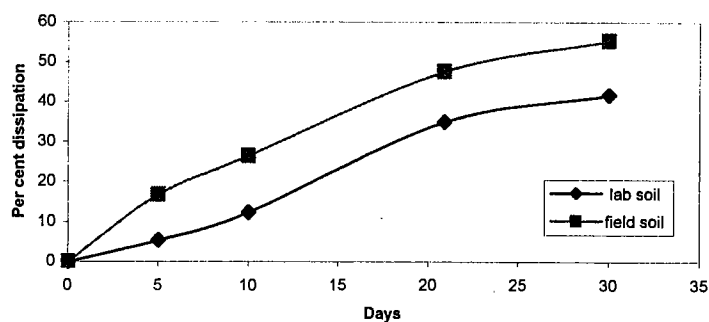
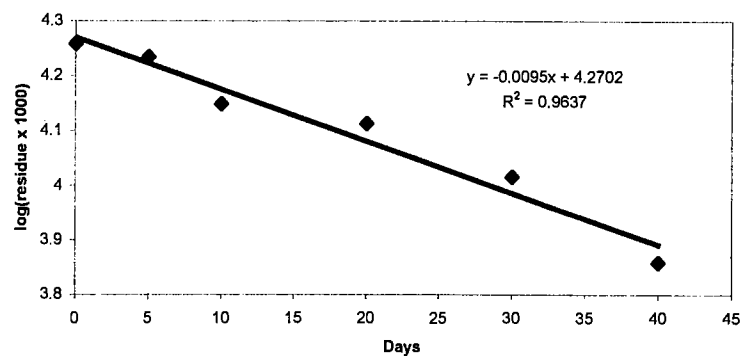


Fig.20c Logarithmic plots of imidacloprid in soil under laboratory condition



Endosulfan was dissipated quite faster in the laboratory soil. The initial residue of $8.687 \mu\text{g g}^{-1}$ declined gradually to 7.249, 5.820, 2.412 and 2.051 in 5, 10, 10 and 30 days after incubation, respectively (Table 33c and Fig. 21a).

Table 33c. Residues and per cent dissipation of endosulfan in soil under laboratory condition treated @ $10 \mu\text{g g}^{-1}$

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Dissipation (%)
	R ₁	R ₂		
0	8.250	9.123	8.687 $\pm$ 0.617	-
5	7.372	7.125	7.249 $\pm$ 0.175	16.55
10	5.750	5.890	5.820 $\pm$ 0.099	33.00
20	2.322	2.502	2.412 $\pm$ 0.127	72.33
30	2.120	1.982	2.051 $\pm$ 0.097	76.39
SEm $\pm$			0.23	
CD at 5%			0.76	
$Y = -0.0648X + 3.2125,$ $R^2 = 0.8393,$ $T_{1/2} = 4.7 \text{ days},$				

When the fungicide chlorothalonil investigated in laboratory soil for the dissipation study after the treatment at 10 ppm, the initial concentration of the pesticide was found to be 9.911 ppm. This concentration declined gradually over the elapse of time. There were 7.853, 6.224, 5.209, 4.182 and 2.434 ppm residue in soil after 5, 10, 20, 30 and 40 days after incubation of the soil, respectively.

Unlike pendimethalin and Imidacloprid, endosulfan dissipated at higher rate in the initial 10 days of incubation (33.00%). The trend was further proliferated with the dissipation of 72.33 per cent of parent compound within 20 days. After 30 days there was 2.051 ppm endosulfan residue in the soil depicted 76.39 per cent dissipation.

Fig.21a Persistence of endosulphan in soil under laboratory condition

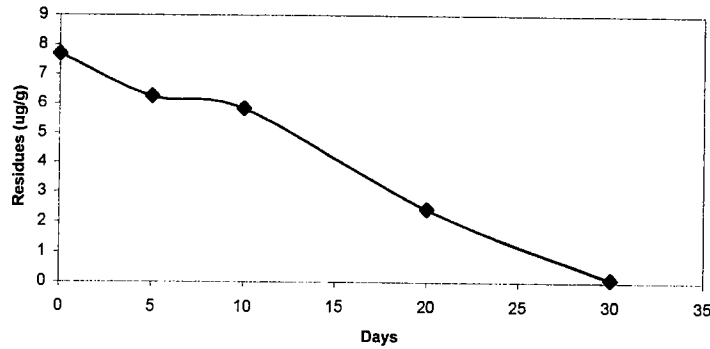


Fig.21b Dissipation of endosulphan in soil under field and laboratory condition

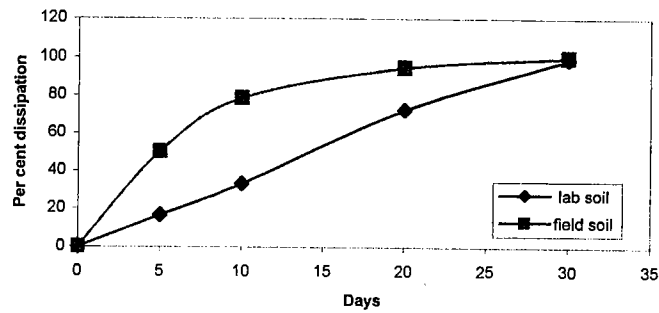
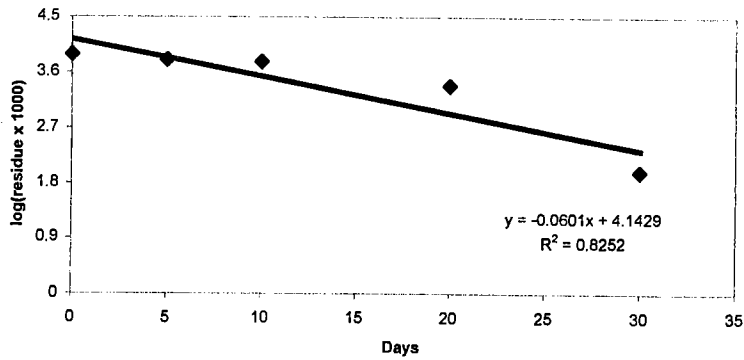


Fig.21c Logarithmic plots of endosulphan in soil under laboratory condition



Chlorothalonil dissipation trend was nearly similar to pendimethalin and imidacloprid (Fig. 22b). The initial 5 days marked 20.76 per cent dissipation from soil. This followed with 37.2 per cent dissipation in the next 5 days and 47.44 per cent in subsequent 10 days. At the end of 30 days, 42.2 per cent chlorothalonil residue persisted in alluvial soil under laboratory.

Table 33d. Residues and per cent dissipation of chlorothalonil in soil under laboratory condition treated @ 10 $\mu\text{g g}^{-1}$

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Dissipation (%)
	R ₁	R ₂		
0	9.899	9.923	9.911 $\pm$ 0.017	-
5	7.776	7.929	7.853 $\pm$ 0.108	20.76
10	6.175	6.272	6.224 $\pm$ 0.069	37.20
20	5.272	5.145	5.209 $\pm$ 0.090	47.44
30	4.110	4.253	4.182 $\pm$ 0.101	57.80
SEm $\pm$			0.06	
CD at 5%			0.17	
Y = -0.0119X+0.9596,				
R ² = 0.9548,				
T _{1/2} = 25.3 days				

The logarithmic plots of pesticides residues versus time obtained by fitting the regression equations has been presented in (Fig. 19c, 20c, 21c and 22c). The plots indicated that the disappearance of parent compound fitted a first orders kinetics decay curve suggesting that the rate of dissipation of pesticides was proportional to the concentration of pesticide remaining in soil at the given time. The slope of the curve, correlation coefficient, rate constant and half-life of the pesticides as obtained from the regression equation are summarized in respective tables. The half-life of pendimethalin, imidacloprid, endosulfan and chlorothalonil under laboratory condition as estimated by best fit was found to be 27.1, 31.7, 4.7 and 25.3 days, respectively.

Fig.22a Persistence of chlorothalonil in soil under laboratory condition

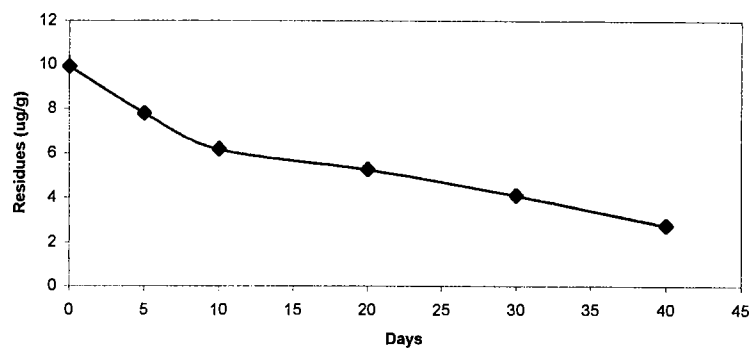


Fig.22b Dissipation of chlorothalonil in soil under field and laboratory condition

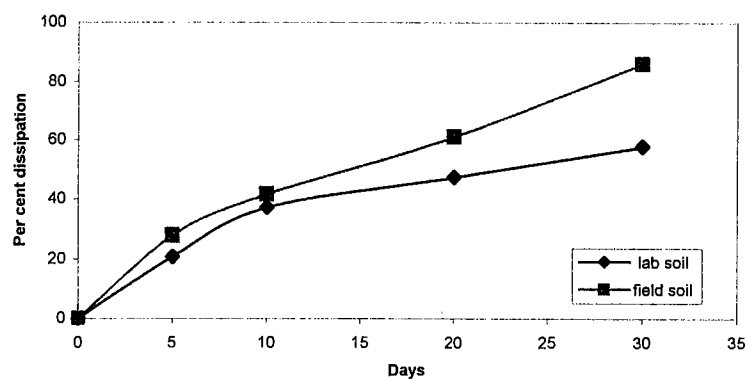
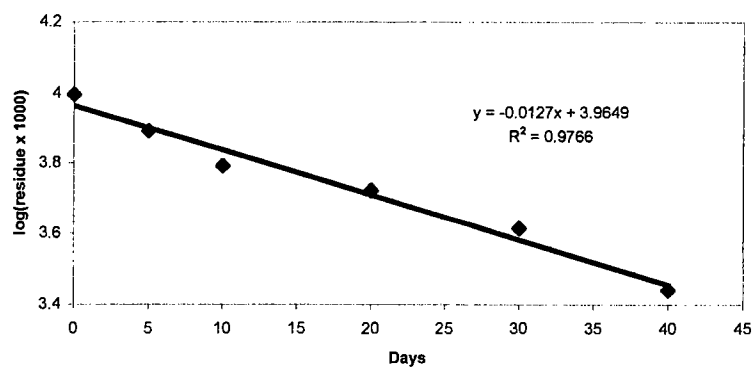


Fig.22c Logarithmic plots of chlorothalonil in soil under laboratory condition



4.2.4. Dissipation of pesticide in soil : field versus laboratory

It is evident from the results (Table 34a to 34d) that dissipation of pesticides in soil was rapid in the field as compared to laboratory conditions.

In case of pendimethalin 48.33 per cent herbicide dissipated under the field condition in 10 days. Whereas 25.83 per cent dissipated under the laboratory condition during the same period. After one month of pendimethalin application 29.17 per cent residues persisted in field against 45.62 per cent in laboratory condition (Table 34a).

Table 34a. Dissipation of pendimethalin under field and laboratory conditions

Interval (days)	Residues (per cent)		Dissipation (per cent)	
	Field	Laboratory	Field	Laboratory
0	100.00	100.00	-	-
5	77.21	83.82	22.79	16.18
10	51.67	74.17	48.33	25.83
20	33.82	57.76	66.18	42.24
30	29.17	45.62	70.83	54.38

There was 36.90 per cent imidacloprid dissipation under field condition vis a vis 22.24 per cent dissipation under laboratory condition after 10 days. At the end of experimental period 55.35 per cent compound dissipated from the field whereas 42.75 per cent dissipation in laboratory condition left 57.25 per cent residue.

Table 34b. Dissipation of imidacloprid under field and laboratory conditions

Interval (days)	Residues (per cent)		Dissipation (per cent)	
	Field	Laboratory	Field	Laboratory
0	100	100	-	-
5	79.70	94.62	20.3	5.38
10	63.10	77.76	36.9	22.24
20	55.54	71.72	44.46	28.28
30	44.65	57.25	55.35	42.75

Endosulfan also dissipated at much higher rate from the field condition than laboratory. Only 33.00 per cent dissipation occurred in first 10 days from laboratory soil which was much lower than field soil (50.17 per cent). After 20 days experiment endosulfan left 27.77 per cent residue in laboratory, in contrast there was 20.00 per cent residue after 20 days in the field soil.

Table 34c. Dissipation of endosulfan under field and laboratory conditions

Interval (days)	Residues (per cent)		Dissipation (per cent)	
	Field	Laboratory	Field	Laboratory
0	100.00	100.00	-	-
5	68.58	83.45	31.42	16.55
10	49.83	67.00	50.17	33.00
20	20.00	27.77	80.00	72.23
30	0.00	23.61	100.00	76.39

Chlorothalonil also showed slower trend of dissipation in lab soil than field soil. After 5 days of experiment 27.78 per cent dissipation occurred from the field soil and 20.76 per cent in laboratory soil which left 72.22 and 79.24 per cent residues, respectively. After one month, 86.11 per cent chlorothalonil dissipated from the field soil whereas 57.80 per cent dissipation was observed in the lab soil.

Table 34d. Dissipation of chlorothalonil under field and laboratory conditions

Interval (days)	Residues (per cent)		Dissipation (per cent)	
	Field	Laboratory	Field	Laboratory
0	100.00	100.0	-	-
5	72.22	79.24	27.78	20.76
10	58.33	62.80	41.67	37.20
20	38.89	52.56	61.11	47.44
30	13.89	42.20	86.11	57.80

The half-lives in soil estimated by best fit was found for field and laboratory conditions, respectively (Table 34e).

Table 34e. Half-lives of different pesticides in soil under field and laboratory conditions

	Pesticides	Half-life (days)	
		Field condition	Laboratory condition
1.	Pendimethalin	16.8	27.1
2.	Imidacloprid	26.9	31.7
3.	Endosulfan	4.3	4.7
4.	Chlorothalonil	11.2	25.3

Both biological and non-biological agencies appeared to be responsible for the degradation of pesticides in soil. Besides environmental conditions (rainfall, humidity and temperature) all the dissipation agencies viz., adsorption, leaching, uptake by plants, chemical, photochemical and microbial degradation seemed to play sufficient role in disappearance of pesticides from field soil following its application to tomato crop. The role of environmental forces is ruled out in the laboratory experiments and pesticide losses due to adsorption on soil colloids, chemical hydrolysis, photochemical and for microbial degradation appear dominant and demand confirmation, if possible.

4.3. Evaluation of interaction of pesticides/mixtures with soil and microbes

4.3.1. Studies on adsorption of pesticides in soil

Adsorption studies were carried out in IARI alluvial (Typic Ustochrept soil). Aqueous solution containing different concentration of pesticides were added separately to the soil and equilibrated. After equilibration the amount of pesticide left in the solution phase was determined by GLC/HPLC. The difference between initial amount added and the amount left in the solution phase after equilibration was taken as amount adsorbed on the soil.

4.3.1.1. Individual pesticides

4.3.1.1.a. Pendimethalin

The initial concentration, equilibrium concentration, amount adsorbed by one gram of soil and per cent adsorption of pendimethalin are presented in Table 35a.

Table 35a. Initial concentration, equilibrium concentration and imidacloprid adsorbed on IARI alluvial soil

Initial concentration ($\mu\text{g mL}^{-1}$)	Equilibrium concentration ($\mu\text{g mL}^{-1}$)	Pendimethalin adsorbed ($\mu\text{g g}^{-1}$ soil)	Pendimethalin adsorbed (% of added amount)
0	0.000	0.000	0.00
1	0.273	7.27	72.70
5	1.142	35.28	70.56
10	3.450	65.5	65.50
15	5.677	93.23	61.49
20	8.213	117.87	58.94
25	11.262	137.38	54.95

$$Y = 0.8212X + 1.5346, R^2 = 0.994$$

The data of adsorption of pendimethalin on soil (Table 35a) indicated that the total amount of pendimethalin adsorption increased with increasing initial concentration from 5 to $25\mu\text{g mL}^{-1}$ of equilibrium solution ($50\mu\text{g}$ to $250\mu\text{g g}^{-1}$ soil). The amount of pendimethalin adsorbed in soils varied from 7.27 to $137.38\mu\text{g g}^{-1}$.

To ascertain the relationship of pendimethalin adsorption on soil as a function of equilibrium concentration of pendimethalin in the soil solution, help of adsorption isotherm was taken. For this a graph of equilibrium concentration of pendimethalin versus the amount of pendimethalin adsorbed was plotted (Fig. 23a). The plot was also L-type adsorption

isotherm for IARI soil, indicating a high affinity of soil particles for adsorbate molecule. The pendimethalin adsorption ranged between 54.95 to 72.70 per cent (average 64.13 per cent) in IARI alluvial soil. The per cent adsorption, decreased with increasing concentration of pendimethalin solution. At 25 $\mu\text{g mL}^{-1}$ concentration only 54.95 per cent pendimethalin was adsorbed.

Mithyantha (1973) and Garg (1982) also observed similar results while making an adsorption studies of carbofuran in soil. The findings was also conform with the adsorption study of flufenacet by Gupta *et al.* (2001).

4.3.1.1.b. Imidacloprid

The initial concentration, equilibrium concentration, amount adsorbed by one gram of soil and per cent adsorption of imidacloprid are presented in Table 35b.

Table 35b. Initial concentration, equilibrium concentration and imidacloprid adsorbed on IARI alluvial soil

Initial concentration ($\mu\text{g mL}^{-1}$)	Equilibrium concentration ($\mu\text{g mL}^{-1}$)	Imidacloprid adsorbed ($\mu\text{g g}^{-1}$ soil)	Imidacloprid adsorbed (% of added amount)
0	0.000	0.000	0.00
1	0.237	7.630	76.30
5	1.592	34.080	68.16
10	3.272	67.280	67.28
15	5.552	94.480	62.99
20	8.353	116.470	58.24
25	12.525	124.750	49.90

Y = 0.7369X+1.6425
R² = 0.9842

Perusal of data in Table 35b revealed that the total amount of imidacloprid adsorbed increased with increasing initial concentration from 5 to 25 $\mu\text{g mL}^{-1}$ of equilibrium concentration (50 μg to 250 $\mu\text{g g}^{-1}$ soil).

The amount of imidacloprid adsorbed in soils ranged from 7.63 to 124.75 $\mu\text{g g}^{-1}$ soils. Like pendimethalin, adsorption of imidacloprid on soil was

ascertained with the help of an adsorption isotherms where adsorption was function of equilibrium concentration. The graph was plotted with equilibrium concentration of imidacloprid versus the amount of imidacloprid adsorbed in soil (Fig. 24a). These plots were L-type adsorption isotherms.

The imidacloprid adsorption ranged between 49.90 to 76.30 per cent (average 63.81 per cent). The per cent adsorption decreased with increasing concentration of imidacloprid solution. At the highest concentration of $25\mu\text{g mL}^{-1}$ 49.9 per cent imidacloprid was adsorbed in IARI alluvial soil.

4.3.1.1.c. Chlorothalonil

The initial concentration, equilibrium concentration, amount adsorbed by one gram of soil and per cent adsorption of chlorothalonil are presented in Table 35c.

Table 35c. Initial concentration, equilibrium concentration and chlorothalonil adsorbed on IARI alluvial soil

Initial concentration ($\mu\text{g mL}^{-1}$)	Equilibrium concentration ($\mu\text{g mL}^{-1}$)	Chlorothalonil adsorbed ($\mu\text{g g}^{-1}$ soil)	Chlorothalonil adsorbed (% of added amount)
0	0.00	0.00	0.00
5	2.643	23.57	47.14
10	5.232	47.68	47.68
15	9.105	58.95	39.30
20	13.987	60.13	30.07
25	18.232	67.68	27.07

Y = -0.6566X+1.4359
R² = 0.9408

The results revealed that the total ammount of chlorothalonil adsorbed increased with increasing initial concentration from 5 to $25\mu\text{g mL}^{-1}$ of equilibrium solution ($50\mu\text{g}$ to $250\mu\text{g g}^{-1}$ soil). Amount of chlorothalonil adsorbed in soil varied form 23.57 to $67.68\mu\text{g g}^{-1}$. To ascertain the relationship of chlorothalonil adsorption on soils as a function of equilibrium

Fig.23a Freundlich adsorption isotherm of Pendimethalin in soil

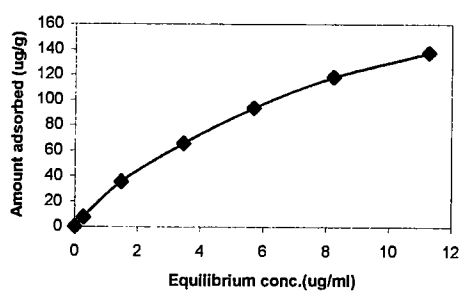


Fig.23b Logarithmic plot of adsorption of pendimethalin in soil

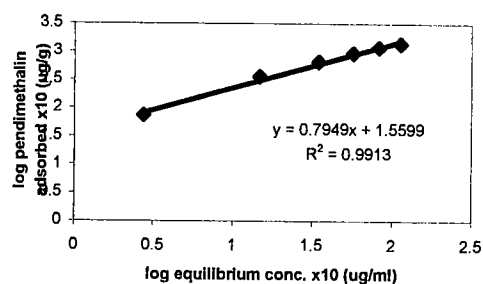


Fig.24a Freundlich adsorption isotherm of Imidacloprid in soil

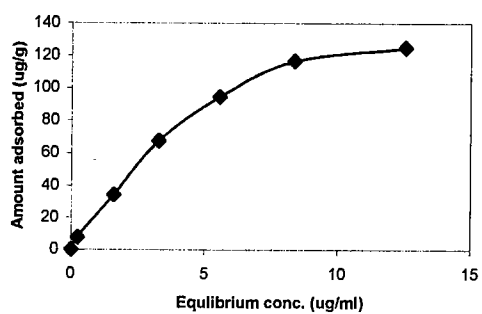


Fig.24b Logarithmic plot of adsorption of Imidacloprid in Soil

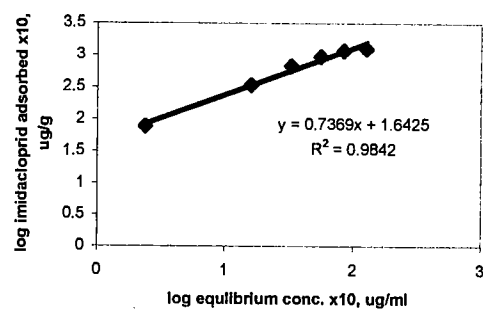


Fig.25a Freundlich adsorption isotherm of Chlorothalonil in soil

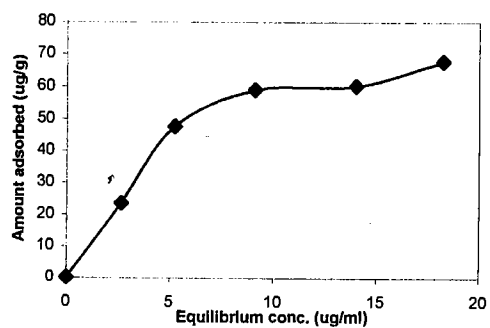
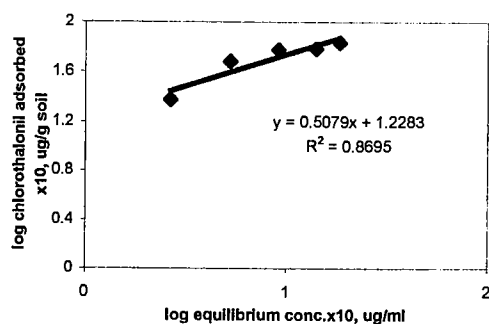


Fig.25b Logarithmic plot of adsorption of chlorothalonil in soil



concentration of pendimethalin in soil solution, help of adsorption isotherms was taken. For this a graph of equilibrium concentration of chlorothalonil versus the amount of herbicide adsorbed on soil was plotted (Fig. 25a). These plots were L-type adsorption isotherms.

The chlorothalonil adsorption ranged from 27 to 48 per cent. The per cent adsorption, however, decreased with increasing concentration of chlorothalonil in solution. At $25 \mu\text{g mL}^{-1}$ concentration, only 27.07 per cent chlorothalonil was adsorbed. The L-type adsorption isotherms indicated a high affinity of soil particles for adsorbate (solute) molecules.

4.3.1.2. Adsorption of mixture of pendimethalin, imidacloprid and chlorothalonil in soil

Since three of the pesticides used in the module used to apply in soil, there is a obvious demand for the adsorption pattern of the individual and mixture of compounds. To study the possible changes in adsorption of the mixture, adsorption study was carried out in the laboratory. The same IARI alluvial soil (Typic Ustochrept) was taken as in the cases of individual pesticides. Aqueous solution containing different concentration of pesticides were added in the same test tube soil and equilibrated. Only the amount of the pesticide was taken as one third of the individual one (except imidacloprid). After equilibration, amount of pesticide left in the solution phase was determined by GLC/HPLC or GLC followed by HPLC. The difference between initial amount added and the amount left in the solution phase after equilibration was taken as amount adsorbed on the soil.

The initial concentration, equilibration concentration, amount adsorbed by one gram of soil and per cent adsorption of the components, of the mixture are presented in Table 35d. The Freundlich adsorption isotherm of individual component of the mixture and their logarithmic plots are presented in Fig. 26, 27 and 28.

Table 35d. Initial concentration, equilibrium concentration and pesticide adsorbed on IARI alluvial soil

Pesticide component in mixture	Initial concentration ($\mu\text{g mL}^{-1}$)	Equilibrium concentration ($\mu\text{g mL}^{-1}$)	Pesticide adsorbed ($\mu\text{g g}^{-1}$ soil)	Pesticide adsorbed (% of added amount)
Pendimethalin	0.00	0.00	0.00	0.00
	0.33	0.11	2.20	66.67
	1.67	0.57	11.5	68.86
	3.33	0.93	24.0	72.07
	5.00	1.95	30.5	61.00
Imidacloprid	0.0	0.00	0.00	0.00
	1.0	0.37	6.30	63.00
	5.0	1.56	34.40	68.80
	10.0	3.52	64.80	64.80
	15.0	6.85	81.50	54.33
Chlorothalonil	0.0	0.00	0.00	0.00
	0.33	0.12	2.10	63.64
	1.67	0.47	12.00	71.86
	3.33	1.02	23.10	69.37
	5.00	2.18	28.20	56.40

The results revealed that the total amount of each pesticide adsorbed increased with increasing initial concentration. Amount of pendimethalin added in soil varied from 2.2 to 30.5 $\mu\text{g g}^{-1}$, whereas the amount adsorbed for chlorothalonil and imidacloprid ranged from 2.1 to 28.2 and 6.3 to 81.5 $\mu\text{g g}^{-1}$, respectively. To ascertain the relationship of the pesticide adsorption soil as a function of equilibrium concentration in solution, help of adsorption was taken. For this graph of equilibrium concentration of pendimethalin versus the amount of pesticide adsorbed on soil was plotted (Fig. 23a). The plots were L-type adsorption isotherm.

Here, the per cent adsorption of individual component spread a narrow range, though the per cent adsorption decreased with increasing concentration. At the highest concentration for imidacloprid 15 $\mu\text{g mL}^{-1}$, adsorption was 81.5 per cent; where as at the highest concentration of 5 $\mu\text{g mL}^{-1}$, 28.2 and 30.5 $\mu\text{g g}^{-1}$, chlorothalonil and pendimethalin were adsorbed, respectively.

Fig.26a Freundlich adsorption isotherm of pendimethalin in mixture

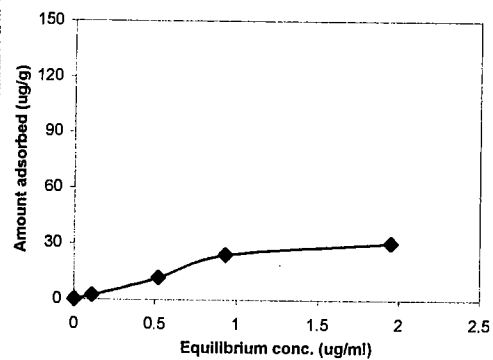


Fig.26b Logarithmic plot of adsorption of pendimethalin in mixture

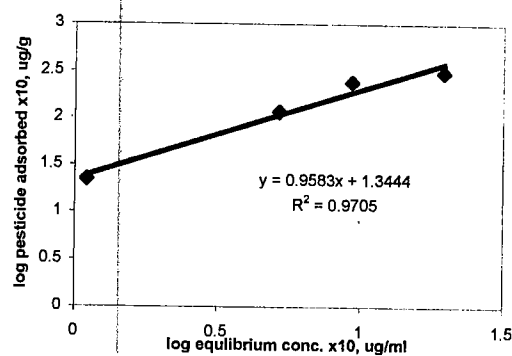


Fig.27a Freundlich adsorption isotherm pattern of imidacloprid in mixture

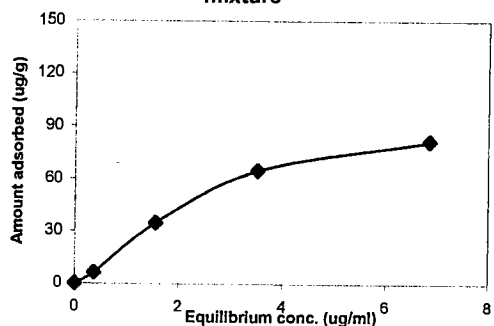


Fig.27b Logarithmic plot of adsorption pattern of imidacloprid in mixture

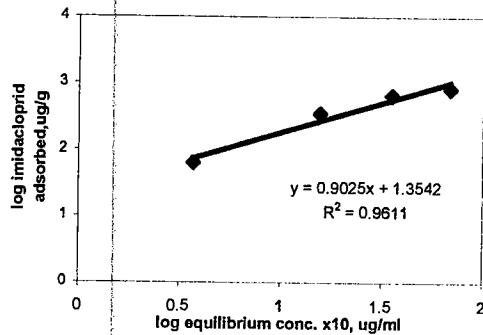


Fig.28a Freundlich adsorption isotherm of chlorothalonil in mixture

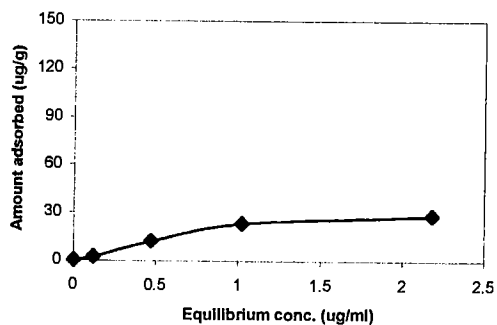
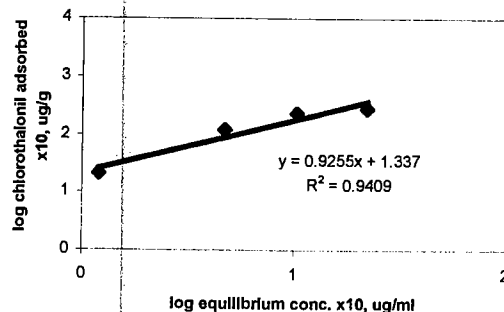


Fig.28b Logarithmic plot of adsorption of chlorothalonil in mixture



The adsorption isotherm obtained fitted well with the Freundlich equation when plotted on a log-log scale (Fig. 23b, 24b and 25b). The values of Freundlich constants (K and n) $1/n$ and $\log K$ for individual and component mixture are presented in Table 35e.

The ' K ' values were highest for the imidacloprid (43.90) followed by pendimethalin (34.20) and chlorothalonil (16.92) and in IARI alluvial soil when applied alone. There was significant difference in the extreme of pesticide adsorption. In case of mixture the ' K ' values for the respective pesticides were 22.60 and 22.08 and 21.73, respectively. So, in case of mixture similar/equal adsorption of all the three pesticides occurred on IARI soil. The intensities of adsorption ($1/n$) were maximum for pendimethalin (0.8212), medium for imidacloprid (0.7369) and minimum for chlorothalonil (0.5079).

Both organic matter and clay contents of the soil appeared to be responsible for the adsorption. As IARI soil contained moderate amount of organic carbon (0.34 per cent) and clay (17.2) dominated by Kaolinite type of mineral, there may be clay-humus complexes in the adsorption surface of the clay.

Table 35e. Freundlich adsorption isotherm constants for individual and mixture components of pesticides

Pesticides	Freundlich adsorption constants					
	Regression equation	$\log K$	K	$1/n$	n	r
I. Alone						
Pendimethalin	$Y = 0.8212X + 1.5346$	1.534	34.20	0.8212	1.218	0.997
Imidacloprid	$Y = 0.7369X + 1.6425$	1.642	43.90	0.7369	1.357	0.992
Chlorothalonil	$Y = 0.5079X + 1.2283$	1.228	16.92	0.5079	1.969	0.932
II. Mixture						
Pendimethalin	$Y = 0.9583X + 1.344$	1.344	22.08	0.9583	1.044	0.985
Imidacloprid	$Y = 0.9025X + 1.3542$	1.354	22.60	0.9025	1.108	0.980
Chlorothalonil	$Y = 0.9255X + 1.537$	1.337	21.73	0.9255	1.080	0.970

In the experiment it was observed that adsorption was high at low concentration and then decreased with increasing concentration. This may be because of moderate solubility of the pesticides, a state where the surface was completely covered with pesticides was never reached. Sato *et al.* (1987) also made similar observations for butachlor.

It may be noted that the soils used in the present study were relatively low in organic matter. This would enable other factors like clay content to influence adsorption. Pionke and DeAngelis (1980) reported that use of K_{oc} to predict herbicide adsorption fails if the ratio of clay to organic carbon exceeds 30 to 40. Gerstl (1984) also noticed better correlation between oxamyl adsorption with clay than with organic matter and their results were attributed to low organic matter content of the soil for the study. Wahid and Sethunathan (1978) and Kumari and Singh (1994) also reported that adsorption of pesticides is almost entirely on organic surfaces if the soils contain above 2% organic matter but adsorption was significantly low on clay surface or inorganic surface if the soils contained below 2% organic matter.

The experimental data were not found to fit well in the Langmuir isotherm probably for the reasons that the requirements of the Langmuir equation were restrictive for heterogenous soils which consisted of different types of surfaces and in which multiple bonding and/or multimolecular adsorption occurred. That is the adsorbing soil surfaces were not homogenous and were considered unique and complex adsorbing surfaces incorporating several newer features like clay-organic interactions etc. and adsorption probably occurred at different sites through several types of bonding mechanism. Similar observations have been made by Bailey and White (1970), Freed and Haque (1973) and Sato *et al.* (1987) for

chloroacetanilide herbicides. Further, it was pointed out by Moreals and van Blade (1979) that every deviation from linearity of Langmuir equation indicated the occurrence of atleast two different retention mechanism.

4.3.2. Effect of pesticides on microorganisms

4.3.2.1. Degradation of pesticides

4.3.2.1.1. Rate kinetics of pesticides in sterile and non-sterile soil

a. Individual pesticides

Non biological degradation of pesticides (applied in soil) was studied in sterilized alluvial soil by destroying the microbial activity of soil by autoclaving and comparing it with that of the non-sterile alluvial soil. The amount of pesticides recovered from sterile and non-sterile alluvial soils treated at $10 \mu\text{g g}^{-1}$ level (imidacloprid $20 \mu\text{g g}^{-1}$ level) at different time intervals are presented in Table 36a to 36h for pendimethalin, imidacloprid and chlorothalonil, respectively.

Table 36a. Residues and per cent dissipation of pendimethalin in sterile alluvial sterilized soil treated with pendimethalin @ $10 \mu\text{g g}^{-1}$

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Degradation (%)
	R ₁	R ₂		
0	9.987	9.961	9.974 $\pm$ 0.018	-
5	8.592	5.575	8.584 $\pm$ 0.012	13.59
10	7.938	7.992	7.965 $\pm$ 0.038	20.14
20	6.217	6.723	6.470 $\pm$ 0.358	35.13
30	6.027	5.937	5.982 $\pm$ 0.064	40.02
SEm $\pm$			0.68	
CD at 5%			2.05	
Y = 0.0074X+0.9801				
R ² = 0.9614				
T _{1/2} = 40.7 days				

Table 36b. Residues and per cent dissipation of pendimethalin in non-sterile alluvial soil treated with pendimethalin @ 10 $\mu\text{g g}^{-1}$

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Degradation (%)
	R ₁	R ₂		
0	9.272	9.125	9.986 $\pm$ 0.104	-
5	7.320	8.102	7.711 $\pm$ 0.553	22.78
10	6.270	7.375	6.823 $\pm$ 0.781	31.67
20	5.282	5.343	5.313 $\pm$ 0.043	46.80
30	4.365	4.029	4.197 $\pm$ 0.238	57.97
SEm $\pm$			0.31	
CD at 5%			0.93	

$$Y = -0.0119X + 0.9689$$

$$R^2 = 0.9983$$

$$T_{1/2} = 25.3 \text{ days}$$

The initial residues of 9.974 ppm dissipated to 8.584, 7.965, 6.470 and 5.982 ppm in 5, 10, 20 and 30 days, respectively under sterile condition whereas under non-sterile condition, the initial residues of 9.199 ppm dissipated to 7.711, 6.823, 5.313 and 4.197 ppm in 5, 10, 20 and 30 days, respectively. The graphs of disappearance of pendimethalin at different intervals under two different conditions depicted in Figure 33a Indicated a gradual loss of herbicide under sterilized condition and a very rapid loss under non-sterile condition. At the end of the experimental period (30 days) 59.08 and 54.62 per cent pendimethalin remained under sterilized and non-sterilized conditions, respectively.

The rate of degradation of pendimethalin in sterile and non-sterile soil followed first-order kinetics, that is, there was a linear relationship between the time of incubation and logarithm concentration of pendimethalin remaining in soil (Fig. 33b). Half-lives of pendimethalin under sterile and

non-sterile soils calculated from the slope of the degradation curve are presented in Table 36i.

The decomposition was relatively slow under sterile condition with half-life 40.7 days and quite rapid under non-sterile condition with half-life of 27.1 days. In the absence of biomass activity, it is presumed that sterile soil may behave like normal soils in their adsorption characteristics. In general the degradation under sterilized condition was about two times as slow as under non-sterilized condition in the soil under investigation as shown in Figure 33b.

Persistence of pendimethalin increased from 42.02 per cent in non-sterilized to 57.97 per cent in sterile alluvial soil in 30 days indicating a positive role of soil microbes on degradation of pendimethalin. This was because, in non-sterile soil, biological and non-biological agencies are responsible for degradation of pendimethalin whereas in sterile soil, only biological agencies were involved in degradation of pendimethalin. Therefore, it could be concluded that 17.95 per cent higher degradation in non-sterile soil was due to soil microorganisms.

The initial residues of imidacloprid 18.793 ppm dissipated to 17.556, 15.218, 14.263, 13.284 and 12.342 ppm in 5, 10, 20, 30 and 40 days, respectively under sterile conditions; whereas under the non-sterile condition, the initial residues of 19.235 ppm dissipated to 17.120, 14.069, 12.977, 10.358 and 7.231 ppm in 5, 10, 20, 30 and 40 days, respectively. The graphs of disappearance of imidacloprid at different intervals under two different conditions depicted in Fig. 33c indicated a gradual loss of imidacloprid under sterilized condition and a very rapid loss under non-sterile condition. At the end of the experimental period (40 days) 65.67 and 37.59

Fig.29a Persistence of pendimethalin in sterile and non-sterile soil

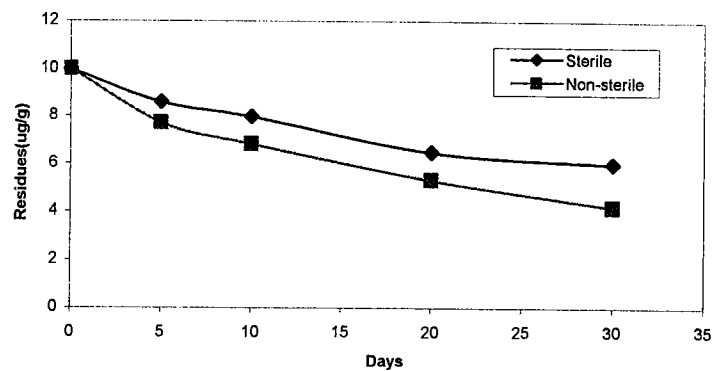


Fig.29b Dissipation of pendimethalin in sterile and non-sterile soil

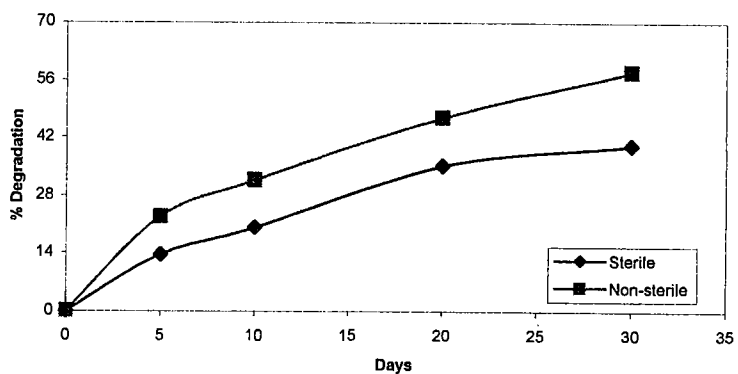
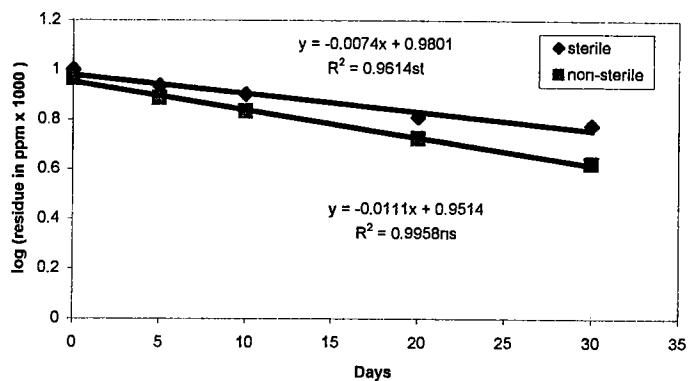


Fig.29c Logarithmic plots of pendimethalin in sterile and non-sterile soil



per cent imidacloprid remained under sterilized and non-sterilized conditions, respectively (Fig. 33c).

Table 36c. Residues and per cent dissipation of imidacloprid in sterile alluvial soil treated with imidacloprid @ 20 $\mu\text{g g}^{-1}$

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Degradation (%)
	R ₁	R ₂		
0	19.360	18.226	18.793 $\pm$ 0.802	-
5	17.213	17.899	17.556 $\pm$ 0.485	6.58
10	15.324	15.112	15.218 $\pm$ 0.150	19.02
20	14.136	14.390	14.263 $\pm$ 0.180	24.10
30	13.012	13.556	13.284 $\pm$ 0.385	29.31
40	12.315	12.369	12.342 $\pm$ 0.038	34.33
SEm $\pm$			0.29	
CD at 5%			1.05	
Y = -0.0044X+1.2554				
R ² = 0.9341				
T _{1/2} = 68.4 days				

Table 36d. Residues and per cent dissipation of imidacloprid in non-sterile alluvial soil treated with imidacloprid @ 20 $\mu\text{g g}^{-1}$

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Degradation (%)
	R ₁	R ₂		
0	19.023	19.447	19.235 $\pm$ 0.300	-
5	17.297	16.943	17.120 $\pm$ 0.250	11.00
10	14.135	14.003	14.069 $\pm$ 0.093	26.12
20	12.102	13.582	12.977 $\pm$ 1.046	32.53
30	9.785	11.919	10.358 $\pm$ 1.508	46.15
40	7.015	7.447	7.231 $\pm$ 0.305	62.41
SEm $\pm$			0.59	
CD at 5%			2.14	
Y = -0.0099X+1.2815				
R ² = 0.9686				
T _{1/2} = 27.1 days				

Fig.30a Persistence of imidacloprid in sterile and non-sterile soil

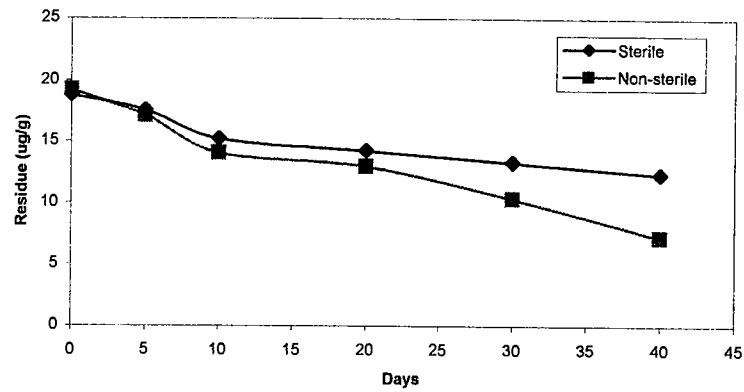


Fig.30b Dissipation of imidacloprid in sterile and non-sterile soil

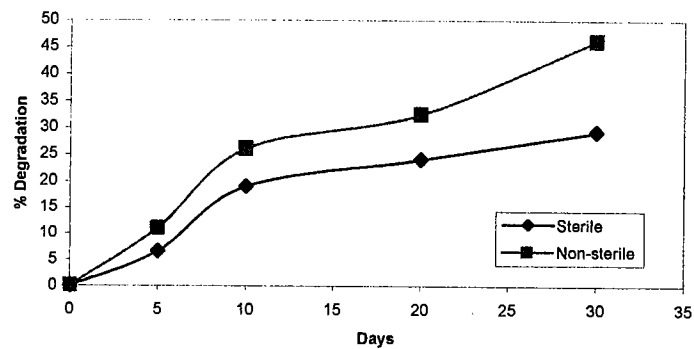
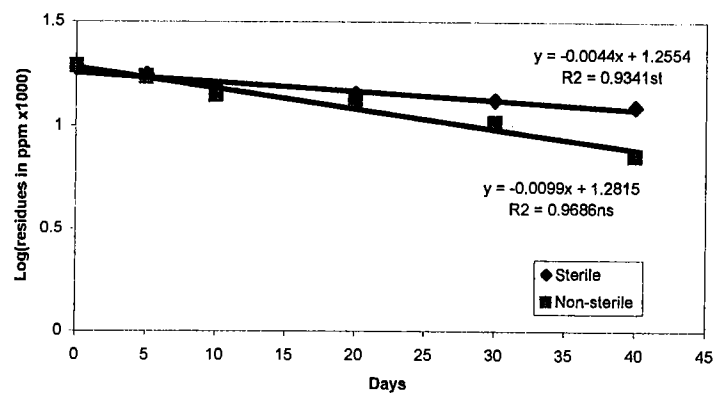


Fig.30c Logarithmic plots of imidacloprid in sterile and non-sterile soil



In the sterile condition, the initial residue of endosulfan was 9.226 ppm which was dissipated to 8.814, 7.851 and 7.115 ppm in 5, 10 and 20 days, respectively. In the non-sterile soil the initial deposit was 8.867 ppm and dissipated to 7.249, 5.820 and 2.412 ppm in 5, 10 and 20 days, respectively. The graphs of disappearance of endosulfan under two different conditions is given in Fig. 33e which indicate a gradual loss of insecticide under sterilized condition and a very rapid loss in non-sterile condition.

Table 36e. Residues and per cent dissipation of endosulfan in sterile alluvial soil treated with endosulfan @ 10 $\mu\text{g g}^{-1}$

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Degradation (%)
	R ₁	R ₂		
0	9.326	9.125	9.266 $\pm$ 0.142	-
5	8.820	8.808	8.814 $\pm$ 0.008	4.88
10	7.846	7.856	7.851 $\pm$ 0.007	15.27
20	7.123	7.107	7.115 $\pm$ 0.011	23.21
30	6.883	7.017	6.950 $\pm$ 0.095	25.00
SEm $\pm$			0.05	
CD at 5%			0.22	
Y = -0.0059X+0.9668				
R ² = 0.970				
T _{1/2} = 51.0 days				

Table 36f. Residues and per cent dissipation of endosulfan in alluvial non-sterile soil treated with endosulfan @ 10 $\mu\text{g g}^{-1}$

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Degradation (%)
	R ₁	R ₂		
0	8.250	9.123	8.867 $\pm$ 0.617	-
5	7.372	7.125	7.249 $\pm$ 0.175	16.55
10	5.750	5.890	5.820 $\pm$ 0.099	44.51
20	2.322	2.502	2.412 $\pm$ 0.127	72.23
30	2.120	1.982	2.051 $\pm$ 0.037	76.39
SEm $\pm$			0.21	
CD at 5%			0.82	
Y = -0.0648X+3.2125, R ² = 0.8393, T _{1/2} = 4.7 days				

Fig.31a Persistence of endosulphan in sterile and non-sterile soil

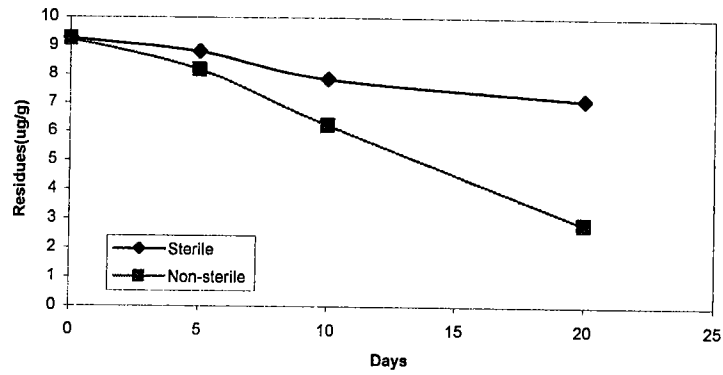


Fig.31b Dissipation of endosulphan in sterile and non-sterile soil

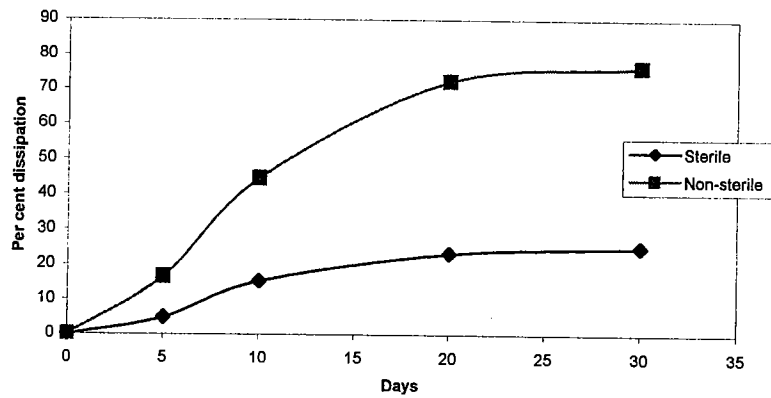
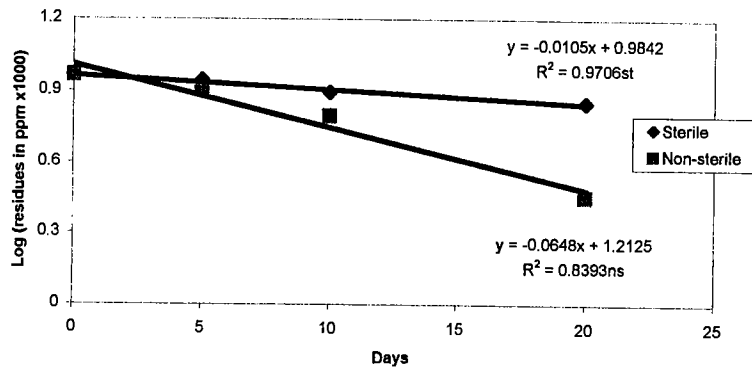


Fig.31c Logarithmic plots of endosulphan in sterile and non-sterile soil



At the end of the experiment period (30 days) 75.00 and 23.61 per cent endosulfan residue remained under sterilized and non-sterilized conditions, respectively (Fig. 33e).

The initial residue of chlorothalonil was 9.864ppm and dissipated to 9.319, 7.951, 7.441 and 6.204 in 5, 10, 20 and 30 days, respectively whereas under non-sterile condition, the initial residue 9.911ppm dissipated to 5.209 and 4.182 ppm in 5, 10, 20 and 30 days, respectively (Fig. 33g). The graphs of disappearance of chlorothalonil under different conditions depicted in Fig. 33g indicated a gradual loss of the fungicide under sterilized condition and a very rapid loss under non-sterile condition. At the end of the experiment (30 days) 62.9 and 42.2 per cent chlorothalonil remained under sterile and non-sterile conditions, respectively.

Table 36g. Residues and per cent dissipation of chlorothalonil in sterile alluvial soil treated with chlorothalonil @ 10 $\mu\text{g g}^{-1}$

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Degradation (%)
	R ₁	R ₂		
0	9.785	9.942	9.864 $\pm$ 0.111	-
5	9.297	9.340	9.319 $\pm$ 0.030	5.52
10	8.013	7.886	7.951 $\pm$ 0.092	10.39
20	7.652	7.230	7.441 $\pm$ 0.298	24.56
30	6.283	6.15	6.204 $\pm$ 0.112	37.10
SEm $\pm$			0.11	
CD at 5%			0.32	

$$Y = -0.0066X + 0.9908$$

$$R^2 = 0.9651$$

$$T_{1/2} = 45.6 \text{ days}$$

Fig.32a Persistence of chlorothalonil in sterile and non-sterile soil

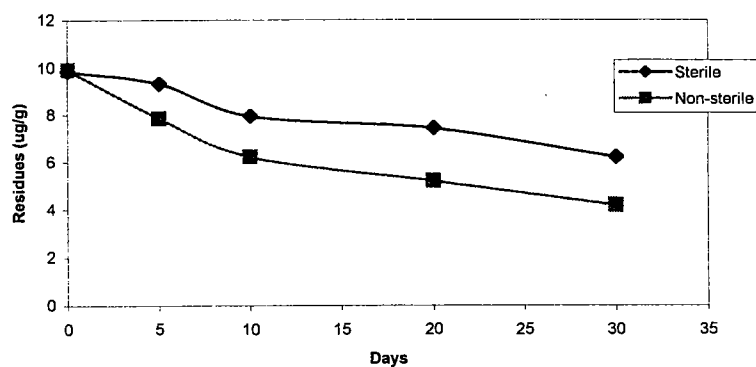


Fig.32b Dissipation of chlorothalonil in sterile and non-sterile soil

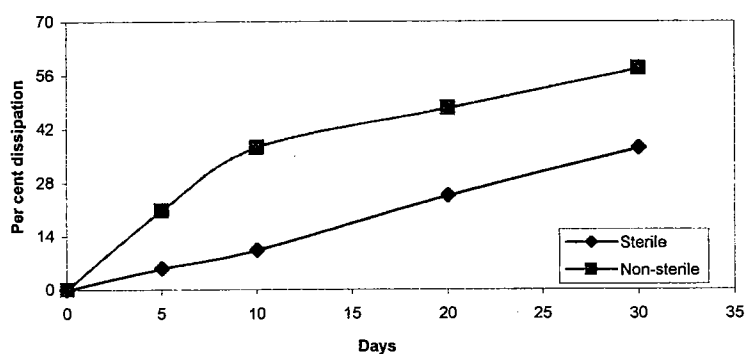


Fig.32c Logarithmic plots of chlorothalonil in sterile and non-sterile soil

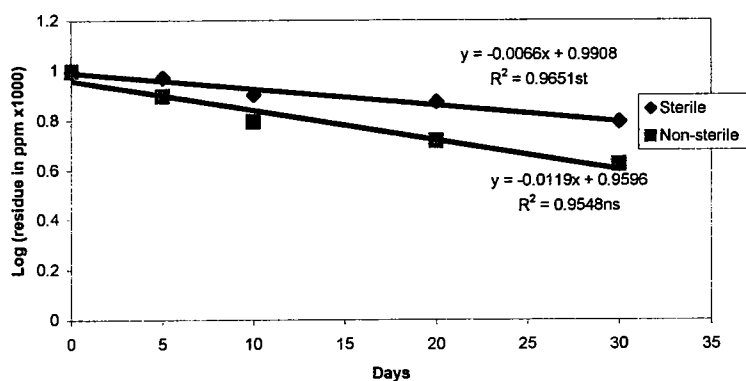


Table 36h. Residues and per cent dissipation of chlorothalonil in non-sterile alluvial soil treated with chlorothalonil @ 10 µg g⁻¹

Interval (days)	Residues (µg g ⁻¹)		Average residues (µg g ⁻¹ ± SD)	Degradation (%)
	R ₁	R ₂		
0	9.899	9.923	9.911 ± 0.017	-
5	7.776	7.929	7.853 ± 0.108	20.76
10	6.175	6.272	6.224 ± 0.069	37.20
20	5.272	5.145	5.209 ± 0.089	47.44
30	4.110	4.253	4.182 ± 0.101	57.80
SEm±			0.06	
CD at 5%			0.21	
Y = -0.0119X+0.9596				
R ² = 0.9548				
T _{1/2} = 25.3 days				

Like pendimethalin the rate of degradation of imidacloprid, endosulfan and chlorothalonil in sterile and non-sterile soil followed first-order kinetics, that is, there is linear relationship between the time of incubation and logarithm of concentration of pesticides remaining in soil (Fig. 33d, 33f, 33h). Half-lives of these pesticides under sterile and non-sterile soils calculated from slope of the degradation curve are presented in Table 36i.

Table 36i. Regression equation, rate constant, correlation co-efficient and half life of pesticides under sterile and non-sterile soil

Pesticide	Condition	Dissipation model	Rate constant (K, days ⁻¹)	Correlation coefficient (r)	Half-life (days)
Pendimethalin	Sterile	Y=-0.0074X+0.9801	0.0170	-0.981	40.68
	Non-sterile	Y= -0.0111+0.9514	0.0256	-0.998	27.12
Imidacloprid	Sterile	Y= -0.0044X+1.2554	0.0101	-0.966	68.41
	Non-sterile	Y= -0.0099X+1.2815	0.0228	-0.984	30.40
Endosulfan	Sterile	Y= -0.0509X+0.9688	0.0135	-0.985	51.01
	Non-sterile	Y= -0.0264X+1.013	0.0608	-0.980	11.40
Chlorothalonil	Sterile	Y=-0.0066X+0.9908	0.0152	-0.982	45.61
	Non-sterile	Y=-0.0119X+0.9596	0.0274	-0.977	25.29

For all the pesticides, the decomposition was relatively slow under sterile condition and quite rapid under non-sterile condition.

In the absence of biomass activity it is presumed that sterile soil may behave like normal soils in their adsorption characteristics. Persistence of pesticides increased in non sterile to sterile alluvial soil indicating a positive role of soil microbes on pesticide degradation. The persistence of pendimethalin was increased from 42.03 to 59.98 per cent, where as for imidacloprid it was 39.97 to 65.67 per cent, for endosulfan 23.61 to 75.00 and chlorothalonil 42.20 to 62.90 per cent. This was because, in non-sterile soil biological and non-biological agencies were responsible for degradation of pesticides, whereas in sterile soil, only non-biological agencies were involved in degradation. Therefore, it could be concluded that the higher degradation in non-sterile soil was due to soil microorganisms.

4.3.2.1.1.b. Pesticide mixture

The initial deposit of pesticides was much less when the degradation study was carried out in the mixture. For their study the added amount of individual components was one fourth of the amount used in the single pesticide.

The initial residues of pendimethalin was 2.192 ppm which was dissipated to 2.006, 1.785, 1.487 and 1.242 ppm in 5, 10, 20 and 30 days, respectively under sterile condition where as under non-sterile condition the initial residues of 2.169 ppm dissipated to 1.770, 1.409, 0.989 and 0.575ppm in 5, 10, 20 and 30 days, respectively (Fig. 33i).

Imidacloprid left 4.849 ppm initial residues when underwent microbial degradation in mixture. This followed with further dissipation to

4.605, 4.3215, 4.112 and 3.875 ppm in 5, 10, 20 and 30 days, respectively under sterile condition whereas under the non-sterile condition, the initial residues of 4.693 ppm dissipated to 3.929, 3.615, 3.326 and 3.038 ppm in 5, 10, 20 and 30 days, respectively (Fig. 33i).

The initial residues of endosulfan was 2.543 and dissipated to 2.274, 2.001 and 1.553 in 5, 10 and 20 days, respectively in sterile condition, whereas in non-sterile condition the initial residues was 2.357ppm and dissipation was 5, 10 and 20 days were 0.986, 0.626 and 0.179 ppm, respectively.

Table 36j. Residues and per cent dissipation of pesticide mixture (pendimethalin + imidacloprid + endosulfan + chlorothalonil) in sterilized soil

Pesticide Component	Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Dissipation (%)
		R ₁	R ₂		
Pendimethalin	0	2.231	2.152	2.192 $\pm$ 0.056	-
	5	1.998	2.013	2.006 $\pm$ 0.011	8.49
	10	1.746	1.823	1.785 $\pm$ 0.054	18.57
	20	1.453	1.520	1.487 $\pm$ 0.047	32.16
	30	1.276	1.223	1.245 $\pm$ 0.037	43.45
Imidacloprid	0	4.875	4.822	4.849 $\pm$ 0.037	-
	5	4.623	4.587	4.605 $\pm$ 0.025	5.03
	10	4.312	4.338	4.325 $\pm$ 0.018	10.81
	20	4.115	4.109	4.112 $\pm$ 0.004	15.20
	30	3.825	3.925	3.875 $\pm$ 0.071	20.09
Endosulfan	0	2.462	2.623	2.543 $\pm$ 0.114	-
	5	2.265	2.282	2.274 $\pm$ 0.012	10.58
	10	2.002	2.000	2.001 $\pm$ 0.001	21.31
	20	1.535	1.531	1.533 $\pm$ 0.003	39.73
	30	1.397	1.405	1.401 $\pm$ 0.005	44.91
Chlorothalonil	0	2.652	2.611	2.632 $\pm$ 0.029	-
	5	2.572	2.581	2.582 $\pm$ 0.006	1.90
	10	2.097	2.092	2.095 $\pm$ 0.004	20.52
	20	2.014	2.018	2.016 $\pm$ 0.003	23.12
	30	1.852	1.880	1.866 $\pm$ 0.019	28.86

Table 36k. Residues and per cent dissipation of pesticide mixture (pendimethalin + imidacloprid + endosulfan + chlorothalonil) in non-sterile alluvial soil

Pesticide Component	Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Dissipation (%)
		R ₁	R ₂		
Pendimethalin	0	2.179	2.158	2.169 $\pm$ 0.015	-
	5	1.757	1.782	1.770 $\pm$ 0.018	18.40
	10	1.412	1.405	1.409 $\pm$ 0.005	35.04
	20	0.986	0.992	0.989 $\pm$ 0.004	54.40
	30	0.583	0.567	0.575 $\pm$ 0.011	73.49
Imidacloprid	0	4.762	4.623	4.693 $\pm$ 0.098	-
	5	3.983	3.875	3.929 $\pm$ 0.076	16.28
	10	3.622	3.608	3.615 $\pm$ 0.009	22.97
	20	3.315	3.337	3.326 $\pm$ 0.016	29.12
	30	3.015	3.061	3.038 $\pm$ 0.032	35.27
Endosulfan	0	2.526	2.187	2.357 $\pm$ 0.240	-
	5	0.989	0.982	0.986 $\pm$ 0.005	58.17
	10	0.632	0.619	0.626 $\pm$ 0.010	73.44
	20	0.1732	0.1848	0.179 $\pm$ 0.008	92.39
	30	0.129	0.135	0.132 $\pm$ 0.004	94.40
Chlorothalonil	0	2.549	2.478	2.514 $\pm$ 0.050	-
	5	2.321	2.383	2.352 $\pm$ 0.044	6.44
	10	1.876	2.090	1.983 $\pm$ 0.151	21.12
	20	1.638	1.672	1.655 $\pm$ 0.024	34.16
	30	1.470	1.484	1.477 $\pm$ 0.009	41.23

Chlorothalonil was also degraded in sterile and non-sterile soil. The degradation in non-sterile soil was at much higher rate than sterile condition. The initial residue of chlorothalonil of 2.632 ppm dissipated to 2.582, 2.095, 2.016 and 1.866 ppm in 5, 10, 20 and 30 days, respectively under sterile condition whereas under non-sterile condition, the initial residues of 2.514

ppm dissipated to 2.352, 1.983, 1.6556 and 1.477 ppm in 5, 10, 20 and 30 days, respectively (Fig. 33i).

The rate of degradation of the pesticides in sterile and non-sterile soils followed first-order kinetics, that is, there was a linear relationship between the time of incubation and logarithm of concentration of pesticides remaining in soil. Half lives of the pesticides as mixture component under sterile and non-sterile soils calculated from the slope of the degradation curve are presented in Table 33I.

Persistence of pendimethalin in mixture increased from 26.51 per cent in non-sterile soil to 56.55 per cent in sterile alluvial soil whereas in case of individual compound the persistence was increased from 42.03 per cent in non-sterilized soil to 59.98 per cent in sterile soil. Thus, persistence of pendimethalin is 5.33 per cent less in case of mixture.

Table 36I. Regression equation, slope, rate constant, correlation coefficient and half-lives of pesticides as mixture component under sterile and non-sterile soil

Pesticide	Condi- tion	Regression equation	Rate constant (K, days ⁻¹)	Correlation coefficient (r)	Half-life (days)
Pendimethalin	Sterile	$Y = -0.0082X + 0.3396$	0.0189	-0.999	36.7
	Non-sterile	$Y = -0.0188X + 3.3424$	0.0433	-0.996	16.2
Imidacloprid	Sterile	$Y = -0.0081X + 0.676$	0.0187	-0.953	37.2
	Non-sterile	$Y = -0.0185X + 3.606$	0.0426	-0.892	16.3
Endosulfan	Sterile	$Y = -0.0106X + 0.4167$	0.0244	-0.950	28.4
	Non-sterile	$Y = -0.1393X + 3.6599$	0.3208	-0.952	12.2
Chlorothalonil	Sterile	$Y = -0.0116X + 0.438$	0.0267	-0.987	26.0
	Non-sterile	$Y = -0.0306X + 3.3863$	0.0705	-0.990	9.8

Persistence of imidacloprid in mixture was also increased from 64.73 per cent in non-sterile to 79.91 per cent in sterile alluvial soil in 30 days indicating a positive role of soil microbes on degradation of imidacloprid. In non-sterile condition the individual pesticide persisted to 37.59 per cent to

Fig.33a Persistence of pendimethalin in sterile and non-sterile soil when applied as mixture

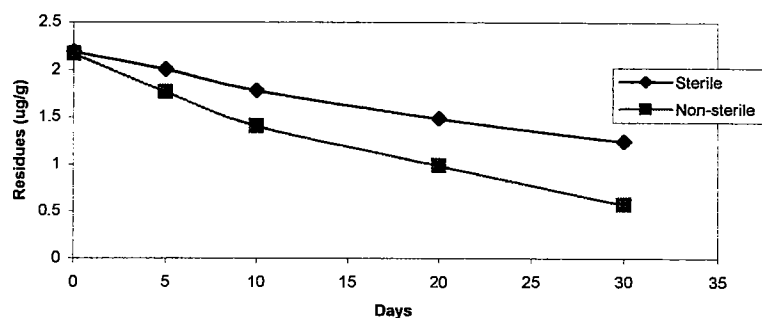


Fig.33b Logarithmic plots of pendimethalin residues in sterile and non-sterile soil when applied as mixture

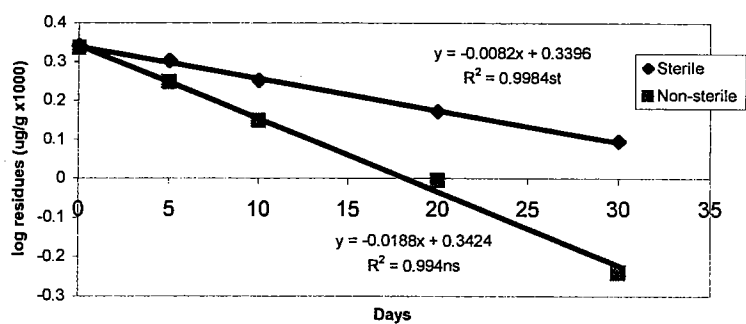


Fig.33c Persistence of imidacloprid in sterile and non-sterile soil when applied as mixture

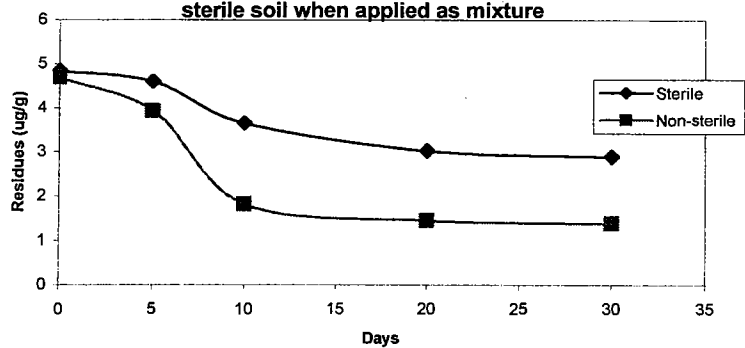


Fig.33d Logarithmic plots of imidacloprid residues in sterile and non-sterile soil when applied as mixture

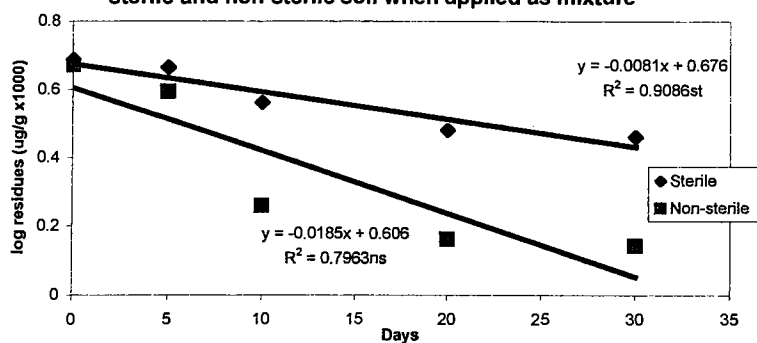


Fig.33e Persistence of endosulphan in sterile and non-sterile soil when applied as mixture

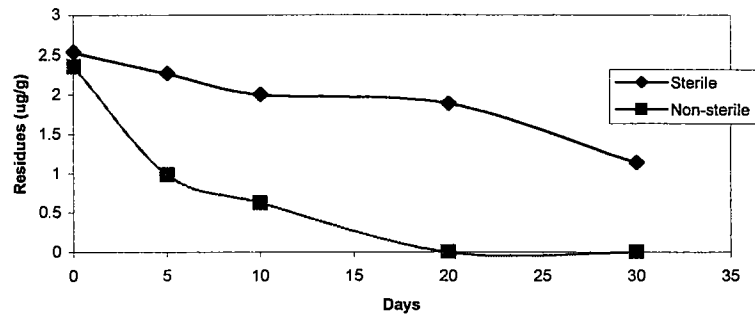


Fig.33f Logarithmic plots of endosulphan residues in sterile and non-sterile soil when applied as mixture

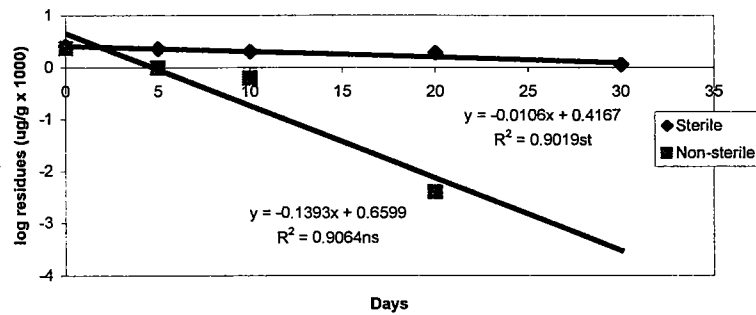


Fig.33g Persistence of chlorothalonil in sterile and non-sterile soil when applied as mixture

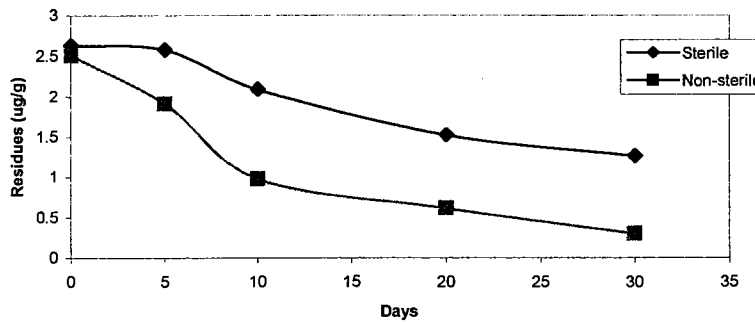
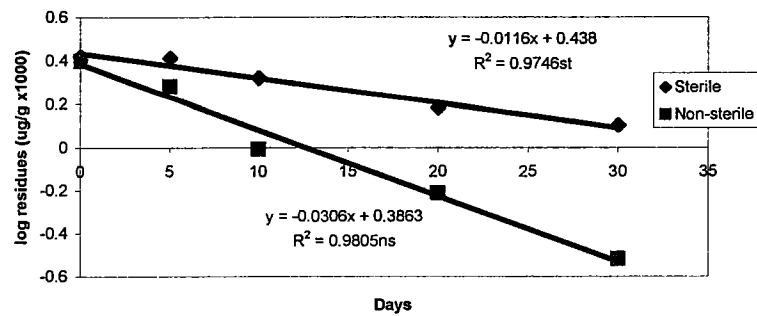


Fig.33h Logarithmic plots of chlorothalonil residues in sterile and non-sterile soil when applied as mixture



65.67 per cent in sterile soil indicating a positive role of soil microbes on degradation of imidacloprid. Thus persistence of pesticides in soil lose high when applied alone as compared to mixture.

In the non-sterile soil endosulfan, as a mixture component persisted 7.11 per cent and in sterile soil persistence was 60.27 per cent in 20 days. In contrast the individual insecticide persistence was increased from 27.77 per cent in non-sterile to 76.79 per cent in sterile alluvial soil in 20 days implicating this response of microbial degradation.

As a mixture component chlorothalonil persisted 58.77 per cent in non-sterile soil, where as in the sterile soil, the persistence was 71.14 per cent, after 30 days vis a vis the individual pesticide persistence was 42.20 and 62.90 per cent, respectively. Thus the persistence was higher in case of mixture than as an individual fungicide indicating higher persistence in the mixture than individual one.

The higher persistence of pesticides in sterile soil in contrast to non-sterile one, indicated the microbial activity on the pesticide moiety. In the non-sterile soil, biological and non-biological agencies were responsible for degradation of pesticide whereas in sterile soil, only non-biological agencies were involved in degradation. Therefore, it could be concluded that the higher degradation in non-sterile soil was due to activity of soil microorganisms which act upon the pesticide for their carbon source.

4.3.2.1.1.c. Biological degradation of pesticides in soil

The biological degradation of pesticides in soil estimated from the difference in degradation under non-sterilized and sterilized conditions have been presented in Table 36m to Table 36q and Fig. 33j.

Table 36m. Biological degradation of pendimethalin in soil

Day	Degradation under non-sterile condition (per cent)	Degradation under sterile condition (per cent)	Biological degradation (per cent)
0	0.00	0.00	0.00
5	22.78	13.59	9.19
10	31.67	20.14	11.53
20	46.80	35.13	11.67
30	57.97	40.02	17.95

The biological degradation of all the four pesticides, individually and as component of mixture increased with the passage of time. After 5 days of incubation period 9.19 and 9.91 per cent dissipation of pendimethalin due to biological degradation was occurred in individual and mixture application, respectively. Which gradually increase to 11.53, 11.67 and 17.95 per cent in case of individual herbicide and 16.47, 22.34 and 30.04 per cent in case of mixture component after 10, 20 and 30 days of incubation, respectively.

Although after 5 days of incubation, the degradation of imidacloprid was merely 4.42 per cent, degradation was increased gradually with the elapse of time. The biological degradation of imidacloprid after 10 days increased to 7.10 per cent which gradually reaches to 8.52, 16.84 and 25.70 per cent after 20, 30 and 40 days, respectively.

Table 36n. Biological degradation of imidacloprid in soil

Day	Degradation under non-sterile condition (per cent)	Degradation under sterile condition (per cent)	Biological degradation (per cent)
0	0.00	0.00	0.00
5	11.00	6.58	4.42
10	26.12	19.02	7.10
20	32.53	24.01	8.52
30	46.15	29.31	16.84
40	60.03	34.33	24.70

The degradation of endosulfan through biological means was at higher rate than imidacloprid. After 5 days of incubation, through the degradation was not so high (12 per cent), it reaches to higher degradation in 20 days. The 29.24 per cent dissipation in 10 days, increased to 49.02 per cent in 20 days. On the other hand endosulfan when used as mixture of with other three pesticides degradation was slightly lower than pesticides degradation was slightly higher than individual pesticide. The degradation of endosulfan as mixture component was 15.27, 32.13 and 52.66 per cent in 5, 10 and 20 days, respectively.

Table 36o. Biological degradation of endosulfan in soil

Day	Degradation under non-sterile condition (per cent)	Degradation under sterile condition (per cent)	Biological degradation (per cent)
0	0.00	0.00	0.00
5	16.55	4.88	11.67
10	44.51	15.27	29.24
20	72.23	23.21	49.02
30	76.39	25.00	51.39

The degradation pattern of chlorothalonil through biological means was poorest one. Although initially there was faster degradation (15.24 per cent in 5 days), the rate was much slower as the time (days) elapsed. There was 17.81, 22.88 and 20.70 per cent biological degradation of chlorothalonil in 10, 20 and 30 days, respectively.

Table 36p. Biological degradation of chlorothalonil in soil

Day	Degradation under non-sterile condition (per cent)	Degradation under sterile condition (per cent)	Biological degradation (per cent)
0	0.00	0.00	0.00
5	20.76	5.52	15.24
10	37.20	19.39	17.81
20	47.44	24.56	22.88
30	57.80	37.10	20.70

Degradation of chlorothalonil was also slow in the mixture. After 5 days of incubation period 4.54 per cent dissipation of chlorothalonil was due to biological degradation which gradually increased to 11.04 and 12.37 per cent after 20 and 30 days of incubation, respectively.

Table 36q. Biological degradation of pesticide mixture in soil

Pesticide component	Day	Degradation under non-sterile condition (per cent)	Degradation under sterile condition (per cent)	Biological degradation (per cent)
Pendimethalin	0	0.00	0.00	0.00
	5	18.40	8.89	9.51
	10	35.04	18.57	16.47
	20	54.40	32.16	22.34
	30	73.49	43.45	30.04
Imidacloprid	0	0.00	0.00	0.00
	5	16.28	5.03	11.25
	10	22.97	10.81	12.16
	20	29.12	15.20	13.92
	30	35.27	20.09	15.18
Endosulfan	0	0.00	0.00	0.00
	5	58.17	10.58	15.27
	10	73.44	21.31	32.13
	20	92.39	39.73	52.66
	30			
Chlorothalonil	0	0.00	0.00	0.00
	5	6.44	1.90	4.54
	10	21.12	20.52	0.60
	20	34.16	23.12	11.04
	30	41.23	28.86	12.67

Fig.33i Biological degradation of Pesticide mixture in Sterile and Non-sterile soil

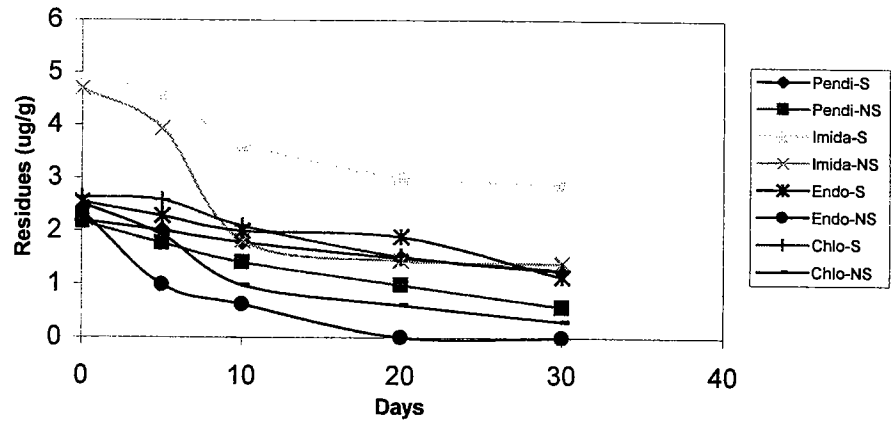
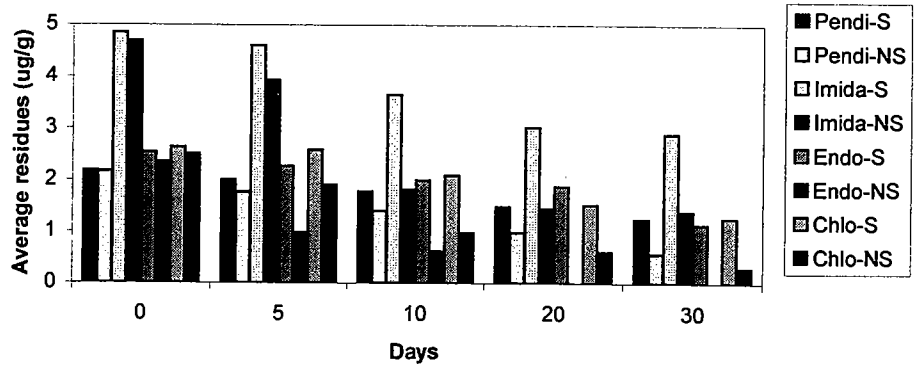


Fig.33j Biological degradation of Pesticide mixture in Sterile and Non-sterile soil



From the experiment it was visualized that biological degradation of individual pesticides was pronounced during first 10 days except imidacloprid. The degradation then reached at a plateau in 20 days and again increased in subsequent 10 days time. For the mixture, the degradation showed nearly similar trends. The last 10 days showed declining degradation for the mixture of pesticides.

The aforesaid experiment indicated that the biological activities of soil biomass was more pronounced in early stage of incubation period. At the later stages, however, the degradation could be the non-biological nature. The results suggested that because of more conducive conditions prevailing in alluvial soil, i.e., moderate alkalinity (pH 7.2), low clay content (16.2 per cent) and low cation exchange capacity [7.21 Cmol (p⁺) kg⁻¹ soil], the microbial population proliferated under the condition which subsequently degraded pesticides in alluvial soil.

4.3.2.1.2. Pesticides degradation in broth

Investigations on the degradation rate kinetics of the pesticide was carried out using liquid broth Czapek-Dox Medium (CDM) to determine the role of soil microbial population in pesticide degradation. The average pesticide present in liquid broth, after different days of interval are presented in Table 37a to 37e.

The initial concentration of pendimethalin was found to be 1.975 µg mL⁻¹ which dissipated to 1.650, 1.435, 1.270 and 0.675 µg mL⁻¹ after 5, 10, 20 and 30 days of inoculation, respectively. The corresponding concentrations during the same time intervals were 1.85, 1.70, 1.52 and 0.97 µg mL⁻¹, respectively, when pendimethalin was applied as mixture component with three other pesticides.

Table 37a. Rate kinetics of pendimethalin in broth

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Degradation (%)
	R ₁	R ₂		
0	1.97	1.98	1.975 $\pm$ 0.007	-
5	1.63	1.67	1.650 $\pm$ 0.028	16.45
10	1.45	1.42	1.435 $\pm$ 0.021	27.34
20	1.268	1.272	1.270 $\pm$ 0.003	35.70
30	0.678	0.672	0.675 $\pm$ 0.004	65.82
SEm $\pm$			0.01	
CD at 5%			0.03	
Y = -0.0142X + 3.305				
R ² = 0.9231				
T _{1/2} = 21.2 days				

The graph of disappearance of pendimethalin at different intervals of time depicted rapid loss of the herbicide. In a span of 10 days 27.34 per cent of the added pendimethalin was metabolized. This was followed by 8.36 per cent degradation in the next 10 days. At the end of the experimental period (30 days) only 34.18 per cent of applied herbicide remained in the broth representing 65.82 per cent degradation of pendimethalin (Fig. 34a).

The dissipation of pendimethalin was slower in the mixture. There was only 6.09 per cent degradation after 5 days of incubation. The dissipation further increased to 13.71, 22.84 and 50.76 per cent in 10, 20 and 30 days respectively. Thus it can be concluded that degradation of pendimethalin in mixture was 15.06 per cent lesser than the individual degradation (65.82 per cent).

The initial concentration of imidacloprid in liquid broth (CDM) was $19.88 \mu\text{g mL}^{-1}$ when the media was supplemented with the insecticide ($20 \mu\text{g mL}^{-1}$). The residue was dissipated to 7.85, 36.22, 43.11 and 61.02 ppm in 5, 10, 20 and 30 days, respectively (Figure 34c).

Table 37b. Rate kinetics of imidacloprid in broth

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Degradation (%)
	R ₁	R ₂		
0	19.84	19.92	19.88 $\pm$ 0.057	-
5	18.89	17.75	18.32 $\pm$ 0.806	7.85
10	13.29	12.07	12.68 $\pm$ 0.863	36.22
20	11.25	11.36	11.31 $\pm$ 0.078	43.11
30	7.27	8.23	7.75 $\pm$ 0.340	61.02
SEm $\pm$			0.43	
CD at 5%			1.32	
Y = -0.0134X+3.2963				
R ² = 0.952				
T _{1/2} = 22.5 days				

The dissipation curve of imidacloprid at different intervals of time indicated slower rate of degradation initially (7.85 per cent in 5 days) followed by rapid degradation of 28.37 per cent in a span of 5 days. This was followed by slower degradation (6.89 per cent) in the next 10 days. At the end of the experiment, 38.98 per cent of applied insecticide remained in the broth representing 61.02 per cent degradation.

The degradation of imidacloprid was further slower in the mixture. After 5 days of incubation there was 6.01 per cent degradation in the media. This was followed by 15.27, 26.68 and 29.74 per cent degradation in 10, 20 and 30 days interval. Initially there was $9.82 \mu\text{g mL}^{-1}$ residue in the mixture which was dissipated to 6.90 per cent in 30 days depicting 70.26 per cent persistence in soil. In general, the degradation of the insecticide in individual application was much faster than as a mixture component.

The degradation of endosulfan was highest in the broth in 30 days of incubation period (78.39 per cent). Initially there was $1.89 \mu\text{g mL}^{-1}$ endosulfan in the solution which was dissipated to 1.76, 1.40, 0.5 and $0.41 \mu\text{g mL}^{-1}$ in 5, 10, 20 and 30 days of intervals, respectively (Fig. 34e). There was 21.69 per cent endosulfan residue remained at the end of the experiment.

Fig.34a Microbial degradation of pendimethalin in broth

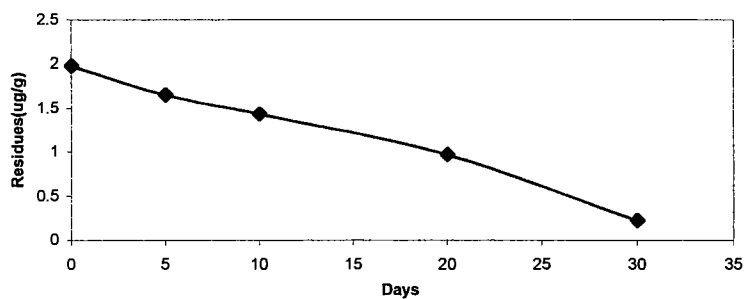


Fig.34b Logarithmic plots of pendimethalin residues in broth

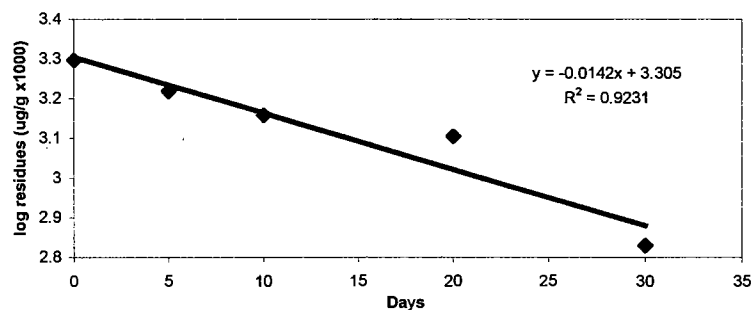


Fig.34c Microbial degradation of imidacloprid in broth

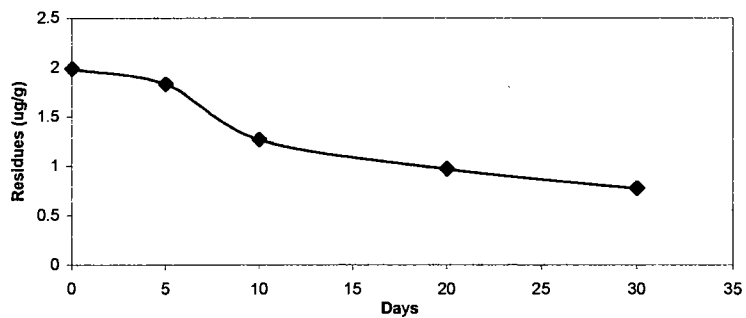


Fig.34d Logarithmic plots of imidacloprid residues in broth

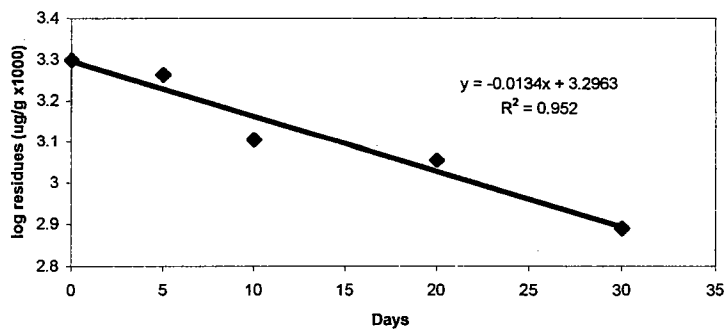


Table 37c. Rate kinetics of endosulfan in broth

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Degradation (%)
	R ₁	R ₂		
0	1.87	1.91	1.89 $\pm$ 0.028	-
5	1.72	1.80	1.76 $\pm$ 0.057	6.89
10	1.42	1.38	1.40 $\pm$ 0.028	25.93
20	0.47	0.52	0.50 $\pm$ 0.035	73.54
30	0.406	0.414	0.410 $\pm$ 0.005	78.39
SEm $\pm$			0.02	
CD at 5%			0.05	
Y = -0.0253X+3.3251,				
R ² = 0.9331,				
T _{1/2} = 11.9 days				

In comparison, the initial residue of endosulfan in the mixture was $1.87 \mu\text{g mL}^{-1}$ and dissipated to 1.86, 1.69, 1.16 and $0.75 \mu\text{g mL}^{-1}$ in 5, 10, 20 and 30 days interval, respectively. At the end of the experiment there was 38.50 per cent endosulfan in the media.

The initial concentration of chlorothalonil was found to be $1.95 \mu\text{g mL}^{-1}$ which dissipated to 1.85, 1.74, 1.63 and $1.48 \mu\text{g mL}^{-1}$ after 5, 10, 20 and 30 days of inoculation, respectively (Fig. 34g). The corresponding concentration during the same time intervals were 1.86, 1.73, 1.65 and $1.54 \mu\text{g mL}^{-1}$, respectively when chlorothalonil was applied as mixture component with three other pesticides.

Table 37d. Rate kinetics of chlorothalonil in broth

Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Degradation (%)
	R ₁	R ₂		
0	1.96	1.90	1.95 $\pm$ 0.014	-
5	1.87	1.85	1.86 $\pm$ 0.014	4.60
10	1.74	1.74	1.74 $\pm$ 0.000	11.79
20	1.68	1.63	1.66 $\pm$ 0.035	14.87
30	1.53	1.48	1.51 $\pm$ 0.035	22.56
SEm $\pm$			0.02	
CD at 5%			0.05	
Y = -0.0036X+2.2861, R ² = 0.9809, T _{1/2} = 83.6 days				

Fig.34e Microbial degradation of endosulphan in broth

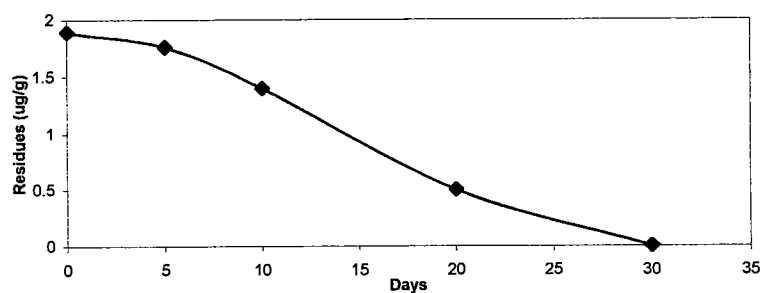


Fig.34f Logarithmic plots of endosulfan in broth

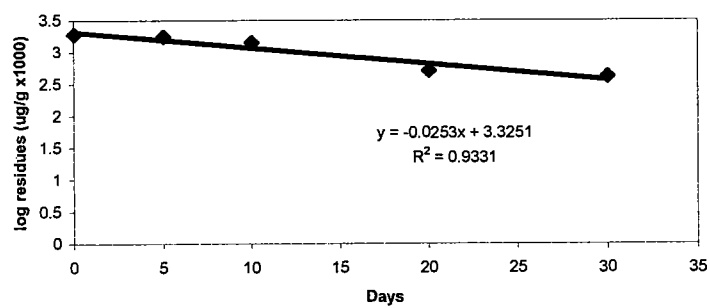


Fig.34g Microbial degradation of chlorothalonil in broth

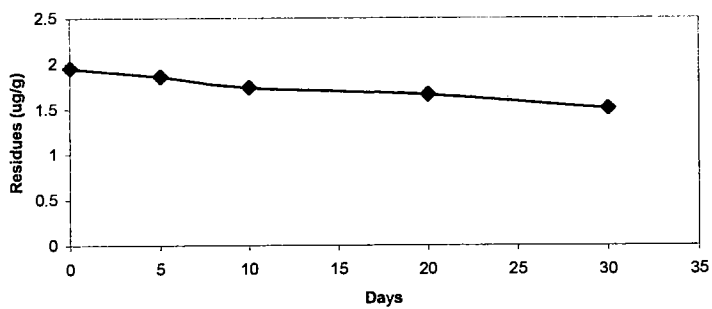
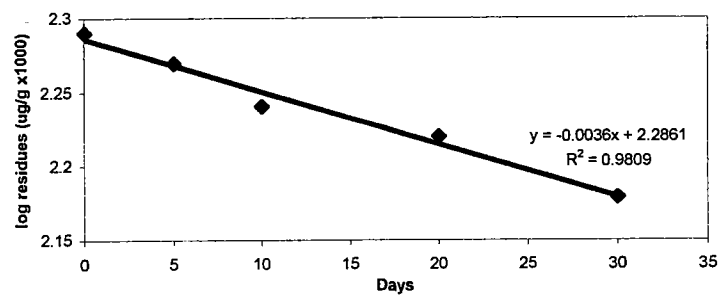


Fig.34h Microbial degradation of chlorothalonil in broth



The graph of disappearance of chlorothalonil at different intervals of time indicated slower loss of fungicide from the media. In the span of 30 days 22.56 per cent of added chlorothalonil was metabolized with 77.44 per cent remaining as residue in the media. Interestingly, in the same span of time interval there was higher residue (80.31 per cent) of chlorothalonil in the media when applied as mixture.

Table 37e. Rate kinetics of pesticide mixture in broth

Pesticide Component	Interval (days)	Residues ($\mu\text{g g}^{-1}$)		Average residues ($\mu\text{g g}^{-1} \pm \text{SD}$)	Degradation (%)
		R ₁	R ₂		
Pendimethalin	0	1.96	1.97	1.97 $\pm$ 0.007	-
	5	1.84	1.86	1.85 $\pm$ 0.014	6.09
	10	1.72	1.68	1.70 $\pm$ 0.028	13.71
	20	1.56	1.47	1.52 $\pm$ 0.064	22.84
	30	0.98	0.95	0.97 $\pm$ 0.021	50.76
Imidacloprid	0	9.86	9.77	9.82 $\pm$ 0.064	-
	5	9.20	9.25	9.23 $\pm$ 0.035	6.01
	10	8.17	8.46	8.32 $\pm$ 0.205	15.27
	20	7.23	7.16	7.20 $\pm$ 0.050	26.68
	30	6.98	6.82	6.90 $\pm$ 0.113	29.74
Endosulfan	0	1.92	1.87	1.87 $\pm$ 0.035	-
	5	1.85	1.86	1.86 $\pm$ 0.007	0.50
	10	1.65	1.72	1.69 $\pm$ 0.042	9.63
	20	1.20	1.12	1.16 $\pm$ 0.057	37.97
	30	0.66	0.78	0.72 $\pm$ 0.085	61.50
Chlorothalonil	0	1.88	1.96	1.92 $\pm$ 0.057	-
	5	1.83	1.88	1.86 $\pm$ 0.035	3.13
	10	1.69	1.76	1.73 $\pm$ 0.050	9.90
	20	1.63	1.67	1.65 $\pm$ 0.028	13.86
	30	1.52	1.56	1.54 $\pm$ 0.028	19.69
SEm $\pm$	Pesticides = 0.021, Interval = 0.024, P x I = 0.048				
CD at 5%	Pesticides = 0.063, Interval = 0.070, P x I = 0.140				

The logarithmic plots of pesticide residue (applied individually and as mixture) versus time obtained by fitting the regression equation have been presented in Figure 34 and 35. The plots indicated that the disappearance of parent compound fitted a first order kinetics decay curve, suggesting that the rate of dissipation of pesticides was proportional to the concentration of the

Fig.35a Microbial degradation of pendimethalin in broth as mixture component

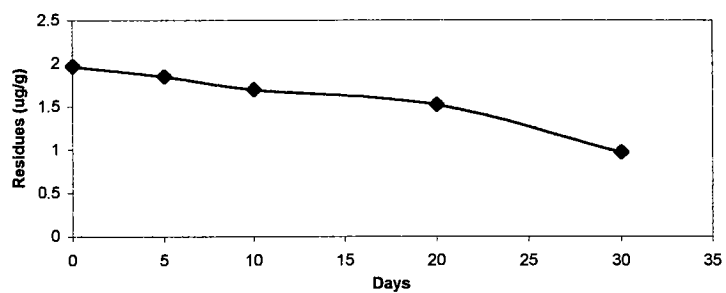


Fig.35b Logarithmic plots of pendimethalin in broth as mixture component

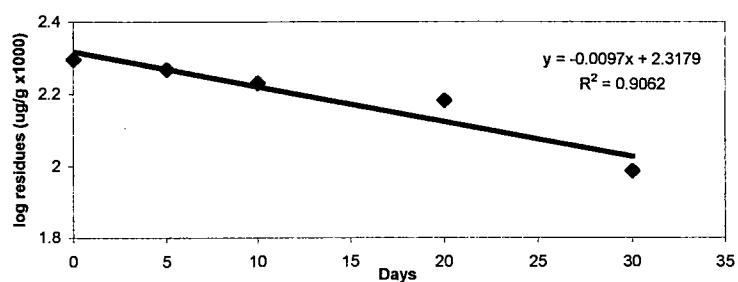


Fig.35c Microbial degradation of imidacloprid in broth

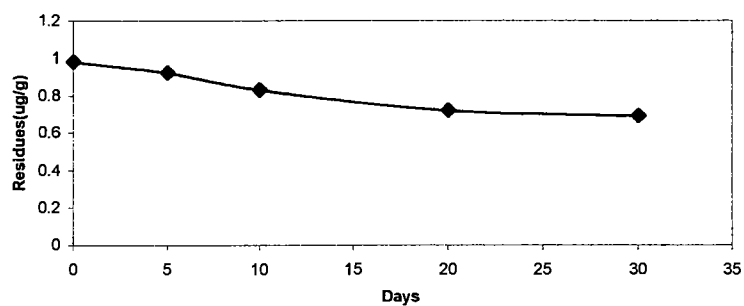


Fig.35d Logarithmic plots of imidacloprid in liquid broth as mixture component

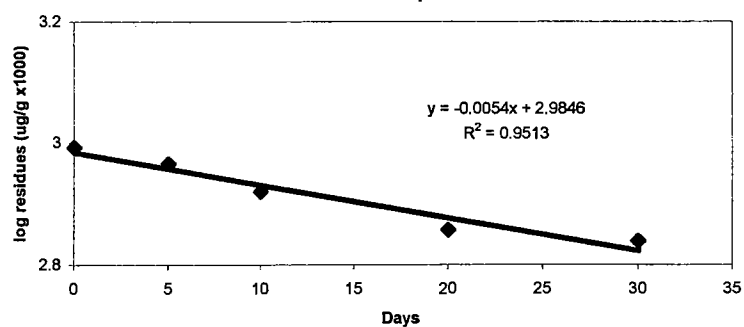


Fig.35e Microbial degradation of endosulphan in broth

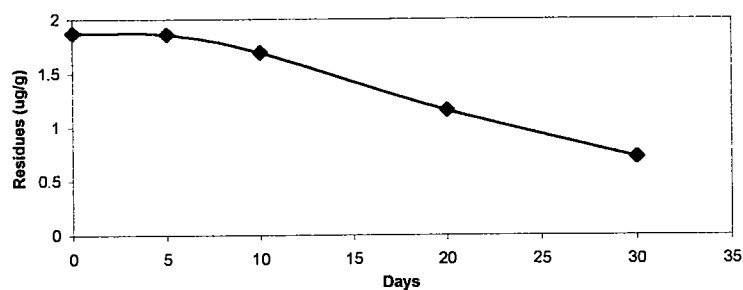


Fig.35f Logarithmic plots of endosulfan residues in broth as mixture component

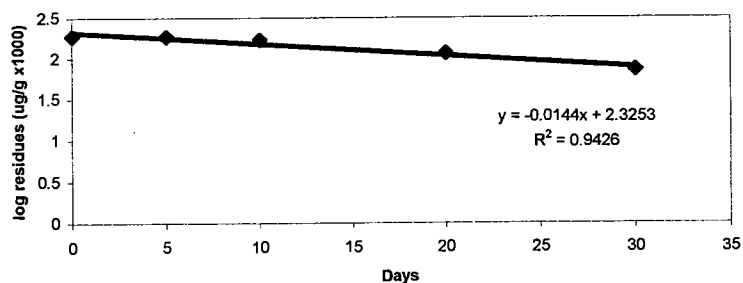


Fig.35g Microbial degradation of chlorothalonil in broth

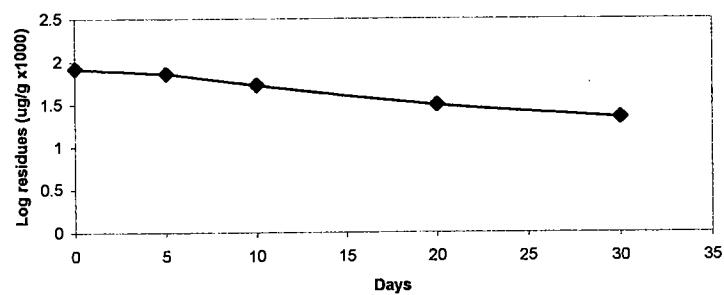
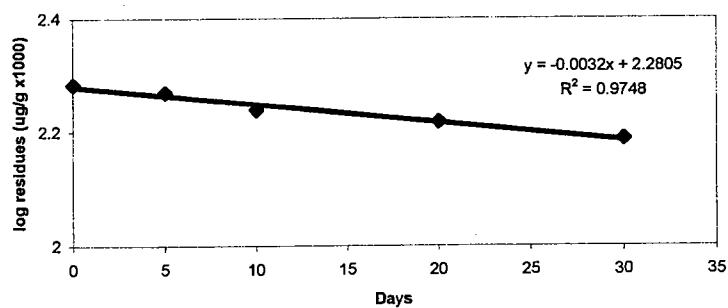


Fig.35h Logarithmic plots of chlorothalonil residues in liquid broth as mixture component



herbicide remaining in soil at a given time. The slope of the curve, correlation coefficient, rate constant and half-lives of the pesticides in liquid broth (CDM) as obtained from the regression equations are summarized in Table 37f.

Table 37f. Regression equation, slope, rate constant, correlation coefficient and half-lives of pesticide in liquid broth

Condition	Pesticide	Dissipation model	Slope	Rate constant-K	Correlation coefficient	Half-life
Alone	Pendimethalin	$Y = -0.0142X + 3.305$	-0.0142	0.0327	-0.960	21.20
	Imidacloprid	$Y = -0.0134X + 3.2963$	-0.0134	0.0309	-0.976	22.46
	Endosulfan	$Y = -0.0253X + 3.3251$	-0.0253	0.0583	-0.966	11.90
	Chlorothalonil	$Y = -0.0036X + 2.2861$	-0.036	0.0083	-0.990	83.61
Mixture	Pendimethalin	$Y = -0.0105X + 0.9842$	-0.0097	0.0223	-0.952	31.03
	Imidacloprid	$Y = -0.0054X + 2.9846$	-0.0054	0.0124	-0.975	55.74
	Endosulfan	$Y = -0.0144X + 2.5253$	-0.0144	0.0332	-0.971	20.90
	Chlorothalonil	$Y = -0.0032X + 2.2805$	-0.0032	0.0074	-0.987	94.06

4.3.2.1.3. Studies on microbial metabolites of pesticides

The transformation of agrochemicals in plants, animals and the environment is described as metabolism, biotransformation or degradation, and information on metabolic pathways, rate of metabolism and distribution is essential for many reasons. The majority of metabolism studies are conducted to provide data on the nature of residue arising from the use of a chemical for submission to regulatory authorities. A knowledge of metabolism, however can provide valuable insight into mode of action or toxicity and can also have a significant impact on novel selection and development. In the case of plants, metabolism studies define the nature of residues in the crop. If particular metabolites are the significant components of the residues then there is a need to detect and analyse them in crop residue trials in addition to parent agrochemicals. In the case of herbicides, a

different rate or route of metabolism occurs in susceptible species (weeds) compared with resistant species (crops), thereby providing a basis for selectivity.

Investigations on the degradation of the pesticides by the mixed fungal culture isolated from field soil treated with pesticides, in liquid medium (Czapek-Dox) was carried out to find out the degradation/transformation products. The fungal culture was allowed to grow in enriched media with pesticides ($250\mu\text{g mL}^{-1}$) as the source of carbon and incubated in BOD at $28\pm 1^\circ\text{C}$ for 20 days. The metabolites formed were identified by co-chromatography using GLC/HPLC technique by comparing with authentic standards and GC-MS.

4.3.2.1.3.a. Microbial metabolites of pendimethalin

The GC-MS analysis of the filter extract on completion of the experiment showed 6 peaks besides the peaks in control sample. Out of these peaks, one peak in each case, was that of unchanged parent compound. The microbial metabolic products were identified by comparison with authentic samples prepared in laboratory and by GC-MS. The retention time, M^+ and mass spectra of the identified products are presented in Table 38.

Table 38. Mass fragmentation pattern and retention (Rt) times of various metabolites of pendimethalin

Structure No.	Retention time (Rt) (min)	M^+ (m/z) (Relative intensity)	Mass fragmentation (relative intensity)
II.	9.180	211(9)	129(4), 115(5), 56(100)
III.	9.629	223(11)	150(10), 149(100), 104(18), 57(32)
IV.	9.913	150(10)	149(100), 121(7), 91(8), 55(53)
V.	13.461	256(5)	253(8), 185(6), 129(19), 55(100)
VI	15.881	165(4)	135(8), 91(11), 57(100), 55(95)
VII	17.476	135(5)	121 (10), 91(15), 57(100)

The mass spectrum of compound **II** showed a molecular ion peak at m/z 211 (9, M^+) (Table 38) with fragment ion peaks at 129(4), 115(5) and 56(100). It was tentatively identified as dealkylated (DA) pendimethalin and was characterized as 2,6-dinitro-3,4-xyldine. The structure of compound **III** was also confirmed by comparison with authentic sample prepared in laboratory and its molecular ion peak was observed at m/z 223 (11, M^+) with fragment ions m/z 150(10), 104(18) and 57(32) and it was identified as N-(1-propyl)-2,6-dinitro-3,4-xyldine. The mass spectrum of **IV** showed a molecular ion peak at m/z 150 (M^+) with fragment ion peak at 149(100), 121(7), 91(8) and 55(53) and it was tentatively identified as xylene-3,4,5-triamine. The metabolite **V** was tentatively identified N-(1-propyl)-4-hydroxy-2,6-dinitro-3,4-xyldine and its mass spectrum showed a molecular ion peak at m/z 256 (M^+ , 5) with fragment ion peaks at 253(8), 185(6), 129(19) and 55(100). Another product was identified as 6-amino-4-nitro-xylene with molecular ion peak was m/z 165(4) and fragment ion peaks at 135(8), 91(11), 57(100) and 55(95).

Thus, a total of six metabolites of pendimethalin were detected and identified in the broth culture. The compound **II** was formed due to cleavage of -N-C bond of amino isopentyl group. Similarly, the removal of -CH₂-CH₃ group from the -CH-(CH₂CH₃)₂ resulted in the formation of metabolite **III** i.e., N-(1-propyl)-2,6-dinitro-3,4-xyldine. The formation of metabolite **IV** was due to the reduction as well as dealkylation of pendimethalin in the presence of soil microflora. The partial dealkylation, followed by hydroxylation in the 4-position of benzene moiety resulted in the formation of metabolite **V**. The formation of the metabolite **V** was in accordance with the earlier report of Barua *et al.* (1990). Metabolite **VI** seems to be formed due to dealkylation,

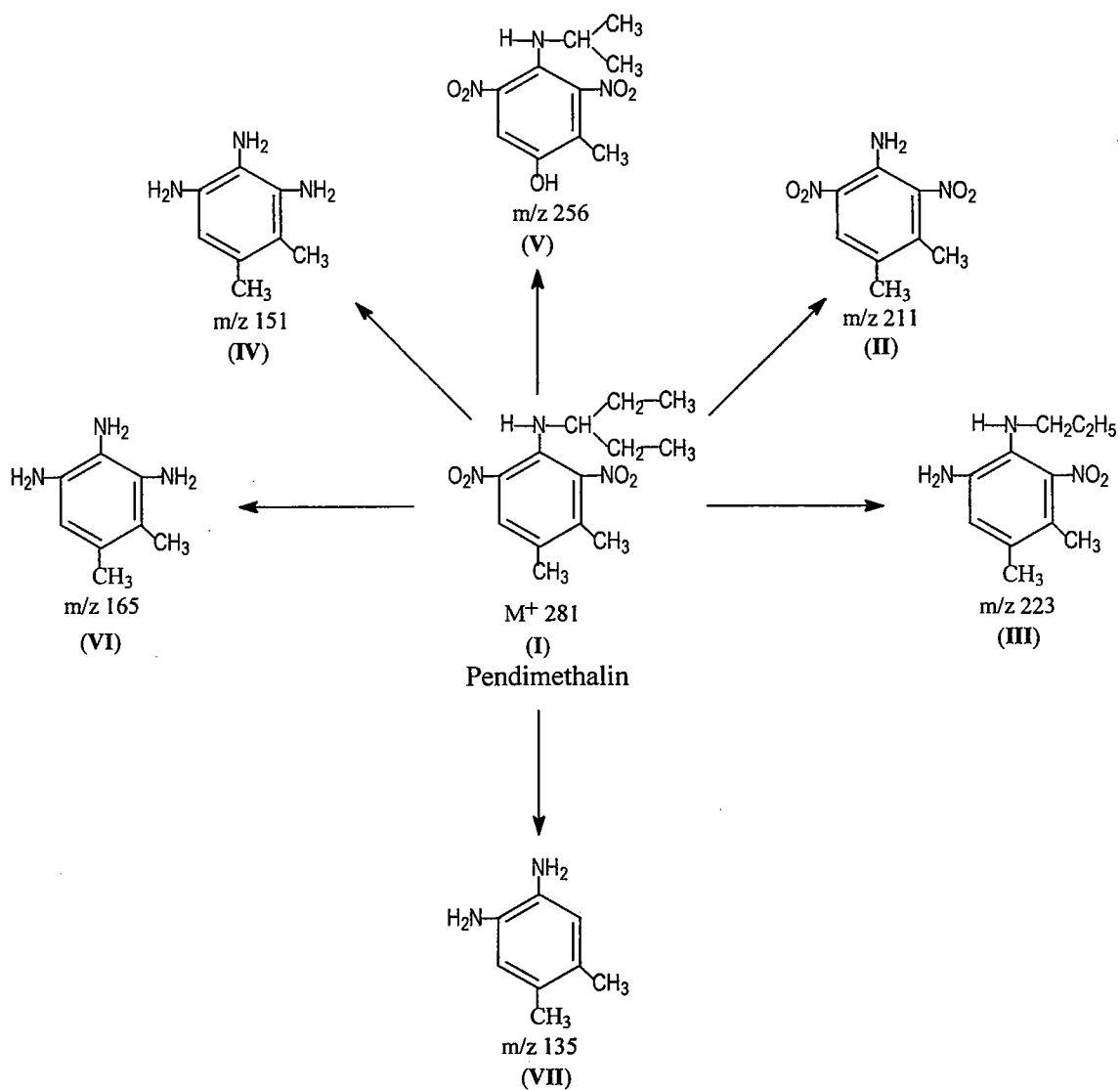


Fig. 35i: Postulated degradation products of Pendimethalin in broth

reduction followed by deamination of the parent pendimethalin. Such compound has also been reported earlier by Kole *et al.* (1994).

4.3.2.1.3b. Microbial metabolites of imidacloprid

Attempts were made to identify the microbial metabolites or transformation products of imidacloprid in liquid broth (CDM). The fungal culture was allowed to grow in enriched media with pesticides ($20 \mu\text{g mL}^{-1}$) as the source of carbon and incubated in BOD at $28 \pm 1^\circ\text{C}$ for 20 days. The metabolites formed were identified by GC-MS. It was found that with the passage of time, as imidacloprid degraded, different metabolites were formed. In other words, the mixed fungal culture metabolised imidacloprid into various transformation products during experimental period.

The GC-MS analysis of the of the filtrate extract indicated 5 peaks. Unchanged parent pesticide was also present as shown by GC-MS. The product identified were microbial metabolites of imidacloprid as they were not present in the respective control samples. The retention time, M^+ and fragmentation of the identified products are presented in Table 39.

Table 39. Mass fragmentation pattern and retention of various metabolites of imidacloprid

Structure No.	Retention time (Rt) (min)	M^+ (m/z) (Relative intensity)	Mass fragmentation (relative intensity)
II.	1.591	211	165(7), 150(100)
III.	12.260	129	129(12), 85(18), 55(100)
IV	15.239	207	167(19), 149(68), 55(100)
V.	8.238	288	207(1), 150(8)
VI.	15.386	185	185(5), 55(100)

The GC-MS of the 20th day extract showed six peaks including one of imidacloprid. Out of these peaks, structures of 5 compounds could be tentatively characterized.

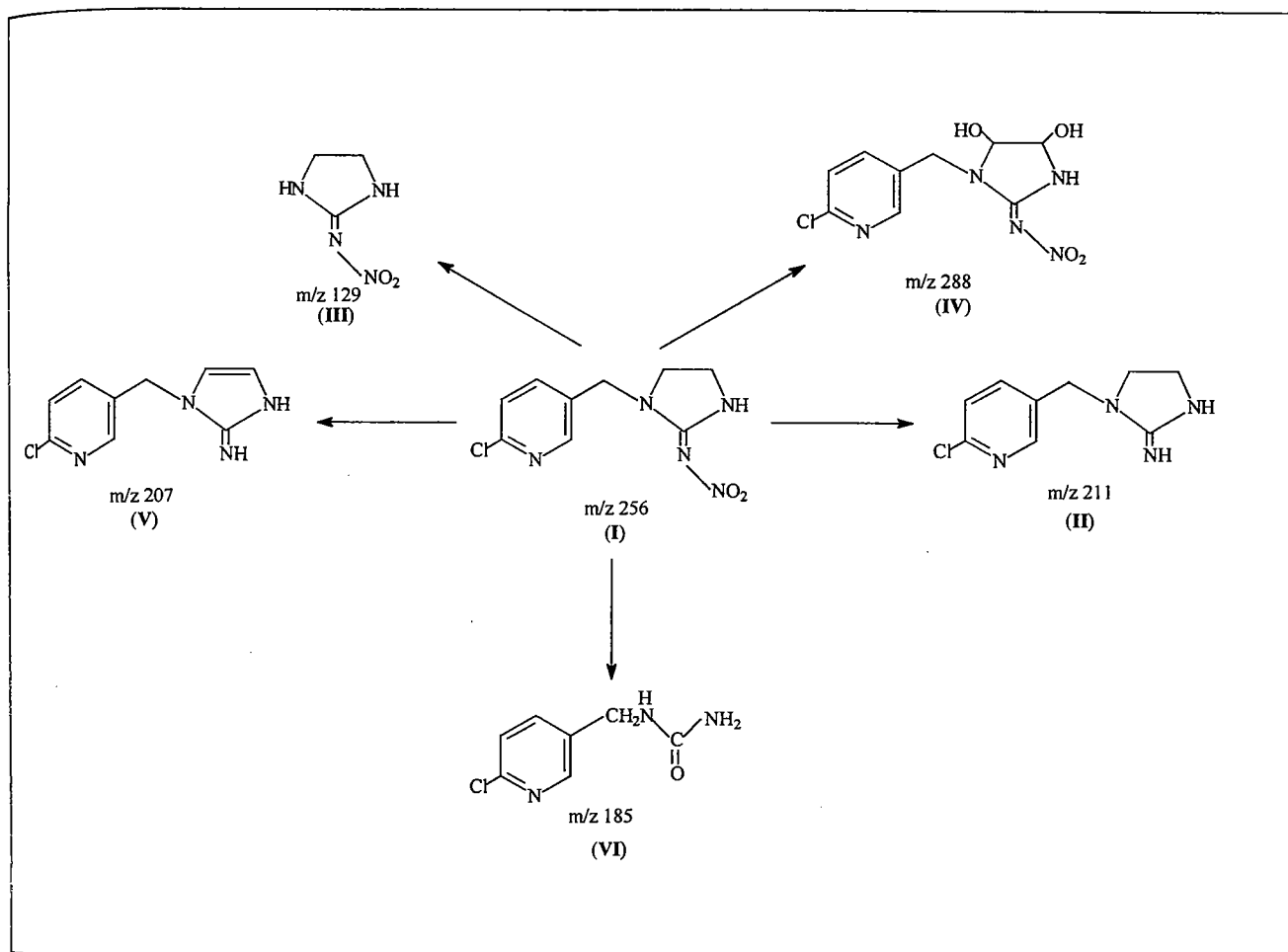


Fig. 35j: Postulated degradation products of imidacloprid in broth

The mass spectrum of compound **II** showed a molecular ion peak at m/z 211 (7, M^+) (Table 39) with fragment ion peaks at 165(7), 150(100). It was tentatively identified as imidacloprid guanidine.

The metabolite **III** was tentatively identified as N-nitroguanidine and its mass spectrum showed a molecular ion peak, at m/z 129 (m/z 12, M^+) with fragment ion peaks at m/z 85(18) and 55(100).

The metabolites **IV** was tentatively identified as dihydro- derivative of imidacloprid guanidine and its mass spectrum showed a molecular ion peak at m/z 207 (M^+) with fragment ion peaks at m/z 167, 149 and 55(100).

Another compound which showed the molecular ion peak at m/z 288 (M^+) with fragment ions at 207(1) and 150(8) was characterized as dihydroxy imidacloprid (**V**).

The mass spectrum of metabolites **VI** showed a molecular ion peak at m/z 185 (M^+) with fragment ion peak at 149 (M^+) with fragment ion peak at 55. It was tentatively identified as 1-(6-chloro-3-pyridyl methyl) urea.

Thus, a total of five metabolites of imidacloprid were detected and identified in the liquid broth.

The formation of these 5 metabolic products indicate the denitration, hydrolysis, dehydrogenation and hydroxylation as the major pathways of degradation of imidacloprid. Denitration products are tentatively identified as **II** by the cleavage of $N-NO_2$ which on dehydrogenation can give product **V**. Hydrolysis of imidacloprid can result in **III** but the second half chloro picolinic acid could not be isolated or indicated in GC-MS..

4.3.2.2. Identification of microorganisms responsible for the degradation of pendimethalin, imidacloprid, chlorothalonil and endosulfan and their mixture

The microorganisms capable of degrading individual pendimethalin, imidacloprid, chlorothalonil, endosulfan and their mixture were isolated and

tested for their ability to degrade pesticide. The mixed culture was obtained from the culture-media where the degradation was experimented. At the final day of experiment an 1 mL of inoculum was taken for the media and inoculated sterilized potato dextrose agar medium (PDA) on petridishes and kept in incubator at $25 \pm 2^\circ\text{C}$ for the 10 days after which the consistent fungi arising therefrom were subcultured and purified by repeated isolations in PDA medium. The purified fungi were sent for identification to Indian Type Culture Collection, Division of Mycology and Plant Pathology, IARI, New Delhi. The microorganisms capable of degradation and per cent degradation of pendimethalin were enlisted in Table 40.

Table 40. Fungal species capable of degrading pesticides alone and its combination

	Pesticides	Average degradation (%)	Fungal species
1.	Pendimethalin (P)	82	<i>Fusarium oxysporium</i> , <i>Aspergillus niger</i> , <i>Aspergillus ochraceous</i> , <i>Aspergillus flavus</i> , <i>Sclerotium rolfsii</i> , <i>Penicillium sp.</i>
2.	Imidacloprid (I)	72	<i>Rhizoctonia bataticola</i> , <i>Penicillium sp.</i> , <i>Aspergillus terreus</i> , <i>Aspergillus sydowii</i> , <i>Paecilomyces variotii</i> , <i>Alternaria alternata</i>
3.	Chlorothalonil (C)	35	<i>Rhizoctonia bataticola</i> , <i>Mucor</i> , <i>Aspergillus terreus</i> , <i>Fusarium solani</i>
4.	Endosulfan (E)	96	<i>Aspergillus niger</i> , <i>Aspergillus terreus</i> , <i>Aspergillus flavus</i> , <i>Penicillium sp.</i>
5.	PICE mixture	42	<i>Trichoderma viridae</i> , <i>Aspergillus terreus</i> , <i>Aspergillus flavus</i> , <i>Penicillium sp.</i>

It is evident from the Table 40 that six species of fungi were responsible for degradation of pendimethalin among them *Aspergillus* was the predominant genera. *Fusarium* and *Sclerotium* also contributed to the degradation of pendimethalin. Barua *et al.* (1990) and Singh and Kulshrestha (1991) reported that similar species responsible for degradation. There was overall 82 per cent degradation of pendimethalin in the broth-culture.

Degradation of imidacloprid was mainly contributed by six fungal species and like, pendimethalin, *Aspergillus* dominated the broth culture. A total of 72% degradation of imidacloprid occurred after 25 days of incubation. The other genera which contributed to the imidacloprid degradation were *Rhizoctonia*, *Penicillium*, *Paecilomyces* and *Alternaria*.

Chlorothalonil was degraded very slowly at the end of the experimental period (25 day). There was merely 35 per cent degradation. The fungal species in the broth-culture identified were *Rhizoctonia*, *Mucor*, *Aspergillus* and *Fusarium*. Probably the slow growth at the early stage of the fungi contributed the reduced degradation of chlorothalonil. Mori *et al.* (1998) evaluated the combinations of bacteria and fungi for the degradation of chlorothalonil and observed a number of mycoflora are responsible for the degradation of chlorothalonil.

The majority (96 per cent) of the endosulfan was degraded within the experimental period (20 day). The result was thus consistent with the faster degradation in the soil. The major fungal genera present there were *Aspergillus* and *Penicillium*.

The microorganisms were capable of degrading mixture of pendimethalin+imidacloprid+chlorothalonil+endosulfan (PICE) in broth-culture. The mixed culture *Trichoderma viridae*, *Aspergillus terreous*,

Aspergillus flavus and *Penicillium* sp. degraded the PICE mixture to the extent of about 42 per cent.

Here one thing was clear from the experiment that there was very few fungal species responsible for PICE mixture degradation and the phytopathogenic fungus species like *Rhizoctonia solani*, *R. bataticola*, *Fusarium oxysporium*, *F. solani*, *Sclerotium rolfsii*, *Alternaria alternata* were not found in the PICE-mixture -broth -culture. The result thus enumerated that there was additive or synergistic effects among the compounds in the mixture which killed the majority of phytopathogenic fungi. Thus application of the mixture gave better result in the field condition.

4.3.3. Effect of pesticides on microbial population

The effect of pesticides on soil microbial population was studied by conducting a field experiment with tomato crop. Pesticides were sprayed in the field at the recommended dose. Soil samples were drawn initially (3 hours after application) and 3, 10, 20 and 30 days after application from two layers i.e., 0-3 cm from both the treated and control plots. Soil was air-dried and a 10g representative soil sample was used for the enumeration of soil fungi and nematode separately by serial dilution technique and selective media.

4.3.3.1. Effect on fungal and nematode population

Viable fungal population in soil growing tomato following pesticides application individually, sequentially and as tank mixture (Table 41a to 41f) indicated a decline in their overall growth.

In the case of pendimethalin treated soil, there was decline of fungal strain from 16.8×10^4 to 13.7×10^4 per gram of rhizosphere (0-3 cm) soil in first 3 days (Table 41a).

Table 41a. Effect of pendimethalin residues on fungal and nematode population in tomato field soil

Days	Fungi ($\times 10^4$ g ⁻¹ soil)*		Nematode ($\times 10^2$ per 500g soil)*	
	Control	Treated	Control	Treated
0	17.3	16.8	28.2	27.3
3	15.6	13.7	21.6	12.2
10	21.2	16.6	19.4	13.4
20	18.2	19.2	18.2	14.2
30	23.2	25.2	24.6	16.7
SEm $\pm$		1.05		1.05
CD at 5%		3.16		3.16

* Mean of three replicates

However, after 10 days population in the upper layer of treated plot were found to be 16.6×10^4 g⁻¹ soil and after 30 days 25.2×10^4 g⁻¹ soil.

The nematode population was 27.3×10^2 per 500 g soil initially, which was decreased to 12.2×10^2 per 500 g soil after 3 days of pendimethalin application. After 3rd days the population build up gradually and on the 10th day, the population reached to 13.4×10^2 per 500g soil. At the end of the experiment (30 days) the nematode population were 16.7×10^2 per 50 g soil.

The data on effect of imidacloprid on fungal and nematode population is presented in Table 41b. The fungal population on imidacloprid treated soil was initially on 14.8×10^4 g⁻¹ soil. After 3 days of treatment the population decreased slightly and reached to 13.2×10^4 g⁻¹ soil. On the 10th day the population increased again to 14.5×10^4 g⁻¹ soil which reached to 20.5×10^4 and 22.8×10^4 g⁻¹ soil on 20 and 30 days, respectively.

The initial nematode population in imidacloprid treated soil slightly lower than pendimethalin treated soil (23.5×10^2 /500g soil). After 3 days of treatment the reduction of the nematode population was merely 4.7 per cent

(22.4×10^2). Within 10 days the increment of the population depicted the low nematotoxicity of imidacloprid. After 30 days, the soil nematode reached to $27.6 \times 10^2/500\text{g}$ soil (17.45 per cent increased).

Table 41b. Effect of imidacloprid residues on fungal and nematode population in tomato field soil

Days	Fungi ($\times 10^4 \text{ g}^{-1}$ soil)		Nematode ($\times 10^2$ per 500g soil)	
	Control	Treated	Control	Treated
0	17.6	14.8	27.6	23.5
3	18.5	12.2	29.3	22.4
10	20.2	14.5	30.5	26.4
20	23.4	20.5	30.6	28.2
30	20.6	22.8	32.3	27.6
SEm $\pm$		0.70		0.39
CD at 5%		2.10		1.17

Application of chlorothalonil in the field soil reduced the fungal population significantly. The initial fungal population $16.8 \times 10^4 \text{ g}^{-1}$ soil was reduced to $6.4 \times 10^4 \text{ g}^{-1}$ soil within 3 days of application. On the 10th day the population decreased further to $5.7 \times 10^4 \text{ g}^{-1}$ soil. The population then increases gradually and reaches to $7.2 \times 10^4 \text{ g}^{-1}$ and $9.5 \times 10^4 \text{ g}^{-1}$ soil, on 20 and 30 days, respectively (Table 41c).

The fungicide, chlorothalonil showed slight depressing effect to the nematode population during the initial stages (0-3 days). The before treatment count 19.2×10^2 nematode, reduced to 16.2 within 3 days application but thereafter the fungal population increased with the passage of time and stabilize within 30 days ($24.8 \times 10^2/500 \text{ g}$ soil).

Table 41c. Effect of chlorothalonil residues on fungal and nematode population in tomato field soil

Days	Fungi ($\times 10^4$ g ⁻¹ soil)		Nematode ($\times 10^2$ per 500g soil)	
	Control	Treated	Control	Treated
0	17.6	16.8	21.7	19.2
3	19.3	9.4	23.2	16.2
10	21.6	5.7	25.6	19.7
20	20.8	7.2	25.8	23.2
30	23.5	9.5	27.6	24.8
SEm $\pm$		0.68		0.82
CD at 5%		2.04		2.47

The data on endosulfan treatment of the tomato field soil (Table 41d) revealed that the fungal population decreases initially due to toxicity of the endosulfan. The before treatment fungal population, 21.2×10^4 g⁻¹ soil reduced to 19.3×10^4 g⁻¹ soil within 3 to 10 days. The population then gradually increases and reached to 17.3×10^4 g⁻¹ soil on 30 days.

The nematode population also reduced in the field soil following endosulfan application. The initial population $17.5 \times 10^2/500$ soil was reduced to 9.8×10^2 and $10.2 \times 10^2/500$ g soil within 3 to 10 days. On the 20th day the nematode count was found to be $16.8 \times 10^2/500$ g soil and further satisfied within 30 days.

Table 41d. Effect of endosulfan on fungal and nematode population in tomato field soil

Days	Fungi ($\times 10^4$ g ⁻¹ soil)		Nematode ($\times 10^2$ per 500g soil)	
	Control	Treated	Control	Treated
0	19.4	21.2	18.6	17.5
3	18.5	19.3	21.4	9.8
10	18.8	13.2	17.5	10.2
20	20.6	16.4	22.6	16.8
30	23.4	17.3	24.5	19.7
SEm $\pm$		0.50		1.29
CD at 5%		1.51		3.88

The viable fungal and nematode population in soil growing tomato following pesticides application either sequentially or as mixture (Table 41e and 41f) showed that the population was higher in the control plots than in the treated plots in both the cases. The fungal population before the sequential treatment (PICE) was $16.2 \times 10^4 \text{ g}^{-1}$ soil which reduced to 12.3×10^4 , 10.6×10^4 , $13.7 \times 10^4 \text{ g}^{-1}$ soil after 3, 10 and 20 days respectively. At the end of the experiment (30 days), the fungal population increased to $18.5 \times 10^4 \text{ g}^{-1}$ soil.

The nematode count before the sequential treatment of (PICE) was found to be 22.2×10^2 which reduced following the treatment to 13.4×10^2 and 9.2×10^2 on 3 and 10 day, respectively. The population then started to build-up and on the 20 days the population was $14.8 \times 10^2/500\text{g}$ soil and finally reached to $18.3 \times 10^2/500 \text{ g}$ soil on 30 days.

From the Table 41e it can be visualized that sequential application of pendimethalin-imidacloprid-chlorothalonil-endosulfan (PICE) had significant depressing effect on the fungi and nematode population at any stage.

Table 41e. Effect of sequential application of pesticides on fungal and nematode population in tomato field soil

Days	Fungi ($\times 10^4 \text{ g}^{-1}$ soil)		Nematode ($\times 10^2$ per 500g soil)	
	Control	Treated	Control	Treated
0	15.4	16.2	21.5	22.2
3	17.8	12.3	19.5	13.4
10	18.2	10.6	23.8	9.2
20	17.6	13.7	24.6	14.8
30	21.5	18.5	27.8	18.3
SEm $\pm$		0.47		0.52
CD at 5%		1.42		1.57

The data of PICE mixture application on fungal nematode population is presented in Table 41f.

Table 41f. Effect of pesticide mixture application on fungal and nematode population in tomato field soil

Days	Fungi ($\times 10^4$ g ⁻¹ soil)		Nematode ($\times 10^2$ per 500g soil)	
	Control	Treated	Control	Treated
0	16.7	15.3	19.7	19.6
3	18.3	12.2	21.4	10.5
10	17.6	18.8	22.5	16.50
20	19.4	10.30	26.3	10.2
30	22.8	14.6	28.4	16.9
SEm $\pm$		0.80		0.45
CD at 5%		2.41		1.36

Perusal of data revealed that application of mixture reduced the fungal and nematode population significantly.

The initial fungal count 15.3×10^4 g⁻¹ soil was reduced to 12.2×10^4 and 8.8×10^4 g⁻¹ soil on 3 and 10 days, respectively. The population was far below than control population. The reduction of microbial population indicated that the cumulative effect of PICE mixtures causes the reduction of fungal count. At the end of experimental period (30 days) fungal population had gone nearly the initial population (14.6×10^4 g⁻¹ soil).

The similar observation was occurred on nematode count. The initial population 19.6×10^2 /500 g soil was reduced to 10.5×10^2 and 6.50×10^2 /500g soil on 3 and 10 days, respectively. The count was far below than the nematode population in control plot. At the end of the experimental period the nematode population was increased to 16.9×10^2 /500g soil which was also much lower than control plot 28.4×10^2 /500g soil population.

4.3.3.2. Effect of treatment of pesticides alone and in combination against soil mycoflora and nematode.

The effect of different pesticides and their sequential and simultaneous application on soil against root knot nematode, *Meloidogyne incognita* and different soil mycoflora was studied by conducting a field trial in Division of Agronomy, IARI, New Delhi where *Tylenchorhynchus vulgaris*, *Rotylenchulus reniformis*, and *Meloidogyne incognita* were the predominant species (Table 42a). Since, the tomato crop is often parasitized by root knot nematode, *Meloidogyne incognita*, the effect on this nematode was studied in detail.

Observation on nematode and plant parameters were taken after 90 days of transplanting. The data on Table 42a and 42b revealed that all the treatments significantly reduced nematode galling, number of eggs masses per gram of root and soil population of juveniles.

Table 42a. Effect of treatments (module I) on predominant nematode species in soil

Treatments	Nematode population/250g soil*					
	Before treatment			At picking (after treatment)		
	<i>T. vulgaris</i>	<i>M. incognita</i>	<i>R. reniformis</i>	<i>T. vulgaris</i>	<i>M. incognita</i>	<i>R. reniformis</i>
T ₁ (P)	850	630	1030	570	525	680
T ₂ (I)	1060	570	860	680	520	780
T ₃ (E)	720	580	970	470	370	690
T ₄ (C)	820	550	1040	230	520	920
T ₅ (P+I+E+C)	890	620	870	250	215	210
T ₆ (P-I-C-E)	930	650	1050	270	280	320
T ₇ (Control)	990	620	1070	1040	670	1230
SEm±	20.70	17.88	10.00	12.66	13.99	19.16
CD at 5%	56.78	49.05	27.43	34.73	38.38	52.56

*Mean of three replications
P+I+C+E = Mixture application
P-I-C-E = Sequential application

Table 42b. Effect of treatments (module II) on predominant nematode species in soil

Treatments	Nematode population/250g soil*					
	Before treatment			At picking (after treatment)		
	<i>T. vulgaris</i>	<i>M. incognita</i>	<i>R. reniformis</i>	<i>T. vulgaris</i>	<i>M. incognita</i>	<i>R. reniformis</i>
T ₁ (P)	1260	820	1470	830	720	940
T ₂ (I)	1050	940	1320	340	280	460
T ₃ (β)	870	750	1250	650	640	870
T ₄ (E+T)	1040	930	1370	390	230	420
T ₅ (T+P)	750	840	890	400	240	460
T ₆ (β + E)	840	860	970	650	720	740
T ₇ (E+T+ β)	950	750	760	300	160	250
T ₈ (E-T- β)	1030	820	1420	360	230	380
T ₉ (Control)	960	950	1050	1040	980	1120
SEm $\pm$	13.98	5.32	12.11	11.99	19.97	15.68
CD at 5%	36.78	13.99	31.85	31.53	52.52	41.24

*Mean of three replications

All the treatments were *at par* in respect of number of galls, but number of egg masses and soil population were reduced to greater extent in mixture and sequential application. The plant growth significantly improved in all the treatments, while in case of imidacloprid, plants initially showed some phytotoxicity but later on picked up growth.

Application of the mixture (in both the cases, PICE and ET β) gave the best observation as the plant growth was highly satisfied there and the number of galls per plant were reduced significantly. The number of juveniles per 500 g soil in this plot were also least (650 J₂/500 g soil).

4.3.3.3. Soil mycoflora

Observation on soil mycoflora in terms of frequency for each of the fungus was also encountered from the rhizosphere of each of the amended

treatment collected at the time of harvest by soil dilution plate method. The isolated fungus were grown on potato dextrose agar (PDA) medium and purified by repeated isolations in PDA medium.

The predominant fungi strain present in the soil amended with pendimethalin were *Sclerotium rolfsii*, *Penicillium* and *Aspergillus flavus*.

Imidacloprid amended soil was predominated by three fungal species *Rhizoctonia solani*, *Fusarium* and *Aspergillus flavus* while endosulfan treated soil contained *Penicillium*, *A. flavus* and *A. terreus*.

R. bataticola, *Aspergillus* and *F. solani* were the predominant species in the plot where chlorothalonil was treated.

Application of mixture reduced the nematode population enhancing the bioagents like *Trichoderma viridae*, *A. terreus* and *A. flavus*. The association of these fungi parasitized the egg masses of root knot nematode, *M. incognita*, thus increased the plant vigour.

4.3.3.4. Interaction of soil mycoflora associated with rhizosphere and rhizoplain of root-knot nematodes *Meloidogyne incognita* infecting tomato

The effect of different pesticides and their combinations on soil mycoflora associated with rhizosphere and rhizoplain of root-knot nematode, *Meloidogyne incognita* infested soil was studied (Table 42c) by conducting the field experiment with the tomato crop. Pesticides were applied in the field as per the recommended dose. Egg masses of *M. incognita* collected from treated plants of the experiment. The galled roots from the affected plant along with the soil adhering it was kept separately in refrigerator. Water dilution technique was followed for the nematode soil while for the mycoflora, potato dextrose agar (PDA) medium was used. The mycoflora present in soil were transferred to the petriplates and incubated in BOD at 25°C±1 in which fungi

Table 42c. Effect of pesticides treatments (module I) on tomato against root knot nematode and soil mycoflora

Sl No.	Treatments	Plant growth parameters				Nematode parameters			Mycoflora
		Height (cm)		Dry weight (g plant ⁻¹)		No. of galls per plant	Egg mass/ g root	Juveniles /500g soil	
		Shoot	Root	Shoot	Root				
T ₁	Pendimethalin	60.2	24.1	13.4	2.43	62	134	1220	<i>Sclerotium rolfsii</i> , <i>Penicillium</i> sp., <i>Aspergillus flavus</i>
T ₂	Imidacloprid	58.3	18.2	13.8	2.45	65	156	1370	<i>Rhizoctonia bataticola</i> , <i>Fusarium solani</i> , <i>Aspergillus flavus</i>
T ₃	Endosulfan	65.3	20.2	13.5	2.52	50	88	980	<i>Penicillium</i> sp., <i>Aspergillus flavus</i> , <i>Aspergillus terreus</i>
T ₄	Chlorothalonil	68.5	26.4	18.2	3.70	48	95	1060	<i>Rhizoctonia bataticola</i> , <i>Fusarium solani</i> , <i>Aspergillus terreus</i>
T ₅	Mixture (P+I+C+E)	75.6	30.6	21.8	5.21	42	60	650	<i>Trichoderma viridae</i> , <i>Aspergillus terreus</i> , <i>Aspergillus flavus</i> .
T ₆	Sequential application of PICE	72.2	28.1	18.8	5.0	48	85	730	<i>Rhizoctonia bataticola</i> , <i>Rhizoctonia solani</i> , <i>Aspergillus flavus</i> .
T ₇	C ₁ (control soil at time of picking)	50.2	17.5	8.2	1.25	70	240	3000	<i>Rhizoctonia bataticola</i> , <i>Sclerotium rolfsii</i> , <i>Aspergillus flavus</i> , <i>Penicillium oxidiu</i> m
T ₈	C ₂ (Control soil before experiment)	-	-	-	-	-	-	1400	<i>Aspergillus flavus</i> , <i>Aspergillus niger</i> , <i>Penicillium</i> spp. <i>Fusarium solani</i>

grew after a week. The fungi from the roots were transferred in PDA slant in test tubes after surface sterilization in 0.01% mercury chloride.

The consistently, occurring fungi arising therefrom were subcultured and purified by repeated isolation in PDA medium. The purified fungi were sent for identification to Indian Type Culture Collection (ITCC), Division of Mycology and Plant Pathology, IARI, New Delhi.

In general, a good number of saprophytic fungi, were isolated. Out of these few are known to be fungal biogents viz. *Trichoderma viridae*, *A. niger*. Which has also been commercialized were encountered at the treatment showing improved plant growth parameters and reduced *M. incognita* both in roots and in soil. Out of these treatments, the one with the mixture was (T₅) showed most promising result in improving the plant growth parameters and also suppression of *M. incognita* population. The treatment showing least effect failed to suppress the nematodes population below minimum threshold level (2 larvae g⁻¹ soil) and also exhibited some fungi like *Sclerotium rolfsii* and *Rhizoctonia bataticola*.

4.4. Investigation on biological compatibility of pesticides/mixtures

4.4.1. Effect of various treatments on different insect-pests and diseases infecting tomato crop in the field

4.4.1.1. Insect pests

Tomato plant are attacked by a number of insects-pests and diseases that reduce the yield and spoil the quality of fruits. The major insect-pests are fruit borer [*Helicoverpa armigera* Hübner (Noctuidae)], white fly [*Bemisia tabaci* Gennadius], Aphids (*Aphis gossypii* Glover), tobacco caterpillar (*Spodoptera litura*) etc.

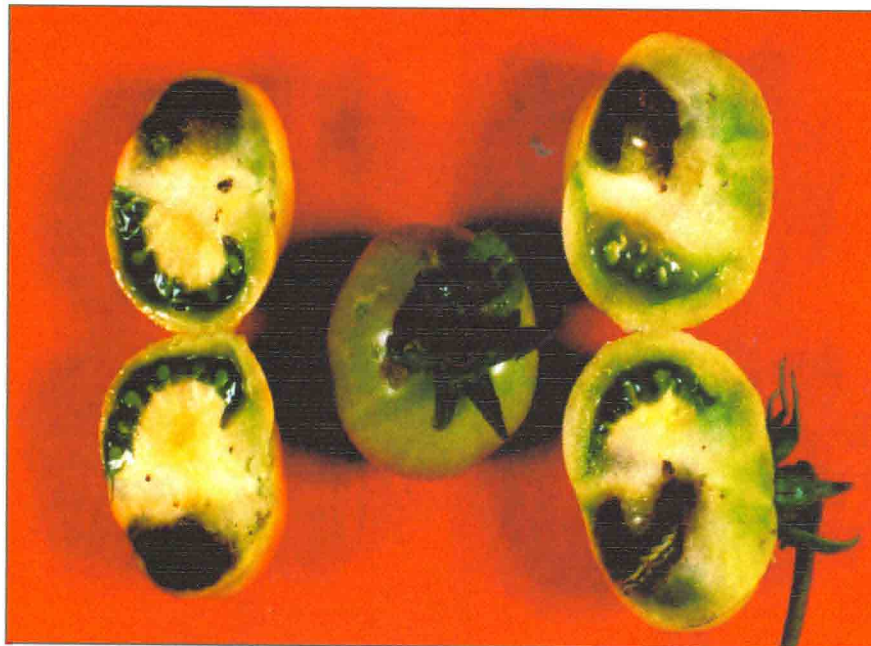
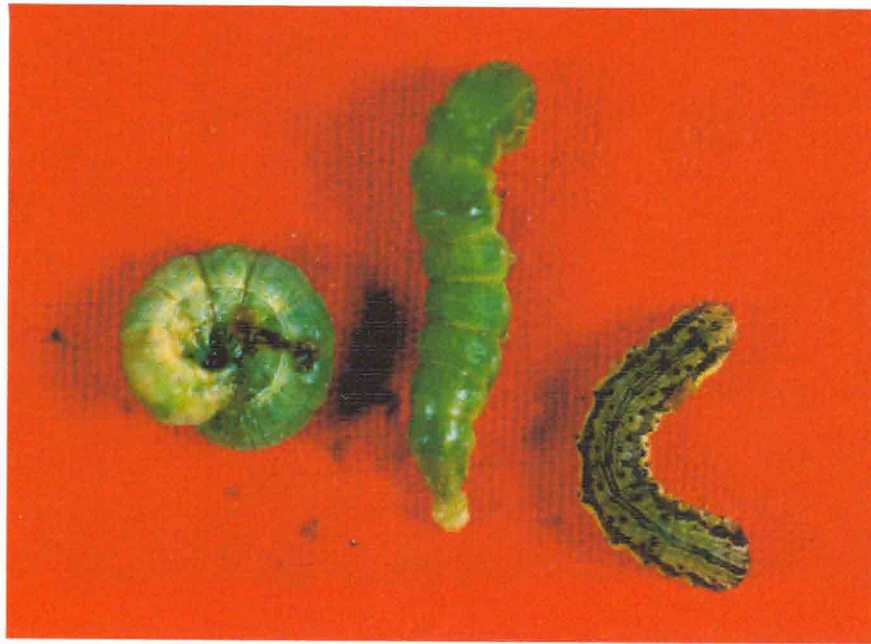


Plate 2. Fruit borer larvae and the damaged tomato fruits



Plate 3. White fly (*Bemisia tabaci*) nymphs feeding on tomato leaf

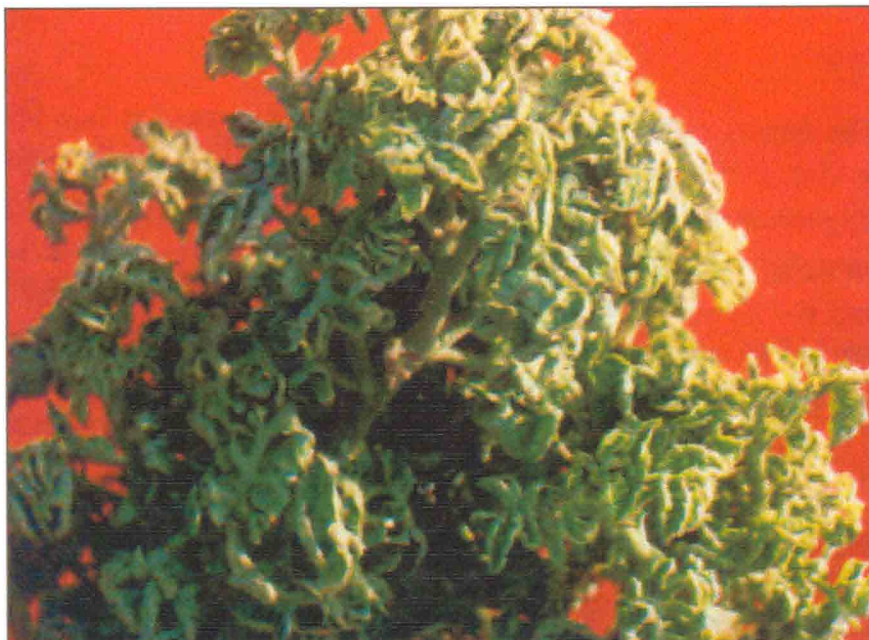


Plate 4. Leaf curl infestation in tomato due to white fly infestation

Helicoverpa armigera Hübner is a major pest of tomato. The young caterpillars feed on tender foliage and in advanced stage (fourth instar onwards) attack the fruits. They bore circular holes and thrust only a part of their body inside the fruit and eat inner contents (Plate 2). If the fruit is bigger in size, it is only partly damaged by caterpillar but subsequently it is invariably invaded by fungi, bacteria etc. and spoiled completely.

Whitefly (*Bemisia tabaci* Gennadius) is a tiny, scale-like insect seen dating about near the plants or crowding in between the veins on ventral surfaces of the leaves (Plate 3) sucking the sap from the infested parts. As a result of their feeding, the affected parts become yellowish, the leaves wrinkle and curl downwards and are ultimately shed. Besides damaging by feeding, these insects also exude honey dew which favours development of sooty mould. In case of severe infestation this black coating is so heavy that it interferes with the photosynthetic activity of the plant resulting in stunted growth. This whitefly also acts as vector, transmitting the leaf curl virus disease (Plate 4), causing severe loss, as all the affected plants are necessarily to be uprooted and destroyed.

Cotton aphid, *Aphis gossypi* Glover, is minor but very common insect-pest in tomato plants. The nymphs and adults, often found in large number suck the cell sap and secrete honeydew, which not only attracts the black ants but also favours the growth of sooty mould fungus giving the plants a sickly appearance and hindering their photosynthetic activity.

Besides these insects, several other insect-pests are reported by several authors. But in our experimental field these species were most predominant. Hence, our study was entitled to control these insect-pests.

There were sixteen treatments of individual/sequential/mixture combinations of pesticides applied onto the tomato crop for management of

various insect-pests. The predominant insect-pests that is fruit borer, white fly and aphids were controlled with the application of various treatments. The observations were taken on 1, 3, 7 and 10 days after spray. In case of root-dip treatments, the observations were taken 75 days after transplanting.

The insect population was observed in the field tagging the five plants per plot (2.5m x 2.5 m). In case of white fly and aphid, the population was taken per 30 cm twig but fruit borer population was observed by taking the total plants (no. of larvae per plant). The data were collected at regular intervals and statistically analysed (Table 43a).

Perusal of the data (Table 43a) revealed that the insect-pest population had decreased following different treatments over the control. Initially high population of aphid was reduced within 3 days of pesticide application and the effect continued to 10 days or more (in some cases). Among the various treatments T_{15} (spray treatment of mixture of endosulfan, triazophos and β -cyfluthrin) proved to be the best against aphids. It reduced the initial population of average 7.33 aphids/twig to 2.67 aphid /twig within 3 days of application. The effect continued till 7 days (Fig. 36a) and 10 days (Fig. 36b) where the value was significantly lower the majority of the treatments. Despite the superior control of aphid through T_{15} the other treatments T_7 (sequential root-dip treatment of imidacloprid followed by chlorothalonil (at transplanting and sequential spray of the imidacloprid - endosulfan - chlorothalonil at flowering), T_9 (foliar spray of triazophos), T_{14} (sequential spray of triazophos-endosulfan and beta-cyfluthrin did not differ significantly which implied the same kind of control of aphids by these treatments.

Table 43a. Effect of pesticide treatments on different insect-pests infecting tomato crop in the field.

Treatments		Insect intensity per 30 cm twig/plant											
		3 days				7 days				10 days			
		AP	WF	FB	AP	WF	FB	AP	WF	FB	AP	WF	FB
T ₁	Foliar spray of imida	17.33	4.00	10.67	8.67	3.00	9.00	9.67	2.33	6.33	7.33	1.67	5.00
T ₂	Foliar spray of endo	16.33	13.33	12.00	8.67	10.33	8.67	11.67	11.67	10.33	13.67	13.67	13.33
T ₃	Foliar spray of imida followed by chloro	17.67	7.33	14.67	13.67	6.67	10.67	6.67	5.33	6.00	9.33	4.67	7.67
T ₄	Foliar spray of imida + chloro	12.00	5.33	11.00	8.00	2.33	5.33	10.67	3.97	7.33	9.00	1.67	6.67
T ₅	Sequential spray of imida-chloro -endo	9.00	4.33	10.67	7.67	4.00	7.67	13.00	3.00	7.33	8.00	2.33	4.00
T ₆	Foliar spray of mixture of imida + chloro + endo	13.00	6.00	12.00	8.00	3.00	3.33	3.67	1.67	3.33	5.33	2.67	2.33
T ₇	Sequential root-dip of imida+chloro (transplanting) and sequential spray imida-chloro-endo (flowering)	10.00	7.00	9.00	6.67	5.33	6.33	7.67	3.33	5.33	6.00	3.33	3.33
T ₈	Root dip in mixture of imida+chloro (at transplanting) and foliar spray of mixture of imida+chloro+endo at flowering	8.00	4.33	5.67	5.00	2.67	13.33	6.67	2.67	3.33	6.67	3.33	2.67
T ₉	Foliar spray of Triazophos	18.00	3.33	10.00	5.67	3.00	10.00	6.67	3.00	8.00	10.67	3.67	12.67
T ₁₀	Foliar spray of b-cyfluthrin	10.00	5.33	5.67	9.00	5.33	4.337	8.00	6.67	5.33	12.33	6.67	7.67
T ₁₁	Foliar spray of mixture of β -cyflu + endo	10.00	10.33	6.33	9.33	8.33	3.33	7.33	7.33	4.00	13.33	8.33	3.33
T ₁₂	Foliar spray of mixture of endo + triazo	13.33	8.33	13.33	8.67	4.33	5.33	16.67	6.00	7.33	10.00	6.33	7.33
T ₁₃	Foliar spray of mixture of β -cyflu + triazo	13.67	11.00	12.33	13.00	3.33	6.67	8.33	3.33	6.67	14.00	4.67	7.67
T ₁₄	Sequential spray of triazo-endo- β -cyflu	8.33	7.00	12.67	5.00	4.33	5.33	7.33	5.00	8.33	5.00	4.33	7.00
T ₁₅	Foliar spray of mixture of endo + triazo + β -cyflu	7.33	9.33	7.67	2.67	4.33	3.00	3.00	5.33	3.67	3.33	5.33	3.67
T _{6(c)}	Control	28.33	13.00	13.33	32.00	17.67	17.33	36.00	22.33	20.33	37.00	24.6	28.67
SEM $\pm$		4.98	1.06	2.07	1.69	1.32	1.24	1.81	1.71	1.76	1.62	1.38	1.49
CD at 5%		5.72	3.06	5.98	4.88	3.81	3.58	5.23	4.94	5.08	4.68	3.98	4.30

AP – Aphid, WF – White fly, FB – Fruit borer

Therefore, triazophos alone gave the same control as the sequential application of imidacloprid-chlorothalonil-endosulfan and triazophos-endosulfan-betacyfluthrin or the mixture application of imidacloprid + chlorothalonil + endosulfan and mixture of endosulfan + triazophos + betacyfluthrin. After 7 day, except T₂, T₄ and T₅ all other treatments gave significantly same control over the aphids (Fig. 36a). On the 10th day, the aphid population was built-up slowly in some of the treatments like T₂, T₈, T₉, T₁₀, T₁₁ and T₁₂. But the remaining treatments gave good control of aphids among which T₁₅ proved to be best.

For the control of white fly, T₁ (foliar spray of imidacloprid) and T₄ (foliar spray of mixture of imidacloprid + chlorothalonil) were found to be two best treatments in tomato crop as both the treatments gave the lowest population of white flies even after 10 days of treatment (1.67 per 30 cm twig). The same treatments also worked better as it lowered down the initial insect-population within 3 days. Although most of the treatments gave good control of the white flies. Some of these did not differ significantly in their activity. Treatment T₁ was *at par* with T₃, T₄, T₅, T₆, T₇, T₈, T₉, T₁₃, T₁₄ and T₁₅ and gave significant control over whitefly. The superior activity of imidacloprid alone (T₁) and in combination with chlorothalonil (T₄) revealed that imidacloprid is very much compatible with the fungicide and did not register any negative impact on its activity. The further revelation of this result was emphasized when the T₄ treatment gave good control of the diseases as well (Table 43b).

The activity of T₇ and T₈ on white fly enumerated the fact that the root dip treatment (at transplanting) followed by foliar spray (sequential or mixture) was also very effective against the insect. The same result revealed that the solution mixture (or sequential) were biologically compatible for the

Fig.36a Effect of treatments on different insect-pests infecting tomato crop in the field 7days after treatment

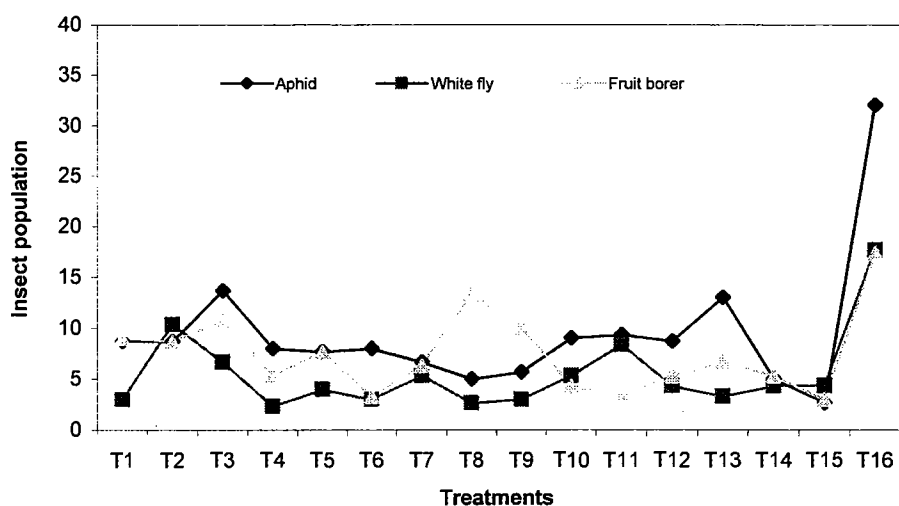
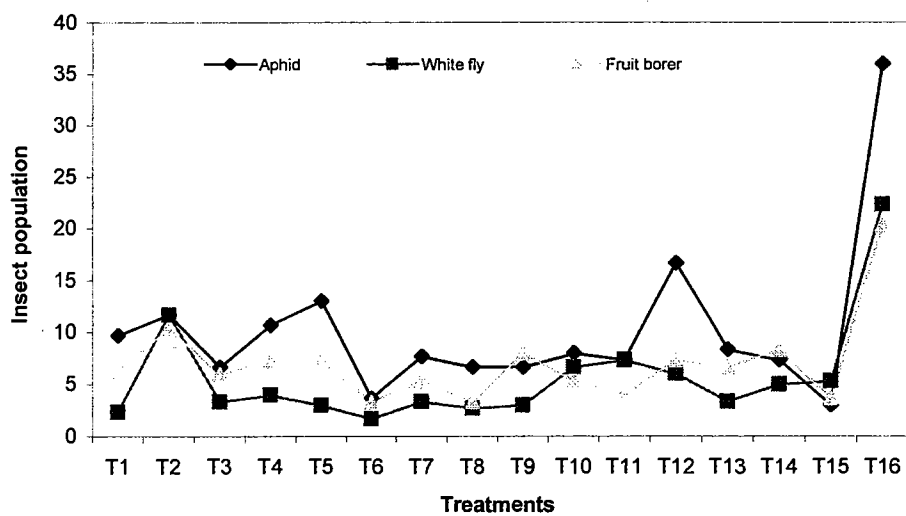


Fig.36b Effect of treatments on different insect-pests infecting tomato crop in the field 10days after treatment



root-dip treatment which had provided the protection of the crop at the early stage of growth. The mixture of β -cyfluthrin and triazophos also gave excellent control of white fly and the result was at par with triazophos alone or sequential or mixture treatment of endosulfan + triazophos + β -cyfluthrin. This result also gave the same revelation that the application of the mixture of endosulfan + triazophos + β -cyfluthrin can be very much effective to take the challenge of the control of leaf curl virus (transmitted by white flies).

The infestation of fruit borer in the tomato was checked by the application of different treatments. When the same sixteen treatments were tried for the control of the worm, the study revealed that, all the treatments were better over the control (check) and gave significantly better reduction of the pest-species. Among all the treatments T_6 (foliar spray treatment of imidacloprid + chlorothalonil + endosulfan) and T_8 (Root dip in mixture of imidacloprid + chlorothalonil at transplanting and foliar spray treatments of mixture of imidacloprid + chlorothalonil + endosulfan at flowering) gave best control of the borers. Although imidacloprid and endosulfan controlled the insect-pests, the presence of chlorothalonil in the spray solution did not affect the activity rather it helped to control the diseases in the field. The both T_6 and T_8 treatments, higher activity against the insect further revealed the fact that the two (imidacloprid + chlorothalonil) or three (imidacloprid + chlorothalonil + endosulfan) pesticide formulation mixture did not affect their activity in the field condition. Therefore, they are biologically compatible and can be recommended for the use in the farmers field. Among the other treatments T_1 , T_5 , T_7 , T_{11} and T_{15} gave the significant control against *Helicoverpa* and result was *at par* with the best treatment T_6 and T_8 . The other treatments like T_2 , T_3 , T_4 , T_9 , T_{10} , T_{12} , T_{13} and T_{14} gave significantly better result than control but the activity was lower than the previous case.

The sequential or mixture application of imidacloprid –chlorothalonil – endosulfan registered a similar observation of borer control in the field. The higher activity have further revealed the biological compatibility of these compounds in mixture.

The sequential or mixture application of endosulfan-triazophos beta-cyfluthrin also registered an excellent control against borer and hence recommended for the mixture application as it saved time, labour and cost.

4.4.1.2. Diseases

Of the tomato diseases, damping off (*Rhizoctonia* sp., *Phytophthora* sp., *Pythium* sp.), early blight (*Alternaria solani*), late blight (*Phytophthora infestans*) and buck-eye rot (*Phytophthora parasitica*) were very much common in our experimental field.

The pre-emergence damping-off of the seedlings and stem of the seedling decays at the soil surface due to shrinking of the cortical tissue of the hypocotyl and finally it collapses due to shrinking of the tissues of the stem near the ground.

The early and late blight (Plate 5) can be destructive at any stage of plant growth and results in defoliation, drying off of twigs and premature fruit drop depending on severity. In severe attacks the losses may be as high as 78 per cent (Nagaraja *et al.*, 2000).

Buck-eye rot is one of the most important fungal disease and affects mostly the green fruits.

Since the wilt is mainly problematic in nursery, our observation was concerned to 3 main diseases *viz.*, early blight, late blight and buck-eye rot.

The sprays of chlorothalonil and its combination with insecticide (Table 43b) were applied in the field to see the biological compatibility of the



Plate 5. Early blight (*Alternaria solani*) and late blight infestation on tomato leaf

Table 43b. Effect of pesticide treatment on different diseases infecting tomato crop in the field

Treatment	Diseases scoring on											
	3 days				7 days				10 days			
	EB	LB	BERT	EB	LB	BERT	EB	LB	BERT	EB	LB	BERT
T ₁ Foliar spray of chloro	12.67	9.00	5.33	8.33	7.00	5.00	4.33	3.33	3.00	8.00	5.67	5.00
T ₂ Foliar spray of imida followed by chloro	13.00	8.33	5.67	9.00	5.00	7.33	10.67	5.33	6.67	12.33	7.00	5.67
T ₃ Foliar spray of mixture of imida + chloro	10.67	9.67	7.00	7.67	5.00	6.67	4.33	4.67	5.67	5.67	5.67	4.67
T ₄ Sequential spray of imida-chloro -endo	10.33	10.33	7.00	10.00	9.67	6.67	6.00	7.00	6.33	9.67	7.67	5.00
T ₅ Foliar spray of mixture of imida + chloro + endo	11.00	8.00	6.00	7.67	9.33	5.67	6.00	4.00	5.67	8.67	6.67	5.00
T ₆ Sequential root-dip of imida+chloro (transplanting) and sequential spray imida-chloro -endo (flowering)	10.00	8.67	6.33	8.33	5.00	5.33	6.00	3.67	4.67	8.67	3.00	6.00
T ₇ Root dip in mixture of imida+chloro (at transplanting) and foliar spray of mixture of imida+chloro+endo at flowering	11.33	9.33	6.33	7.33	5.67	5.33	4.67	2.33	5.67	6.00	2.33	6.33
T ₈ (c) Control	17.67	13.33	9.00	21.33	19.67	12.33	28.67	25.33	14.67	32.67	65.67	17.33
SEm±	1.01	1.65	1.47	1.72	1.62	1.27	1.14	1.28	1.16	2.07	1.20	1.78
CD at 5%	3.06	5.0	4.46	5.22	4.91	3.85	3.46	3.88	3.52	6.28	3.64	5.40

EB = Early blight, LB = Late blight, BERT = Buck eye rot

fungicide. The sprays were given treatmentwise at 15 days interval starting from the first incidence of the diseases. The root dip treatments were given at the time of transplanting. Disease scoring was done at the interval of 3, 7, 10 and 15 days after last spray. The data so generated were analysed and critical difference was worked out for drawing the inferences. The graphical presentation of disease on 7 and 10 days has been presented in Fig. 37a and Fig. 37b.

It is evident from the Table 43b that the disease was initially higher and decreased after the treatment effect persisted upto 10 days and then gradually increased. Data on the perceptible decreament of the disease was observed on the 10th day (Fig. 37b). Application of chlorothalonil, in combination with imidacloprid, and the root dip treatment in mixture of these two at transplanting followed by foliar spray at flowering were significantly superior to the control as they recorded the lowest infestation of 4.33 and 4.67, respectively. However, they were *at par* with T₃ (foliar application of mixture imidacloprid + chlorothalonil), T₄ (sequential application of imidacloprid –chlorothalonil and endosulfan), T₅ (mixture, application of imidacloprid + chlorothalonil + endosulfan), T₆ (sequential root –dip of imidacloprid followed by chlorothalonil (transplanting) and sequential spray of imidacloprid, chlorothalonil followed by endosulfan and T₇ (root dip treatments of mixture of imidacloprid + chlorothalonil and foliar spray of mixture of imidacloprid +chlorothalonil + endosulfan).

Therefore, chlorothalonil retained its activity when it was applied as mixture with imidacloprid or endosulfan. Moreover the consistent results of

Fig.37a Effect of treatments on different diseases infecting tomato crop in the field 7 days after treatment

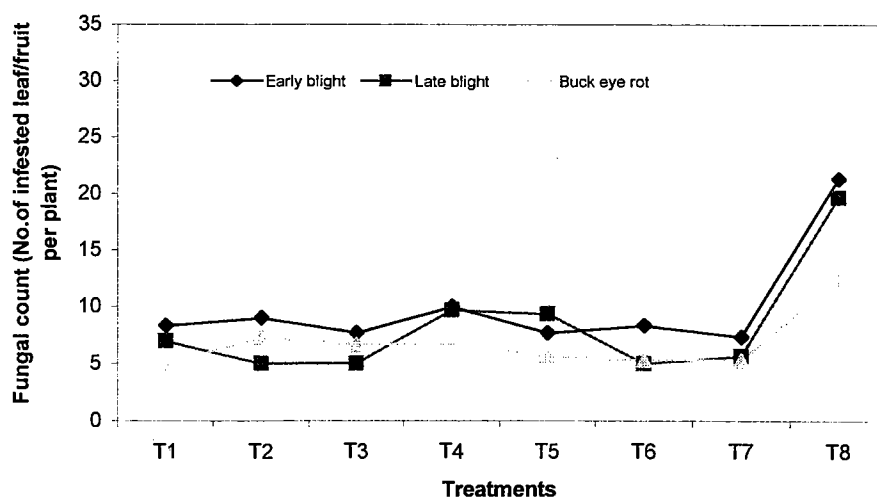
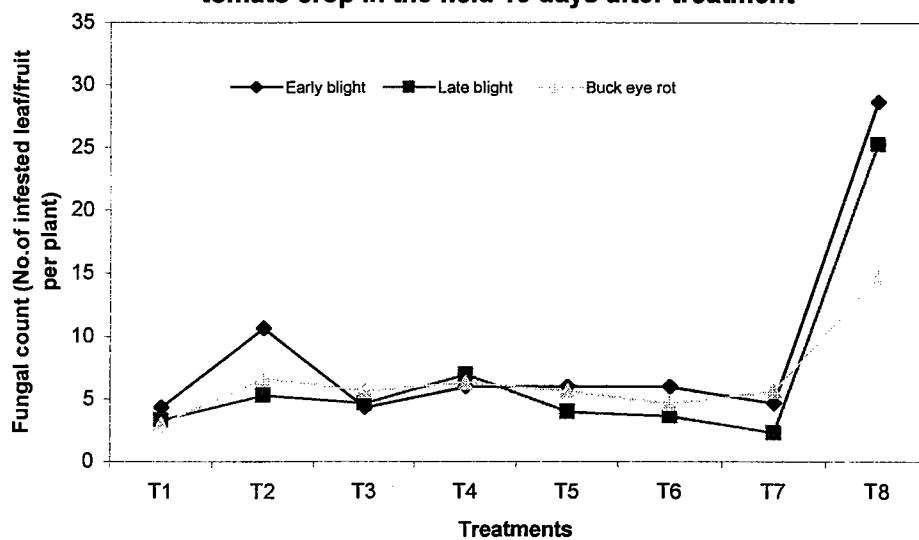


Fig.37b Effect of treatments on different diseases infecting tomato crop in the field 10 days after treatment



root dip application at transplanting followed by foliar spray revealed the fact, that imidacloprid and chlorothalonil can be mixed together and can be used for the root-dip treatment for the crops which suffers initial set back due insects like aphids, jassids or diseases like wilt or root rot. Hence, chlorothalonil is biologically compatible with the imidacloprid.

4.4.1.3. Effect of root-dip treatment on insects pests and disease infesting tomato crop

Root dip treatment of the tomato saplings in insecticide (imidacloprid) or fungicide (chlorothalonil) or in the formulation mixture is *in vogue* for the control of insects pests and disease.

In the present study, root dip treatment of tomato seedling with imidacloprid, sequential simultaneous dipping in imidacloprid and chlorothalonil mixture did not register significant reduction of pests population in the tomato crop (Table 43c). However, the decreament of aphid population following root dip in imidacloprid and chlorothalonil mixture. The study further emphasized the earlier results of favourable interaction or bio-compatibility of imidacloprid with chlorothalonil.

In case of fruit borer and whitefly, the results showed the same revelation of better suppression of pests in treated plot than control but the treatment themselves did not differ significantly. Only the sequential application left higher borer population enumerated in the later stage, as the concentration of the insecticides in plant system decreased ostensibly.

The same treatments (Table 43d) when viewed on the disease control aspects, it was revealed that T_2 and T_3 i.e., sequential and simultaneous application of imidacloprid and chlorothalonil registered better suppression



Plate 6. Weed infestation in tomato field

Table 43c. Effect of root-dip treatment on insect-pests infecting tomato crop in the field

Treatment	Aphid			Whitefly			Fruit borer		
	R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean	
T ₁ Root-dip treatment of tomato seedling with imidacloprid	8	6	8	7.33	3	3	2	2.67	4 6 7 5.67
T ₂ Sequential root-dip of imidacloprid followed by chlorothalonil	17	9	13	13.00	2	3	4	3.00	8 7 7 7.33
T ₃ Seedling root-dip treatment in mixture of imidacloprid +chlorothalonil	9	8	6	7.67	4	3	3	3.33	5 6 5 5.33
T ₄ Control	15	17	10	14.00	5	4	5	4.67	10 9 8 9.00
SEm±				1.60				0.46	0.66
CD at 5%				5.54				1.59	2.28

Table 43d. Effect of root-dip treatment on diseases infecting tomato crop in the field

Treatment	Early blight			Late blight			Buck eye rot		
	R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean	
T ₁ Root-dip treatment of tomato seedling with chlorothalonil	4	3	3	3.33	3	3	4	3.33	0 2 2 1.33
T ₂ Sequential root-dip of imidacloprid followed by chlorothalonil	3	3	2	2.67	3	4	5	4.00	3 4 3 3.33
T ₃ Seedling root-dip treatment in mixture of imidacloprid +chlorothalonil	2	2	1	1.67	5	4	5	4.67	3 2 2 2.33
T ₄ Control	5	6	5	5.33	7	8	12	9.00	3 5 6 4.67
SEm±				0.25				0.66	0.58
CD at 5%				0.86				2.28	2.01

of diseases than control. The treatment T_1 and T_2 did not differ significantly implies the fact that the sequential application of imidacloprid followed by chlorothalonil was at par with the chlorothalonil individual application. Therefore, sequential application of insecticide followed by fungicide did not hamper the activity of the fungicide. However, the better control of early blight was achieved through the application of imidacloprid and chlorothalonil, this might be as a consequence of overactivity of chlorothalonil in presence of imidacloprid.

In case of late blight, all the three treatments did not differ significantly. Therefore, the study revealed the same result of better biological compatibility of imidacloprid and chlorothalonil mixture.

The treatments showed least activity against buck eye rot. Except the individual application of chlorothalonil, not a single treatment showed better result on the diseases. However, other two treatments were also fairly good to control the diseases. Thus, the mixture did not register any negative impact of the activity of chlorothalonil.

4.4.1.4. Observation on weeds

4.4.1.4.a. Weed flora

Weed flora in the experimental plot consisted of a mixed population of grass weeds and broad leaf weeds (Plate 6). The important grass weeds were *Panicum repens* L., *Eragrotis tenella* L., *Digitaria sanguinalis* Scop, *Dactylactenium aegyptium* Beauv and *Cynodon dactylon* (L.) Pers. and broad leaf weeds were *Trianthema portulacastrum* L., *Digera arvensis* Forts. *Commelina benghalensis* L., *Convovulus arvensis* L., *Portulaca oleraceae*

L., *Phyllanthus niruri* L., *Euphorbia hirta* and *Tribulus terrestris* L. Among sedges, only *Cyperons rotundus* L. was present in the experimental field. Out of these *Erogrotis tenella* L. amongst the grasses and *Trianthema portulacastrum* L. amongst the broad leaf weeds were the most dominant species. Since weed related data were collected starting from the transplanting of the crop, our observation on weeds were limited to module I pesticides only.

4.4.1.4.b. Weed population

Data pertaining to weed population m^{-2} recorded at 30 and 60 days after transplanting and at harvest as influenced by pendimethalin and without pendimethalin herbicide and weed control is presented in Table 43e and Fig. 38a, respectively. The main plot treatments registered a significant variation in weed population in the tomato field after 30, 60 days and at harvesting. The individual, sequential and simultaneous (mixture) treatments did not marked reduction in grass, broad leaf weed and total weed population. All the sub-plots treatments were nearly *at par* with control except few cases where the initial reduction of the weed species occurred may be due to the phytotoxicity of the insecticides and fungicides on weeds. From the compatibility study it was evident that chlorothalonil and endosulfan was not so stable with pendimethalin. The result was further emphasizes by the quick growth of the weed population. The less persistence of pendimethalin contributed to the significant increase of weed species as compare to other species. Maximum reduction in weeds after 30 days was recorded under treatment eleven (T_{11}). One hand weeding at 45 days was found to be the best treatment in respect to the weed management.

Table 43e. Effect of treatments on weed population (No./m²) at different growth stages

Treatments	Weed population (No./m ²)*		
	30 DAT	60 DAT	At harvest
I. Main plot treatments			
With pendimethalin	2.26	2.91	4.99
Without pendimethalin	4.75	5.98	8.21
SEm±	0.05	0.04	0.63
CD (p = 0.05)	0.29	0.26	3.82
II. Sub-plot treatments			
T ₁ = Foliar spray of imidacloprid	3.77	5.07	7.15
T ₂ = Foliar spray of endosulfan	3.75	5.07	7.30
T ₃ = Foliar spray of chlorothalonil	3.68	4.92	6.93
T ₄ = Sequential root-dip treatment of imidacloprid followed by chlorothalonil	3.95	5.12	7.08
T ₅ = Root dip treatment of seedling in imidacloprid+chlorothalonil mixture	4.12	5.17	7.18
T ₆ = Sequential spray treatment of imidacloprid followed by chlorothalonil	3.75	4.82	6.72
T ₇ = Spray treatment of mixture of imidacloprid + chlorothalonil	3.72	4.98	4.48
T ₈ = Sequential spray of imidacloprid, chlorothalonil followed by endosulfan	4.27	4.60	6.28
T ₉ = Spray treatment of mixture of imidacloprid +chlorothalonil + endosulfan at flowering	3.90	4.33	6.17
T ₁₀ = T ₄ at transplanting + T ₆ at flowering	3.57	4.27	6.00
T ₁₁ = T ₅ at transplanting + T ₇ at flowering	3.75	3.92	11.65
T ₁₂ = One hand weeding (30 DAT)	1.75	3.23	4.92
T ₁₃ = Weed free	0.7	0.70	0.70
T ₁₄ = Control	4.42	6.02	7.88
SEm±	0.13	0.12	1.57
CD (P = 0.05)	0.37	0.35	4.46

*Data in table are square root transformed of original values

The observations on weed population after 60 days and at harvest were similar to the 30 days observations except some sub-plots treatments. All the sub-plots treatments did not mark reduction in weed population as compared to control treatment. Although some treatments were found significantly controlling weeds, but that could be due to lowering of activity of pendimethalin due to the interaction of other applied insecticide or fungicide.

At the harvest the (90 days) observation on weed population further emphasized the results of 30 and 60 days observation. The main plot treatments i.e., with pendimethalin and without pendimethalin showed significant difference on weed population. Not a sub-plot treatments were found to be highly satisfactory for the controlling of weeds. The relative lowering of weed population in the sub-plots may be attributed to the incompatibility of pendimethalin in soil the environment of other pesticides. Pendimethalin application followed by one hand weeding at 45 days was found to be most effective treatment in bringing down the weed population in the tomato field at the time of harvest.

4.4.1.4.c. Dry matter accumulation

Data on dry weight of weeds (g m^{-2}) recorded at 30 and 60 days after transplanting and at picking of fruits (90 days) as influenced by the application of pendimethalin and other pests control measures are presented in Table 43f and Fig. 38b.

Table 43f. Effect of treatments on weed dry weight (gm^{-2}) at different growth stages

Treatments	Weed dry weight		
	30 DAT	60 DAT	At harvest
I. Main plot			
With pendimethalin	1.00	1.75	1.99
Without pendimethalin	2.26	2.65	2.98
SEm $\pm$	0.01	0.02	0.00
CD ($p = 0.05$)	0.03	0.11	0.02
II. Sub-plot			
T ₁ = Foliar spray of imidacloprid	1.64	2.32	2.63
T ₂ = Foliar spray of endosulfan	1.72	2.33	2.63
T ₃ = Foliar spray of chlorothalonil	1.75	2.31	2.3
T ₄ = Sequential root-dip treatment of imidacloprid followed by chlorothalonil	1.91	2.33	2.68
T ₅ = Root dip treatment of seedling in imidacloprid+chlorothalonil mixture	1.83	2.41	2.68
T ₆ = Sequential spray treatment of imidacloprid followed by chlorothalonil	1.86	2.23	2.65
T ₇ = Spray treatment of mixture of imidacloprid + chlorothalonil	1.62	2.30	2.62
T ₈ = Sequential spray of imidacloprid, chlorothalonil followed by endosulfan	1.94	2.30	2.59
T ₉ = Spray treatment of mixture of imidacloprid+chlorothalonil+endosulfan at flowering	1.60	2.22	2.56
T ₁₀ = T ₄ at transplanting + T ₆ at flowering	1.77	2.21	2.59
T ₁₁ = T ₅ at transplanting + T ₇ at flowering	1.52	2.19	2.53
T ₁₂ = One hand weeding (30 DAT)	0.89	2.35	2.59
T ₁₃ = Weed free	0.70	0.70	0.70
T ₁₄ = Control	2.03	2.51	2.73
SEm $\pm$	0.02	0.04	0.10
CD ($P = 0.05$)	0.06	0.10	0.25

*Data in table are square root transformed of original values

Fig.38a Effect of treatments on weed population (No./m²) at different growth srages of tomato

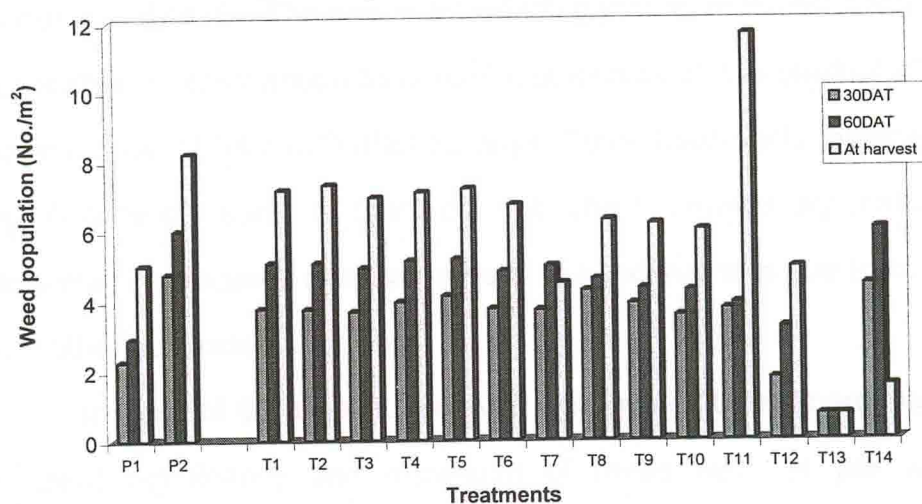
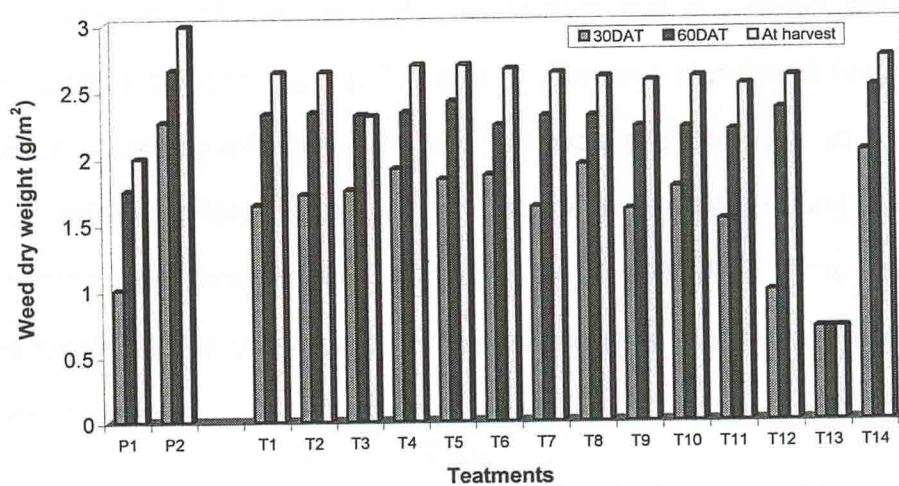


Fig.38b Effect of treatments on on weed dry matter(g/m²) at different growth srages of tomato



Application of pendimethalin influenced dry matter accumulation by weeds (decreased) significantly and increased value was recorded under without pendimethalin plot.

The sub-plot treatments did not curtail dry matter accumulation by weeds significantly. The one hand weeding plot gave lowest accumulation of dry matter. In comparison to control plot treatment T₈ contributed maximum weed matter (1.94 g m⁻²) after 30 days. Some treatments themselves differ significantly but some of them did not. The increased dry matter in these plots may be because of lesser activity of pendimethalin due to its interaction with other pesticides.

In case of 60 days observation, the sub-plot treatments itself did not differ significantly and maximum of these were at par with control treatments.

The significant difference among the main-plot treatments at the time of harvest depicted the efficiency of pendimethalin over without pendimethalin non-treatments for dry matter accumulation by weeds.

Majority of the pesticide treatments did not show any significant reduction. However, T₈, T₉, T₁₀ and T₁₁ caused significant reduction in dry matter accumulation over control. This was perhaps due to application of PICE mixture (Pendimethalin + imidacloprid + chlorothalonil + endosulfan) which caused simultaneous phytotoxicity on the weed flora. The exposure chemicals at the early stage of growth caused significant decrease in the weed species.

4.5. Effect of various treatments on the yield of tomato

Data on the effect of herbicide and combinations of insecticide, fungicide treatment on tomato yield have been presented in Table 44 and Fig. 39. The significant difference in the value of P₁ and P₂ revealed that the

pre-emergence application of pendimethalin (P_1) @ 1.00 kg ha^{-1} in tomato field gave significantly higher fruit yield than the non-pendimethalin plot (P_2). The treatment T_{13} (Root dip in mixture of imidacloprid + chlorothalonil (at transplanting) and foliar spray of mixture of imidacloprid + chlorothalonil + endosulfan at flowering) was proved to be best in the yield aspect. It gave significantly higher yield than any other treatments in the experiment. The treatments T_{11} (Foliar spray of mixture of imidacloprid + chlorothalonil + endosulfan), T_{12} (Sequential root-dip of imidacloprid + chlorothalonil (transplanting) and sequential spray imidacloprid-chlorothalonil-endosulfan (flowering)), T_{18} (Foliar spray of mixture of β -cyfluthrin + triazophos) and T_{20} (Foliar spray of mixture of endosulfan + triazophos + β -cyfluthrin) remaining statistically *at par* with each other and gave second best fruit yield. This is due to and reduction in weed growth due to pendimethalin application T_9 , T_{15} and T_{19} was also statistically *at par* in respect of the tomato yield. Individual application of imidacloprid (T_1) chlorothalonil (T) and endosulfan (T) gave significantly lower yield than T_9 , T_{11} , T_{12} , T_{13} , T_{15} , T_{16} , and T_{22} . This signifies the fact that the application of pesticide combination reduced the weeds, insects and diseases in the tomato field. There was significant difference in the spray and root dip treatment of imidacloprid and imidacloprid.

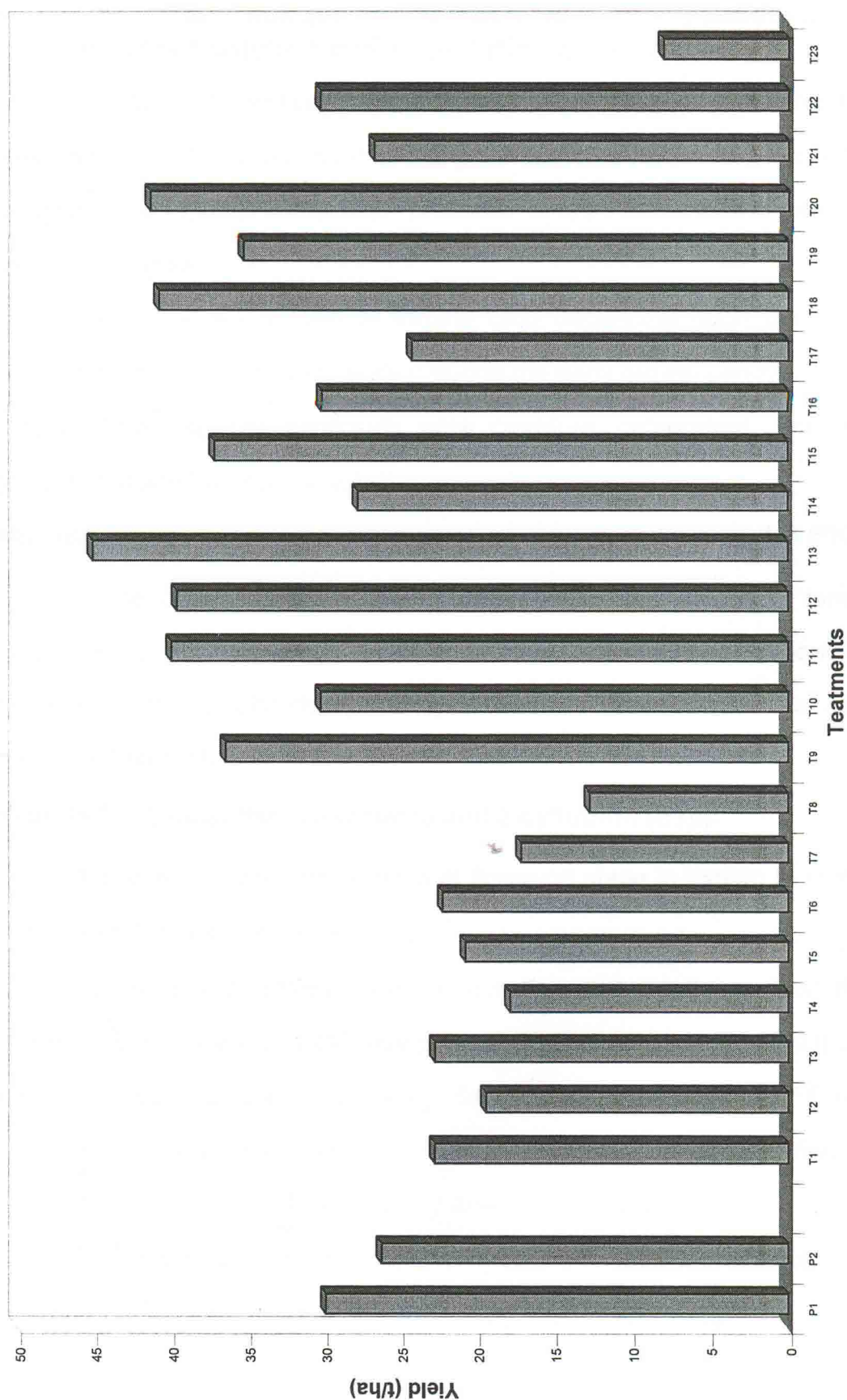
This is due to the longer duration of the tomato crop. The residues analysis revealed that after 75 days there was no imidacloprid in chlorothalonil residues in the fruit sample. However, root dip treatment gave significantly higher yield than control probably due to early protection of the crops from pests and diseases and weed control efficacy of pendimethalin even after 90 days. Bairambekov and Valeeva (1994), Rapparini and Campagna (1996) have also reported effective weed control and higher yield with pendimethalin in tomato. However, Pandey and Padhiar (1998) and

Reddy *et al.* (1999) reported that pre-emergence application of pendimethalin (@ 1.0 kg ha⁻¹) followed by one hand weeding 45 days after transplanting resulted in a higher fruit yield of tomatoes compound with either herbicide alone or with one hand weeding. Our finding was much similar to the findings of Reddy *et al.* (1999).

Table 44. Effect of treatment on yield of tomato

Treatment no.	Treatments	Yield (t ha ⁻¹)
T ₁	Foliar spray of imidacloprid	22.75
T ₂	Root-dip treatment of imidacloprid	19.39
T ₃	Foliar spray of chlorothalonil	22.75
T ₄	Root-dip treatment of chlorothalonil	17.81
T ₅	Foliar spray of endosulfan	20.75
T ₆	Sequential root dip treatment of imidacloprid followed by chlorothalonil	22.21
T ₇	Sequential spray of imidacloprid followed by chlorothalonil	17.11
T ₈	Root-dip treatment in mixture of imidacloprid + chlorothalonil	12.66
T ₉	Foliar spray of mixture of imidacloprid + chlorothalonil	36.44
T ₁₀	Sequential spray of imidacloprid, chlorothalonil followed by endosulfan	30.23
T ₁₁	Foliar spray of mixture of imidacloprid + chlorothalonil + endosulfan	39.99
T ₁₂	Sequential root-dip of imidacloprid + chlorothalonil (transplanting) and sequential spray imidacloprid-chlorothalonil – endosulfan (flowering)	39.62
T ₁₃	Root dip in mixture of imidacloprid + chlorothalonil (at transplanting) and foliar spray of mixture of imidacloprid + chlorothalonil + endosulfan at flowering	45.09
T ₁₄	Foliar spray of Triazophos	27.79
T ₁₅	Foliar spray of β -cyfluthrin	33.17
T ₁₆	Foliar spray of mixture of β -cyfluthrin + endosulfan	30.98
T ₁₇	Foliar spray of mixture of endosulfan + triazophos	24.27
T ₁₈	Foliar spray of mixture of β -cyfluthrin + triazophos	40.82
T ₁₉	Sequential spray of triazophos-endosulfan- β -cyfluthrin	35.28
T ₂₀	Foliar spray of mixture of endosulfan + triazophos + β -cyfluthrin	41.39
T ₂₁	One hand weeding	26.75
T ₂₂	Weed free	30.79
T ₂₃	Control	7.95
	SEm $\pm$	1.33
	CD at 5%	3.73

Fig.39 Effect of different teatments on the yield of tomato fruits



4.6. Chemical interactions/compatibility of pesticides in standard solution and formulation cock-tail

Interaction of pesticides when applied sequentially or simultaneously were evaluated by mixing and incubating the analytical/technical compounds in organic solvents, formulation products and/or formulations products diluted with water.

Pesticides are applied in tomato crop depending upon the insect pests, diseases and weed infestation during the early growth period or at the time of flowering. The pesticides were therefore, categorized into two groups as follows (in two modules).

Module I : Pendimethalin, imidacloprid, chlorothalonil and endosulfan (PICE)

These pesticides are applied during early growth stages of tomato crop to protect it from weeds, white fly, aphids, diseases and lepidopteran pests, respectively. Chemical stability studies were conducted if this could be applied as mixture of 2, 3 or 4.

Module 2. : Endosulfan, Triazophos and β -cyfluthrin (ET β).

These insecticides are applied at flowering stage in tomato to protect mainly from lepidopteran pests.

Pendimethalin, chlorothalonil, endosulfan and β -cyfluthrin and their mixture were analysed in GC using electron capture detector (ECD) and triazophos was analysed in GC using flame thermoionic detector (FTD). In case of mixture of imidacloprid with other mentioned pesticides, first the sample was injected in GC followed by analysis in HPLC.

The initial concentration of pendimethalin, imidacloprid, chlorothalonil and endosulfan were 0.2, 5.0, 0.4 and 0.4 $\mu\text{g mL}^{-1}$, respectively.

4.6.1. Compatibility of analytical/technical compounds in organic solvents.

The standard solution of mixture of pendimethalin and imidacloprid, 0.2 and 5.0 $\mu\text{g g}^{-1}$ of respective components were present upto 10 days after incubation without any alteration (Table 45a). Hence, it was concluded that there was no interaction between imidacloprid and pendimethalin and that imidacloprid can be safely applied as seedling root-dip in tomato crop at the time of transplanting.

In case of imidacloprid and chlorothalonil mixture, the respective concentrations 10 days after incubation were 5.0 and 3.9 $\mu\text{g mL}^{-1}$. There was slight decreament of the concentration of chlorothalonil but the concentration of imidacloprid remained intact.

When the fungicide chlorothalonil was mixed with insecticide endosulfan their concentrations remained the same after 10 days of incubation period. But mixing with pendimethalin caused slight reduction of the recovery of endosulfan connoted the possible interaction between them. Pendimethalin and chlorothalonil mixture revealed the concentration of 0.18 and 0.39 $\mu\text{g mL}^{-1}$, respectively after 10days of incubation while most stability was found in the mixture of endosulfan and imidacloprid. There was no reduction in the concentration even after 10 days of incubation.

In case of mixing three pesticides, all the three compounds showed their stability in the mixture solution. There was hardly any change recorded in the concentrations of all the components. Therefore, analytical mixture solution of three pesticides was considered stable in the normal situation.

When the four components were mixed and analysed the data revealed that 'four-mixture' components were also stable. The concentration

of pendimethalin, imidacloprid, chlorothalonil and endosulfan were 0.18, 4.9, 0.40 and 0.38 $\mu\text{g mL}^{-1}$, respectively after 10 days of incubation (Table 45a).

Table 45a. Stability of pesticides mixture in organic solvent on 10th days of incubation at 25±1°C

Pesticide mixtures	Initial conc. ($\mu\text{g mL}^{-1}$)	Concentration ($\mu\text{g mL}^{-1}$) on 10 th day			
		Pendi	Imida	Chloro	Endo
Pendi+Imida	0.2+5.0	0.20	5.0	-	-
Imida+Chloro	5.0+0.4	-	5.0	0.39	-
Chloro+Endo	0.4+0.4	-	-	0.4	0.39
Endo+Pendi	0.4+0.2	0.20	-	-	0.38
Pendi+Chloro	0.2+0.4	0.18	-	0.39	-
Imida+Endo	5.0+0.4	-	5.0	-	0.4
Pendi+Imida+Chloro	0.2+5.0+0.4	0.19	5.0	0.40	-
Imida+Chloro+Endo	5.0+0.4+0.4	-	4.9	0.38	0.37
Chloro+Endo+Pendi	0.4+0.4+0.2	0.18	-	0.39	0.38
Endo+Pendi+Imida	0.4+0.2+5.0	0.19	4.9	-	0.36
Pendi+Imida+Chloro+Endo	0.2+5.0+0.4+0.4	0.18	4.9	0.4	0.38

In case of module-II (Endosulfan + Triazophos + β -cyfluthrin) the mixture of three insecticide in the technical solution were stable hence there was no interaction between them. The data on compatibility in Table 45b revealed that the mixture of two pesticides viz., endosulfan + triazophos, triazophos+ β -cyfluthrin and β -cyfluthrin + endosulfan were stable at room temperature. There was hardly any change in the initial concentration on 10th day after the incubation. In case of mixture of three compounds viz., endosulfan + triazophos + β -cyfluthrin there was slight reduction which was nearly at par with the two components mixture.

Table 45b. Concentration ($\mu\text{g mL}^{-1}$) of pesticide mixture of analytical solution on 10th day of incubation at 25±1°C

Mixtures	Initial conc. ($\mu\text{g mL}^{-1}$)	Concentration on 10 th day ($\mu\text{g mL}^{-1}$)		
		Endosulfan	Triazophos	β -cyfluthrin
Endo+Triazo	0.4+0.5	0.400	0.498	-
Triazo+ β -Cyf	0.5+0.4	-	0.519	0.400
β -Cyf+Endo	0.4+0.4	0.398	-	0.396
Endo+Triazo+ β -Cyf	0.4+0.5+0.4	0.395	0.497	0.398

4.6.2. Compatibility of pesticides in formulation cock-tail

It is general practice with farmers to sue commercial pesticide as 'tank-mix', if their time of application coincide. These are diluted with water for spray.

For compatibility studies the formulation were mixed (at field rate) diluted (at field spray rate) with water and incubated at room temperature. Samples were extracted at different intervals and analysed by GC.

Therefore, the stability studies with commercial pesticide mixture gave the better insight of the pesticide interaction. When pendimethalin and imidacloprid formulation (Stomp® and Confidor®) were mixed and extracted in solvent, the analysis showed that the initial concentration of pendimethalin and imidacloprid were 0.189 and 4.950 $\mu\text{g mL}^{-1}$, respectively. The 3rd day analysis gave the concentration for respective compound decreased to 0.011 and 0.008 $\mu\text{g mL}^{-1}$ (Table 45c). During next seven days the concentration further decreased to 0.167 and 4.875 $\mu\text{g mL}^{-1}$ explaining the slow degradation of pesticides in formulation mixture. At the end (10 day) of the experiment the per cent decrement of concentrations of the two pesticides were 11.64 and 1.52, respectively.

Mixture of imidacloprid and chlorothalonil in the formulation showed to some extent stability. The initial concentrations were 4.942 and 0.397 $\mu\text{g mL}^{-1}$, respectively which further decreased to 4.931 and 0.395, 4.902 and 0.385 $\mu\text{g mL}^{-1}$, in 3 and 7 days, respectively. After 10 days the decrement of the concentrations were 0.81 for imidacloprid and 3.02 per cent for chlorothalonil.

When chlorothalonil and endosulfan formulations (Kavach® and Thiodan®) were mixed and analysed after 0, 3 and 10 days the respective concentrations for chlorothalonil and endosulfan are given in Table 45c.

The data showed that the concentration of pesticides decreased substantially after days. While the decrement for chlorothalonil was 25.88 per cent. The reduction for endosulfan was 18.73 per cent, respectively.

When endosulfan and pendimethalin formulation were mixed, the analysis revealed the initial active ingredient (a.i.) were 0.396 and 0.192 $\mu\text{g mL}^{-1}$, respectively. The concentration of a.i. decreased over time and after 10 days the concentrations were 0.219 and 0.172 $\mu\text{g mL}^{-1}$, respectively.

Mixture of chlorothalonil and pendimethalin in formulation also showed the deplement of a.i. in different time intervals. The initial concentrations were 0.392 and 0.197 $\mu\text{g mL}^{-1}$, while after 10 days the concentrations were 0.306 and 0.165 $\mu\text{g mL}^{-1}$, respectively indicating possible degradation in the formulation mixture. Thus mixture of chlorothalonil and pendimethalin is not stable in nature.

In case of mixture of three products, the most stable formulation mixture was imidacloprid + chlorothalonil + endosulfan (ICE), followed by pendimethalin + imidacloprid + chlorothalonil (PIC), Endosulfan + pendimethalin + imidacloprid (EPI) and chlorothalonil + endosulfan + pendimethalin (CEP).

In case of mixture of four pesticides (PICE) the concentrations of different mixture-component are presented in Table 45c. From the data presented it is very clear that the concentration of pendimethalin decreased by 23.47 per cent after 10 days. The corresponding decrement for imidacloprid, chlorothalonil and endosulfan during same interval were 12.09, 19.68 and 24.94 per cent, respectively. The formulation was quite stable upto 10 days. Thus from the experiment it could be concluded that the PICE mixture shows it activity upto 10 days in the field condition.

In case of module II three pesticides formulation mixtures (endosulfan, triazophos and β -cyfluthrin) and were mixed and kept for compatibility studies. The remaining a.i. at different time intervals of three different pesticides combinations were analysed by GLC and presented in Table 45c (module II).

From the table it is revealed that, when endosulfan and triazophos formulations were mixed as in the laboratory, the degradation is not so fast. The initial concentration of endosulfan was $0.382 \mu\text{g g}^{-1}$ which decreased to 0.372 and $0.359 \mu\text{g g}^{-1}$ in 3 and 10 days, respectively. The concentration of triazophos in the same days interval were 0.456 and $0.420 \mu\text{g g}^{-1}$, respectively (initial concentration was $0.486 \mu\text{g g}^{-1}$). Thus after 10 days there was 6.02 and 13.58 per cent decrement of a.i. for endosulfan, and triazophos, respectively.

The mixture of triazophos and β -cyfluthrin formulation showed that the initial concentrations were 0.488 and $0.392 \mu\text{g mL}^{-1}$, respectively, which decreased to 0.473 and $0.381 \mu\text{g mL}^{-1}$ in 3 days; and 0.462 and $0.345 \mu\text{g mL}^{-1}$ in 10 days, respectively. Thus, after 10 days the a.i. decreament were 5.33 and 12.00 per cent, respectively.

The mixture of beta-cyfluthrin and endosulfan formulation showed partial decrement of the pesticide component. Initial concentrations for beta-cyfluthrin and endosulfan were 0.378 and $0.390 \mu\text{g mL}^{-1}$, respectively. After 3 days, the a.i. depleted to 0.357 and $0.372 \mu\text{g mL}^{-1}$, respectively. At the end of the experiment (10 days) the a.i. of the pesticides were 0.315 and $0.332 \mu\text{g mL}^{-1}$, respectively. Thus the overall deplement of the concentrations were 16.67 and 14.87 per cent, respectively.

In case of 'triple-mixture' of endosulfan, triazophos and beta-cyfluthrin formulation, the initial concentrations were 0.382 (endosulfan), 0.482

(triazophos) and 0.391 (beta-cyfluthrin). On the third day the a.i. were analysed as 0.355, 0.473 and 0.376 $\mu\text{g mL}^{-1}$, respectively.

After 10th day of the experiment, the concentrations were 0.302, 0.413 and 0.325 $\mu\text{g mL}^{-1}$ respectively. Thus the depletion of a.i. were 0.067 (17.54 per cent), 0.069 (14.32 per cent) and 0.066 (16.88 per cent), respectively.

The study of compatibility thus revealed that most of pesticides (in both the module) showed stability in the organic solvent. However, mixture of the formulations of these pesticides did not behaved as in the row. In the module-I mixture of pendimethalin and imidacloprid was showed maximum stability. This was followed by the mixture of imidacloprid and chlorothalonil. Although formulation mixture of chlorothalonil and endosulfan was quite stable but, endosulfan degraded faster in the presence of pendimethalin, chlorothalonil also behaved similarly.

In case of triple mixture the most stability was observed by imidacloprid + chlorothalonil + endosulfan mixture. The other triple mixture formulations were less stable than the first one.

Our study thus revealed that the pre-emergence application of pendimethalin followed by root-dip or spray treatment does not affect the activity of each other. On the hand simultaneous insect-pests and disease control can be achieved by the formulation cock-tail of imidacloprid and chlorothalonil. The same mixture can be used as root-dip treatment of the crop to give early protection from the pests and diseases.

In case of module-II all the three insecticides were stable in the standard solution mixture. However, their formulation mixture were not as stable as the standard solution mixture. The mixture of triazophos and β -cyfluthrin formulation was most stable after 10 days. This was followed by formulation mixture of triazophos and endosulfan and beta-cyfluthrin. In the

triple mixture the maximum depletion of concentration was occurred for β -cyfluthrin (16.88 per cent).

Thus, the stable formulation mixture of triazophos and β -cyfluthrin can be used for the simultaneous control of whitefly and fruit borer in tomato crop. The other mixture were stable at the standard deviation range. Therefore, all three combinations can be well applied for the control of whitefly and fruit borer.

SUMMARY AND CONCLUSION

The present investigation was carried out to evaluate persistence, residual fate and efficacy of mixture of pesticide in tomato (*Lycopersicon esculentum* Mill.) crop. The field experiment was conducted in *rabi* 2000-2001 in the farm at IARI, New Delhi. Initially four pesticides are chosen for the crops, pendimethalin as pre-emergence herbicide, imidacloprid and chlorothalonil as root dip treatment and foliar spray and endosulfan as foliar spray. The different treatments were made by combination of the last three pesticides at the main plots of herbicide. At the later stage of the growth, another two insecticide (β -cyfluthrin and triazophos) along with endosulfan were taken to control the excessive infestation of fruit borers and white flies in tomato. These three insecticides were also applied on the tomato crop at different combinations of them.

The soil of experimental field was sandy loam and alkaline in reaction. The rainfall pattern was quite sporadic during the period of experimentation.

The salient results of various studies are presented below:

A. Standardization of analytical techniques

Gas chromatographic (GC) technique (Shimadzu GC-17A) using 3 per cent OV-17, megabore column (30m x 0.53 mm i.d.) and temperature programming was standardized for resolution of individual pesticides, their mixtures and some of their authentic degradation products. The compounds (pendimethalin, endosulfan, chlorothalonil and β -cyfluthrin) gave sharp peaks in GC on electron capture detector (ECD) and triazophos gave sharp peak in GC fitted with flame thermoionic detector (FTD). The calibration curve was linear for the concentration ranging from 0.1 to 2.0 $\mu\text{g mL}^{-1}$ of each pesticide.

A high performance liquid chromatographic technique (HPLC) using reverse phase C₈ (RP-C₈) column with acetonitrile : water (70:30, v/v) as mobile phase at λ_{max} 270 nm was standardized for resolution of imidacloprid and its authentic degradation products on Hewlett Packard HPLC fitted with photodiode array detector. The sensitivity of the method was 0.25 $\mu\text{g mL}^{-1}$ for imidacloprid.

Recoveries of pesticides from samples (soil, tomato fruits and aqueous) samples ranged between 74-98 per cent in all cases.

B. Studies on persistence, residual fate of pesticides tomato fruits and soil

Persistence of various pesticides in soil and tomato under field conditions was evaluated following application in tomato crop in two modules. Pendimethalin (as pre-emergence), imidacloprid, chlorothalonil and endosulfan were sprayed @ 1.0, 0.020, 0.5 and 0.35 kg a.i. ha⁻¹, respectively alone or sequentially at fruiting. Similarly, endosulfan, triazophos and β -cyfluthrin were sprayed @ 0.35, 0.70 and 0.0125 kg a.i. ha⁻¹, respectively alone or sequentially at fruiting. However, in case of mixture for pesticide quantity were reduced to one-fourth (module I) or one-third (module II) prior application. The residue were monitored in fruits and soil at regular intervals using GC/HPLC technique.

Persistence of the pesticides and their mixture in soil was also studied under laboratory conditions by incubating the treated (@10 $\mu\text{g g}^{-1}$ for all pesticides/mixture except imidacloprid which was treated as 20 $\mu\text{g g}^{-1}$) soil (moisture 16 per cent) at 28 $\pm$ 1°C.

Individual application

Dissipation of pesticides in soil and tomato fruits under field condition exhibited a declining pattern as a function of time. The logarithmic plots of

pesticides residues (individual and each component of the mixture) versus time, obtained by fitting the regression equation indicated that the disappearance of pesticides followed a first order kinetics decay curve in soil and tomato fruits, respectively. The half lives of pendimethalin in soil was 16.8 days while the half-lives of imidacloprid in soil and plants were 26.9 and 3.9 days, respectively. Chlorothalonil and endosulfan had their half-lives in soil as 11.2 and 9.9 days, respectively but ⁱⁿ tomato fruits, their half-lives were 3.5 and 3.3 days, respectively.

Sequential application

In case of sequential application, the half-life of pendimethalin in soil was 17.5 days while the half-lives of imidacloprid in soil 28.1 days. Chlorothalonil had the half-lives of 15.8 days in soil while endosulfan had the same as 9.5 days in soil. Sequential application of these pesticides on tomato fruits yielded half lives of 6.5, 4.7 and 4.1 days for imidacloprid, chlorothalonil and endosulfan, respectively.

Pesticide mixture application

In case of mixture application in soil the half-lives of pendimethalin 15.3 days, imidacloprid, chlorothalonil and endosulfan were 15.3, 25.5, 15.2 and 5.5 days, respectively while in the plants the half-lives of imidacloprid, chlorothalonil and endosulfan were 3.5, 3.1 and 3.2 days.

The residue in the tomato fruit after the sequential application of imidacloprid, chlorothalonil and endosulfan in tomato did not reach below MRL (1 ppm, 5ppm and 2 ppm, respectively) within 3 days of their application. Therefore sequential application is not safe for the consumption of tomato upto 3 days. However, mixture application of imidacloprid + chlorothalonil + endosulfan on tomato crop caused lowering of the residues

below the MRL (one day after application). Therefore, application of mixture was safe for the consumption purpose.

Dissipation of pesticides and their mixture in alluvial soil under laboratory condition also exhibited a first order kinetics. The half-lives of pendimethalin, imidacloprid, chlorothalonil and endosulfan in the laboratory soil was 27.1, 31.7, 4.7 and 25.3 days, respectively.

C. Interaction of pesticides/mixture in soil/microorganisms

C.1. Studies on Adsorption of pesticides/mixture in soil

Studies on the adsorption of pesticides and their mixture were carried out using alluvial soil (IARI, Typic Ustochrept; organic carbon 0.34% and clay 17.2%) with initial concentration ranging from 0 to 25 $\mu\text{g mL}^{-1}$.

The amount of pendimethalin, imidacloprid and chlorothalonil adsorbed on soil ranged from 33.57 to 124.78, 7.63 to 124.75 and 7.27 to 137.38 $\mu\text{g g}^{-1}$, respectively. While in case of mixture the adsorption varied from 2.20 to 30.5, 6.3 to 81.5 and 2.1 to 28.2 $\mu\text{g g}^{-1}$, respectively. Plotting the pesticide adsorbed on soil versus aqueous equilibrium concentration showed that the adsorption isotherm were L-type indicating a high affinity of soil particles for the adsorbate. The 'K' values for adsorption was highest for imidacloprid (43.90) followed by pendimethalin (34.20) and chlorothalonil (16.92) in alluvial soil when applied individually. But in case of mixture, the 'K' values of the respective pesticides declined and were 22.60, 22.08 and 21.73, respectively.

Due to moderate of organic carbon and clay contents the adsorption of the pesticides in the soil was medium. In case of mixture, 'K' value of three pesticides were very much closer.

The amount of pesticides on, was also revealed by intensities of adsorption (1/n) which was maximum for pendimethalin (0.821), medium for

imidacloprid (0.737) and minimum for chlorothalonil (0.508). However, in mixture adsorption intensity of pesticides reduced may be due to over saturation of sites. Reduced adsorption of pesticides in mixture may be beneficial for uptake by plants or for combating soil borne insect pests, diseases of weeds.

C.2. Interaction of pesticides with microbes

C.2.1. Microbial degradation of pesticides

C.2.1.1. Soil

Studies were conducted to evaluate degradation of pesticides/mixture in sterile and non-sterile alluvial soil. Soil was sterilized in an autoclave at a pressure of 15 p.s.i. for 30 minutes. The autoclaved and non-autoclaved soils treated with pesticides ($10 \mu\text{g g}^{-1}$ pesticide/except imidacloprid, $20 \mu\text{g g}^{-1}$) were incubated at $28 \pm 1^\circ\text{C}$ in BOD.

Degradation of pesticides in soil under both sterile and non-sterile conditions exhibited first order rate-kinetics with estimated half lives of : pendimethalin 40.7 and 25.3 days; imidacloprid 68.4 and 27.1 days; endosulfan 51.01 and 11.40 days and chlorothalonil 45.6 and 25.3 days, respectively.

The rate of degradation of pesticides in non-sterile soil was much higher than that in sterile soil. At the end of 30 days, 59.08 and 42.03 per cent pendimethalin remained in soil under sterilized and non-sterilized conditions. While the corresponding residue of imidacloprid, chlorothalonil and endosulfan were 65.67 and 37.59, 62.29 and 42.20, 47.73 and 75.00 per cent, respectively.

In the mixture the estimated half-lives were : pendimethalin 36.7 and 16.2 days; imidacloprid 37.2 and 16.3 days; endosulfan 28.4 and 12.2 days chlorothalonil 26.0 and 9.8 days in sterile and non-sterile soil, respectively.

In case of mixture, after 30 days the residues remained as: pendimethalin 56.55 and 26.51 per cent; imidacloprid 79.91 and 29.55 per cent, chlorothalonil 71.14 and 12.05 per cent ; endosulfan 55.09 and 5.60 per cent in sterile and non-sterile soil, respectively.

Therefore, it can be concluded that the microbial population was responsible for the rapid degradation of pesticides in non-sterile soil. The difference in degradation in non-sterile and sterile soils suggested greater biological degradation of pesticides in the initial stages of incubation than in later stages.

C.2.1.2. In liquid culture media

Investigation on rate kinetics of microbial degradation of pesticides and their mixture were carried out by inoculating rhizosphere soil suspension in nutrient broth (Czapek Dox medium) amended with pesticides ($2 \mu\text{g mL}^{-1}$) and monitoring the residue at 0 (2h), 5, 10, 20 and 30 days after inoculation.

The logarithmic plots obtained by fitting the regression equation indicated disappearance of compound had followed first order decay curve and the estimated half-lives were : pendimethalin 21.20 and 31.03 days, imidacloprid 22.46 and 55.74 days, endosulfan 11.90 and 20.90 days and chlorothalonil 83.61 and 94.06 days for individual and mixture application, respectively .

The degradation of pesticides in individual application was faster than in the mixture and least for chlorothalonil.

Microbial degradation of pesticides in mixture in soil was reduced compared to individual pesticides which was further confirmed for broth kinetics studies.

C.2.1.3. Degradation products of pesticides

Sterilized Czapek Dox medium solution containing pesticides ($20 \mu\text{g mL}^{-1}$) was inoculated with mixed fungal culture (isolated from field soil) and incubated at $28 \pm 1^\circ\text{C}$ in BOD for 20 days.

Endosulfan was degraded to its sulphate after 5 days of incubation. The concentration of sulphate gradually increased upto 15 days and then decreased.

Chlorothalonil was degraded to its hydroxy product in the media which was identified by co-chromatography, IR and NMR.

Analysis of culture filtrate of imidacloprid treated media revealed the formation of five metabolites which have been identified as imidacloprid guanidine (II), N-nitroguanidine (III), dihydro-derivative of imidacloprid (IV), dihydroxy imidacloprid (V) and 1-(6-Chloro-3-pyridylmethyl). Six metabolites more identified from the pendimethalin treated media after 30 days of incubation. The metabolites which tentatively identified as 2,6-dinitro-3,4-xylydine (II), N-(1-propyl)-2,6-dinitro-3,4-Xylydine (III). Xylene-3,4,5-triamine (IV), N-(1-propyl)-4-hydroxy – 2,6-dinitro-3,4-xylydine (V) and 6-amino-4-nitro-xylene (VI).

C.2.2. Identification of microorganisms responsible for the degradation of pesticides/mixture

The microorganisms capable of degrading individual and mixture of pesticides were isolated from field soil from respective treatment, purified by serial dilution technique utilizing expertise in Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi. The predominant genera in all cases were *Aspergillus*. The other important genera for the degradation were *Fusarium*, *Sclerotium*, *Rhizoctonia*, *Alternaria* and *Mucor*.

In case of pesticide mixture, the predominant fungal species identified viz., *Trichoderma viridae*, *Aspergillus terreus* and *A. flavus*. Thus

the pesticide mixture helped to kill the pathogenic fungi without being toxic to the bio-agents in the soil.

C.3. Effect of pesticides/mixture on soil microbial population

The possible deleterious effect of pesticides/mixture, if any, in soil microbial population (bacteria, fungi and nematode) by taking the pesticide treated soil samples and monitoring the microbial population at different intervals using serial dilution method.

Viable fungi population was slightly affected upto 3 days after pesticides application and thereafter, its population gradually increased. The total fungal population were significantly higher in the treated than the control soil at the end of 30 days.

Viable nematode population also had a depressing effect of pesticides upto 10 days and there after, their population in the treated plots increased to initial population or sometimes out numbered the control plot at the end of 30 days.

The sequential and mixture application of pesticides revealed that, the sequential application reduced the microbial population in much higher rate than the mixture application.

Mixture application causes faster degradation of pesticides (due to high microbial population and lower dose of application) than the sequential application.

Application of pesticides enhanced the growth (probably due to lower dose of application) of bioagents in the soil which killed the phytonematodes. Sequential or individual application reduced the mycoflora including bioagents probably due to higher dose and repeated applications.

E. Chemical interaction/compatibility of pesticides/mixtures

In all cases the pesticide in mixture were highly stable in the organic solvents. The formulation mixture (in water) also revealed the desirable (80 per cent) stability at room temperature. Mixing with pendimethalin reduced the stability of pesticides. Imidacloprid was found to be most stable in formulation mixture while endosulfan showed slight degradation in different combinations. Although imidacloprid alone was effective to control aphid and white fly, formulation mixture of imidacloprid + chlorothalonil + endosulfan and mixture of endosulfan + triazophos + β -cyfluthrin also better gave better results. However the mixture of imidacloprid + chlorothalonil and mixture of triazophos + β -cyfluthrin were most suitable formulation cocktail for the control of pests and diseases.

D. Biological interaction/compatibility of pesticides/mixtures

D.1. Insect pests

Aphid population in the tomato field was significantly reduced by the application of mixture of imidacloprid + chlorothalonil + endosulfan and endosulfan + triazophos + β -cyfluthrin. The results in the later case were *at par* with the sequential application.

Foliar spray of imidacloprid + chlorothalonil + endosulfan and, root dip treatment in imidacloprid + chlorothalonil mixture followed by spraying of the same mixture of the vegetative stage of the crop gave the maximum control of white fly. Application of mixture of β -cyfluthrin + triazophos gave similar efficacy against the white fly.

The root dip treatment of tomato seedling in imidacloprid + chlorothalonil mixture followed by foliar spray of imidacloprid + chlorothalonil + endosulfan gave good (> 60%) control of fruit borer. However, mixture of

β -cyfluthrin and endosulfan also gave excellent (80%) control of this pest in the tomato field.

D.2. Diseases

The predominant diseases of tomato crop like early blight and late blight were successfully controlled by chlorothalonil application. However, buck eye rot was least affected by both the foliar and root dip treatment of chlorothalonil.

Mixing with imidacloprid did not reduce the activity of chlorothalonil. The formulation mixture of the two was also stable in the solution. Therefore, mixing of imidacloprid with chlorothalonil can be recommended for the commercial formulation for simultaneous control of pests and diseases in the field.

D.3. Weeds

Eragrotis tenella L. among the grass of weeds and *Trianthema portulacastrum* L. among the broad leaf weeds were the most dominant weeds species throughout the cropping period.

Pendimethalin application significantly reduced both mono and dicot weed population as was evident from the reduced dry weight of the weeds as compared to non pendimethalin and non pesticide treated control plots.

The insecticide and fungicide treatments did not hamper the activity of pendimethalin and in other words were compatible.

Pendimethalin application registered significantly higher yield than non pendimethalin and non pesticide treated control plots treatments.

From the above studies it was concluded that the imidacloprid + chlorothalonil + endosulfan, imidacloprid + chlorothalonil, endosulfan + β -cyfluthrin + triazophos and β -cyfluthrin + endosulfan mixtures of the pesticides, were stable and showed biological compatibility and did not leave

toxic deleterious effect in tomato fruits and on soil microflora. The pesticide mixture application not only improved biological performance but also reduced the cost of cultivation. Thus these pesticide can be recommended for the preparation of single formulation.

The low dose application of pesticides is the key concept today's agriculture for environmental safety which can be achieved through the judicious application of pesticides. Thus, preparation of multipesticide formulation could be one of the important output to maintain the harmony of the nature.

T-7128

BIBLIOGRAPHY

- Adamson AW (1967). *Physical Chemistry of Surfaces*. Inter Science Publishers Inc., New York, p. 747.
- Agnihotri NP, Chatterjee S, Gajbhiye VT and Mohapatra SP (1996) Persistence and movement of endosulfan in soil in a supervised field trial at Farrukhabad. *Pesticide Research Journal* **8**(2) : 152-156.
- Agnihotri NP, Gajbhiye VT, George T and Srivastava KP (1995) Persistence of chlorothalonil on potato and tomato following foliar application to control late blight. *Ann Pl Protec Sci* **3**(2) : 100-104.
- Aguera A, Almansa E, Malato S, Maldonado MI and Fernandez Alba AR (1998) Evaluation of photocatalytic degradation of imidacloprid in industrial water by GC-MS and LC-MS. *Analisis* **26**(7) : 245-251.
- Antonious GF and Bayers ME (1997) Fate and movement of endosulfan under field conditions. *Environmental Toxicology and Chemistry* **16**(4) : 644-649.
- Antonious GF, Bayers ME and Snyder JC (1998). Residues and fate of endosulfan on field-grown pepper and tomato. *Pesticide Science* **54** : 61-67.
- Awasthi N, Manickam N and Kumar A (1997) Biodegradation of endosulfan by a bacterial coculture. *Bulletin of Environmental Contamination and Toxicology* **59**(6) : 928-934.
- Bachlechner G (1989) Method for high performance liquid chromatographic determination of the insecticide NTN. 33893 in soil. Bayer AG-Report No 559/89/001461/I 676.
- Bai D, Lummis SCR, Leicht W, Beer H and Sattelle DB (1991) Actions of imidacloprid and a related nitromethylene on cholinergic receptors of an identified insect motor neuron. *Pestic Sci* **33** : 197-204.
- Bailey GW and White JL (1970) Factors influencing adsorption, desorption and movement of pesticides in soils. *Res Rev* **32** : 29.

- Bairambekov SB and Valleva ZB (1994). A system of herbicide application on tomatoes *Zashchita Rastenii Moskva* 2 : 32-33.
- Balasubramaniam G and Mathan KK (1996) Influence of physico-chemical properties of soils on the degradation of chlorothalonil. *Madras Agricultural Journal* 83(7) : 457-459.
- Balasubramanian G and Mathan KK (1996). Adsorption and desorption of chlorothalonil in soils. *Journal of the Indian Society of Soil Science* 44 (2) : 321-323.
- Balinov I, Balinova A and Nachkova V (1997) Behaviour of some systemic soil applied N-methyl carbamate insecticides in green house tomato plants. *Bulgarian Journal of Agricultural Science* 3(5) : 551-555.
- Barett MR, Lavy TL, Hoffman DW and Smith JR Jr. (1981) Pendimethalin (Prowl) dissipation in flooded and non-flooded soil. Proceedings 34th Annual Meeting Southern Weed Science Society. 247-248.
- Barrett MR (1983) Effect of soil water control on pendimethalin dissipation. *J Environ Qual* 12(4) : 504-508
- Barringer DF Jr., Zulalian J and Blinn RC (1976) Proc Weed Sci Soc Am p. 96.
- Barua AS, Saha J, Chaudhuri S, Chowdhury A and Aditya Chaudhury N (1990) Degradation of pendimethalin by soil fungi. *Pestic Sci* 29(4) : 419-425.
- Battu RS, Joia BS, Singh B, Kang BK, Chahal KK and Singh B (1999) Residues of β -cyfluthrin in cotton seed and cotton lint. *Pesticide Research Journal* 11(2) : 222-224.
- Beauvais SL, Atchison GJ, Stenback JG and Crumpton WG (1999) Use of cholinesterase activity to monitor exposure of chironomids riparius (Diptera, Chironomidae) to pesticide mixture in hypoxic wet land mesocosms. *Hydrobiologia* 416 : 163-170.
- Beena Kumari, Kumar R, Kathpal TS (2001) An improved multiresidues procedure for determination of pesticides in vegetables. *Pestic Res J* 13(1) : 32-35.

- Begliomini A, Pucci C and Morcellini M (1978) Gas chromatographic methods for the determination of endosulfan residues in bees. *Annali della Facolta di Agraria della Universita degli Studi di Perugia*. **32** (1) : 65-77.
- Blab, W (1990) Method for determination of imidacloprid residues in plant materials using high performance liquid chromatography and UV-detection. Bayer AG-Report No. 97/90, Method No. 329/00171/I-904.
- Bogus ER, Watschke TL and Mumma RO (1990) Utilization of solid-phase and reversed phase and ion pair chromatography in the analysis of seven agrochemical in water. *Journal of agric Food Chem* **38**(1) : 142-144.
- Cacho J, Fierro I, Deban L, Vega M and Pardo, R (1999) Monitoring of the photochemical degradation of metamitron and imidacloprid by micellar electrokinetic chromatography differential pulse voltammetry. *Pestic Sci* **50** : 949-954.
- Calvet R (1980) Adsorption-desorption phenomena. In : Interaction between herbicide and the soil (Ed) RJ Hance. Academic Press. London pp. 1-30.
- Capri E, Camisa MG, Flores-Céspedes, F, Glass CR, Gonzalez-Pradas E and Trevisan M (2001) Imidacloprid and pyrimethanil soil sorption. *Agronomie* **21**(1) : 54-64.
- Ceron JJ, Guitierrez-Panizo, C, Barba A and Camara MA (1995) Endosulfan isomers and metabolite residue degradation in carnation (*Dianthus caryophyllus*) by-product under different environmental conditions. *Journal of Environmental Science and Health. Part B. Pesticides Food Contaminants and Agricultural Wastes*. **30**(2) : 221-232.
- Chahal KK, Joia BS, Battu RS and Tria Kang BK (2000) Dissipation of triazophos on cauliflower *Annals of Plant Protection Science* **8**(2) : 278-280.
- Choudhury BK and Awasthi MD (2000) Persistence and degradation of chlorothalonil under different soil moisture regime. *Pestic Res J* **12**(2) : 170-173.

- Codex Alimentarius Commission (2000) Pesticide residue in food-Maximum residue limit . *FAO/WHO* vol. **2B** pp. 373-376.
- Cox L, Koskinen WC and Pau YY (1997) Sorption-desorption of imidacloprid and its metabolites in soils. *Journal of Agricultural Food Chemistry* **45**(4) : 1468-1472.
- Cox L, Koskinen WC, Celis R, Yen PY, Hermosin ML and Cronejo J (1998) Sorption-desorption of imidacloprid. *J Environ Sci Health* **33** : 123-134.
- Cynamid (1993) STOMP Herbicide American Cynamid Company.
- Day OR (1965) Particle fraction and particle size analysis. In : *Method of Soil Analysis Monograph. Am. Soc. Agron. Madisons Wisconsin* pp. 545-567.
- Dhawan AK and Simwat GS (1994) Field evaluation of Prophenophos alone and in combination with Cypermethrin for the control of insect pest during flowering phase. *Pestology*, xvii(5) : 40-43.
- Dikshit AK and Pachauri DC (2000) Persistence and bioefficacy of β -cyfluthrin and imidacloprid on tomato fruits. *Plant Protection Bulletin, Faridabad*, **52**(3-4) : 1-3.
- Dikshit AK, Lal OP and Kumar R (2001) Persistence and bioefficacy of insecticides in okra and sponge gourd. *Journal of Entomological Research* **25**(2) : 131-136.
- Dios CG, Romero E and Sanchez FR (1992) *J Soil Sci* **43** : 99.
- Diwan K, Barevadia TN and Shah PG (1999) Dissipation of pendimethalin and fluchloralin in soil and their residues in onion. *Pesticide Research Journal* **11**(1) : 76-80.
- Doorn C van, Vink M and Poll JM van der (1995) Gas chromatographic determination of chlorothalonil and its metabolite 4-hydroxy-2,5,6-isophthalonitrile (HII) in water. *Chromatographia* **40**(7-8) : 458-462.
- Dureja P and Walia S (1989) Photodecomposition of pendimethalin. *Pestic Sci* **25** : 105-114.

- Economic Survey (2002) Estimated area and production of major horticultural crops. Economic survey, 2001-2002, Government of India, P. 191.
- Etebrian HR (1992) Studies on fusarium wilt of tomato and its chemical control in Varamin area. *Iranian Journal of Agricultural Sciences*, **23**(1) : 1-14.
- Fahmy AR and Miyata, T (1998) *Journal of Pesticide Science*, **23** : 223-229.
- FAO (1989) Maximum residue limit of endosulfan on various commodities. Pesticide Residues in Food. FAO Plant Production and Protection. **100** : 350-351.
- Fernandez-Alba AR, Valverde A, Aguera A, Contreras M and Chiron S (1996) Determination of imidacloprid in vegetable by high performance liquid chromatography with diode array detection. *Journal of Chromatography* **72**(1) : 97-105.
- Foy CL (1993) Trends in herbicide and adjuvant use. Proc. In : *Sym Indian Soc Weed Sci*, Hissar, November 18-20, vol. I, 259-267.
- Freed VH and Haque (1973). In : Pesticide Formulations : Volkenburg V (ed.) Marcel Dekker, New York, Chapter 10, p. 441.
- Fritz R, Kersting E and Kuck KH (1992) Volatilization behaviour of pesticides in field trial. *Proceedings Brighton Crop Protection Conference. Pest and Disease Brighton*, November 23-26, 829-834.
- Gajbhiye VT and Agnihotri NP (1999) HPLC method for analysis of imidacloprid. In : *Green Pesticides Crop Protection and safe evaluation* (Eds. N.P. Agnihotri, S. Walia and V. T. Gajbhiye), Society of Pesticide Science pp. 176-180.
- Gajbhiye VT, Kumar R, Gupta RK and Kalpana (2000) Translocation and persistence of imidacloprid in tomato. *Pesticide Research Journal* **12**(1) : 127-129.

- Galeano T, Guiberteau A and Salinas F (1992). Rapid determination of alpha-endosulfan and beta endosulfan in formulation and potatoes by high performance liquid chromatography. *Letters* **25**(9) : 1797-1804.
- Ganesan T and Lalithakumari D (1992) *Adv Pl Sci* **5** : 398.
- Garcia-Valcarcel AI, Sanchez-Brunete C, Martinez L, Tadeo JL, Gelpi EL and Grimalt JO (1996) Determination of dinitroaniline herbicides in environmental samples by gas chromatography. *Journal of Chromatography* **719**(1) : 113-119.
- Garg PK (1982) Adsorption, desorption and degradation of carbofuran and benidocarb in soil. *Ph.D. Thesis*. PG School, IARI, New Delhi-12.
- Garrido Frenich A, Alartine Z, Vidal JL, Pablos Espada MC, Gil Garcia MD and Arrebola FJ (2000) Comparison of gas chromatography with NPD, MS and Tandon MS-MS in the multiresidue analysis of pesticides environmental waters. *Chromatographia* **52**(9-10) : 614-620.
- GauFang P, YanZhong C, ChunLin F, Jin Jie Zhang, Xue Min Li and Tiesheng Zhao (1995). Modification of AOAC multiresidue method for determination of synthetic pyrethroid residues in fruits, vegetables, and gains, Part I. acetonitrile extraction system and optimization of Florisil clean up and gas chromatography. *Journal of AOAC International* **78**(6) : 1481-1488.
- Gaynor JD, Hamill AS, McTavish DC (1993) Efficacy, fruit residues and soil dissipation of the herbicide metolochlor in processing tomato. *Journal of The American Society for Horticultural Science* **118**(1) : 68-72.
- Gelsomino A, Petrovicova B, Tiburtini S, Maganani E and Felici M (1997) Multiresidue analysis of pesticides in fruits and vegetables by gel permeation chromatography followed by gas chromatography with electron-capture and mass spectrometric detection. *Journal of Chromatography*, **782**(1) : 105-122.

- Gerstl (1984). Adsorption decomposition and movement of oxamyl in soil. *Pestic Sci* **15** : 9-17.
- Gopal M and Mukherjee I (1993) Determination of residues of endosulfan and endosulfan sulfate on egg plant, mustard and chickpea. *Pestic Sci* **37** : 67-72.
- Gopal M, Mukherjee I, Diskhit AK, Roy NK, Nagia DK, Saini ML, Sharma SP and Sanjay Kumar (1988). Persistence of endosulfan on brinjal and gram crop. *Indian J Plant Prot* **16**(12) : 177-183.
- Gopichand K, Ramesh B, Reddy KN, Reddy DDR and Gour TB (1999) Dissipation of endosulfan residues in tomato. In : Green Pesticides Crop Protection and Safety Evaluation (Eds. NP Agnihotri, S Walia and VT Gajbhiye). SPS1, New Delhi-110012.
- Green RE (1994) Pesticide clay water interactions. In : Pesticides in Soil and Water (Eds. W.D. Guenzi). Inc., Publisher Madison, USA pp. 3-37.
- Guerin TF and Kennedy IR (1992) Distribution and dissipation of endosulfan and related cyclodienes in sterile aqueous system implications for studies on biodegradation. *Journal of Agricultural and Food Chemistry*, **40** (11) : 2315-2323.
- Gupta S, Gajbhiye VT and Agnihotri NP (2001) Adsorption-desorption, persistence and leaching behaviour of flufenacet in alluvial soil of India. *Bull Environ Contam Toxicol* **66** : 9-16.
- Hamaker JW and Thompson JM (1972) Adsorption In : Organic Chemical in Soil Environment (Eds. C.A.I. Goring and J.W. Hamaker), Vol. 1. Marcel Dekker, New York pp. 49.
- Harrison RB, Holmes DC, Roburn J and Tatton OC (1967). The fate of some organochlorine pesticides on leaves. *J Sci Food Agric* **18** : 10-15.
- Helling CS (1976) Reviews and analysis, dinitroaniline herbicides in soils. *J Environ Qual* **5**(1) : 1-15.

- Horowitz M (1969) Evaluation of herbicide persistence in soil. *Weed Res* **9** : 314-312.
- Ishii Y, Kobori I, Aroki Y, Kurogachi S, Iwaya K and Kagabu S (1994) HPLC determination of new insecticide imidacloprid and its behaviour in rice and cucumber *J Agric Food Chem* **42** : 2917-21.
- Ismail BS and Kalithasan K (1999) Bioactivity, adsorption and dissipation of pendimethalin in two Malayasian soils *Tropical Agriculture* **76**(4) : 241-245.
- Iwaya K, Maruyama M, Nakanishi H and Kurogochi (1998) Concentration of imidacloprid in rice plant and biological effect on *Nilparvata lugens* (Stal.). *J Pestic Sci* **23** : 419-421.
- Jacques GL and Harvey RG (1979). Adsorption and diffusion of dinitroaniline herbicides in soils. *Weeds Sci* **27**(4) : 450-455.
- Jaglan RS and Yadav PR (1988) Persistence and degradation of endosulfan in and on unprocessed and processed cauliflower curds. *Indian J Ent* **48** (4) : 441-447.
- John C and Schwack W (2001) Determination of cutin-bound residues of chlorothalonil by immunoassay. *Journal of Agricultural and Food Chemistry*, **49**(3) : 1233-1238.
- Jongen MJ, Engle R and Leenheers LH (1991) Determination of the pesticide chlorothalonil by HPLC and UV detection for occupational exposure assessment in green house carnation culture. *Journal of Analytical Toxicology* **15**(1) : 30-34.
- Jury WA (1987) In : *Vadose Zone Modelling of Organic Pollutants*. (Eds.) S Hern, Con SM Melano. Lewish Publishing Chelka MI pp. 177-189.
- Kar SN, Saha T, Banerjee H and Bhattacharyya A (2000) Persistence of chlorothalonil residues in /on mango. *Journal of Interacademia* **4**(2) : 270-273.

- Karageogiev D (1981). A gas chromatographic method for determining triazophos in tree fruits *Gradinarska-i-Lozarska-Nauka* **18**(5) : 3-9.
- Katayama A and Matsumara F (1993) Degradation of organochlorine pesticides, particularly endosulfan, by *Trichoderma harzianum*. *Environmental Toxicology and Chemistry* **12**(6) : 1059-1065.
- Katayama A, Ito T and Ukai T (1997) Ubiquitous capability to substitute chlorine atoms of chlorothalonil in bacteria. *Journal of Pesticide Science* **22**(1) : 12-16.
- Katayama A, Mori T and Kuwastuka S (1995) Abiotic dissipation of chlorothalonil in soil accelerated by amendment with high applications of farm yard manure. *Soil Biology and Biochemistry*, **27**(2) : 147-151.
- Kaur I, Mathur RP, Tandon SN and Dureja P (1998) Persistence of endosulfan (technical) in water and soil. *Environmental Technology*, **19**(1) : 115-119.
- Kim YK, Kim SH and Choi SC (2001) Kinetics of endosulfan degradation by *Phanerochaete chrysosporium*. *Biotechnology Letters* **23**(2) : 163-166.
- Kole RK, Saha J, Pal S, Chaudhuri S and Chowdhury A (1994) Bacterial degradation of the herbicide pendimethalin and activity evaluation of its metabolites **52**(5) : 779-786.
- Koskinen WC, Cox L and Yen PY (2001) Changes in sorption/bioavailability of imidacloprid metabolites in soil with incubation time. *Biology and Fertility of Soils*, **33**(6) : 546-550.
- Kulshrestha G and Singh SB (1992) Influence of soil moisture and microbial activity on pendimethalin degradation. *Bulletin of Environmental Contamination and Toxicology*, **48**(2) : 269-274.
- Kulshrestha G and Singh SB and Yaduraju NT (1999) Persistence, Movement and degradation of pendimethalin following long term use in maize-wheat cropping system. In : Green Pesticides Crop Protection and Safety Evaluation (Eds. NP Agnihotri, S Walia and VT Goybhiye). pp. 198-206.

- Kulshrestha G, Singh SB, Lal SP and Yaduraju NT (2000) Effect of long-term application of pendimethalin : enhanced degradation in soil, *Pest Manage Sci* **56** : 202-206.
- Kumar R (1999). Persistence and efficacy of imidacloprid and alphamethrin against pest complex of mustard (*Brassica campestris* Linn.). *Ph.D. Thesis*, PG School, IARI, New Delhi-12.
- Kumar R, Dikshit AK and Dureja P (2000) Metabolism of imidacloprid in brassica. *Pestic Res J* **12**(1) : 91-95.
- Kumari B, Kumar R, Malik MS, Naresh JS and Kathpal TS (1996). Dissipation of endosulfan and landane on sunflower seeds and cowpea pods. *Pesticide Res J* **8**(1) : 49-55.
- Kumari K and Singh RP (1994). Influence of different factors on the adsorption of phosphamidon on soils. *Toxicol Environ Chem* **42** : 183-190.
- Kwon CS and Penner D (1995) The interaction of insecticides with herbicide activity. *Weed Technology* **9** : 119-124.
- Lawruk TS, Gueco AM, Jourdan SW, Scutellaro AM, Fleeker JR, Herzog DP and Rubio FM (1995) Determination of chlorothalonil in water and agricultural products by a magnetic particle based immunoassay. *Journal of Agricultural and Food Chemistry*, **43**(5) : 1413-1419.
- Lee HD, Park SS, Oh BY and Ryu JD (1997) Development of pesticide fertilizer mixture formulation for simultaneous applications of fungicides and top dressing of fertilizer at ear formation for labour saving cultivation of paddy rice. *RDA Journal of Crop Protection*, **32** (1):8-13.
- Leicht W (1993) Imidacloprid –a chloronicotinyl insecticide. *Pesticide Outlook* **4** : 17-21.
- Lovato LA (1975) Compatibility of the herbicide propanil with insecticides. *Lavoura Arrozeira* **28**(268) : 48-54.

- Luttrell RG, Bell M and Roush RT (1991) Management of pyrethroid resistance in the tobacco budworm : role of insecticide persistence and insecticide mixtures. *Southwestern Entomologist* **15** : 93-110.
- Maier Bode H (1966). Versuche uber die persistenz des insekticides endosulfan in fplozlichen and tierischem organisms. *Meded Landbowhoogesh opzoch stat gent* **31** : 305.
- Malathi P and Jeyarajan R (1995) Persistence of chlorothalonil in potato foliage for late blight control. **25**(3) : 299-300.
- Malathi S, Sriramulu M and Ramesh Babu T (1999) Dissipation of endosulfan on cabbage *Brassica oleracea* var. capitata. *Pestic Res J* **11**(2) : 215-217.
- Malato S, Caceres J, Aguera A, Mezcuca M, Hernando D, Vial J and Fernandez-Alba AR (2001) Degradation of imidacloprid in water by photo-Fenton an TiO₂ photocatalysis at a solar pilot plant: a comparative study. *Environmental Science and Technology* **35**(21) : 4359-4366
- Marei AH, Barringer DF, Zulalian J, Kapoor IP, Gatterdam PE and Blinn RC (1976) *Proc Weed Sci Soc Am* p. 18.
- Martens R (1972) Degradation of endosulfan by soil micro ornganisms. *Schriftenreihe des Vereins fur wasser Boden und Lufthygiene* **37** : 167-173.
- Martens R (1977) Degradation of endosulfan 8,9-¹⁴C in soil under different conditions. *Bulletin of Environmental Contamination and Toxicology* **17**(4) : 438-446.
- Miles Inc. (1993). Imidacloprid : Pesticide leaching potential model. Report No. 105008.
- Miles JRW and Moy P (1979) Degradation of endosulfan and its metabolites by a mixed culture of soil microorganisms. *Bulletin of Environmental Contamination and Toxicology*, **23**(1-2) : 13-19.

- Mitchell LR (1976) Collaborative study of the determination of endosulfan, endosulfan sulphate, tetrasul and teradifon residues in fresh fruits and vegetables. *Journal of the Association of Official Analytical Chemists* **59**(1) : 209-212.
- Mithyantha MS (1973). Studies on retention and persistence of pesticides in soils. *Ph.D. Thesis*. UAS Bangalore, India.
- Monteiro RTR, Hirata R, Andrea-MN-de, Walder JMM and Wiendl FM (1989) Endosulfan ^{14}C degradation in soil. *Revista Brasileira Ciencia-do-solo* **13** (2): 163-168.
- Moreals A and van Bladel R (1979) Soil interaction of herbicide-derived aniline residues; a thermodynamic approach. *Soil Sci* **127** : 1-9.
- Mori T, Fujie K and Katayama A (1998) Bacterial and fungal contributions to chlorothalonil degradation in soil. *Soil Science and Plant Nutrition*. **44**(3) : 297-304.
- Moza PN, Hustert K, Pal S and Sukul P (1992) *Chemosphere* **25** : 1675-82.
- Mukherjee I and Gopal M (2000). Environmental behaviour and translocation of imidacloprid in eggplant, cabbage and mustard, *Pest Management Science* **56** : 932-936.
- Mukherjee I, Gopal M and Kusum (2002). Evaluation of residues of beta-cyfluthrin on cotton. *Bulletin of Environmental Contamination and Toxicology* **69** (1) : 54-58.
- Murakami Y and Tanaka R (1985) Studies on environmental contaminants in food I. Simultaneous determination of diphenyl ether herbicides and TPN (chlorothalonil) in vegetables *Shokuhin Eiseigaku Zasshi* **26**(4) : 343-349.
- Nabarawy IM and Carey WF (1988) Improved method for determination of chlorothalonil and related residues in cranberries. *Journal of Official Analytical chemists* **71**(2) : 358-360.

- Nagaraja A, Naik KS and Anahosur KH (2000). Efficacy of fungicides against early blight of tomato. *Pestology* **14**(1) : 18-20.
- Navalon A, Gonzalez CA, El-Khattabi R, Vilchez JI and Fernandez Alba AR (1997) Determination of imidacloprid in vegetables samples by gas chromatography-mass spectrometry. *Analyst* **122**(6) : 579-581.
- Navarro S, Barba A, Segura JC and Oliva J (2000) Disappearance of endosulfan residues form sea water and sediment under laboratory conditions. *Pest Management Science* **56**(10) : 849-854.
- Nelson JE, Meggitt WF and Penner D (1983) *Weed Sci* **31** : 68-75.
- Nelson, JE (1980) *Diss Abstr Int* **40** : 2924-2928.
- Neog P and Goswami MM (1999) Dissipation of endosulfan residues in/on the fruits of lady's finger. *Journal of the Agricultural Science Society of North East India*. **12**(2) : 262-265.
- Ni HX, He LS, Cao YZ and Jin HN (1989) field experiment on the efficacies of mixed pesticides against wheat pests. *Scientia Agriculture Snica* **22**(2) : 1-6.
- Oi M (1999) Time dependent sorption of imidacloprid in two different soils. *Journal of Agricultural and Food Chemistry* **47**(1) : 327-332.
- Pai AK, Chhonkar PK and Agnihotri NP (1999) Persistence of Pendimethalin and Anilophos in six diverse soils. *Pestic Res J* **11**(1) : 132-137.
- Pal R and Handa SK (1994). Persistence of endosulfan on soybean. *Pesticide Res J* **16**(1) : 87-91.
- Pal S, Moza PN and Kettrup A (1991) *J Agric Food Chem* **39** : 797-800.
- Pandey CS and Padhiar RG (1998). Study on the comparative efficacy of different herbicides in tomatoes (*Lycopersicon esculentum* Mill) under south Gujarat conditions. *Indian Journal of Environment and Toxicology*, **8**(1) : 44-46.

- Pandit GK, Pal S and Das AK (1995) Photocatalytic degradation of pendimethalin in the presence of titanium dioxide 1. *J Agric Food Chem* **43** : 171-174.
- Parochetti, IU and Dec GW (1978) *Weed Sci* **26** : 153-156.
- Parrilla P, Martinez GM, Garrido-Frenich A and Martinez Vidal JL (1997) *Journal of Liquid Chromatography and Related Technologies* **20**(3) : 425-442.
- Pawar SA and Mali AR (1997) Effect of insecticide mixture against *Helicoverpa armigera*. *Pestology* **21**(3) : 36-38.
- Pesticide Manual. (1994). (Ed. Clive Tomlin) 10th Edition. BCPC, The Royal Society of Chemistry. 132 pp. 193.
- Pesticide Manuals (1994). (Ed Clive Tomlin) Tenth edition BCPC; The Royal Society of Chemistry, pp. 591-592.
- Philstrom T, Hellstrom A and Axelsson V (1997). Gas Chromatography analysis of pesticides in water with off-line solid phase extraction. *Analysis Chemica Acta* **353** : 2-3.
- Pionke HB and DeAngelis RJ (1980) Method for distributing pesticide loss in field runoff between the solution and adsorbed phase. In : *CREAMS*. A field scale model for chemical runoff and erosion from agricultural management systems. USDA Conservation Research Report. 26 USDA SEA, Washington DC, p. 607-643.
- Placke FJ and Weber E (1993) Method of determining imidacloprid in plant materials. *Pflanzenschutz Nachr. Bayer* **46**(2) : 109-182.
- Rai AK, Chhonkar PK and Agnihotri NP (2000) Adsorption-desorption of pendimethalin in six soils of India. *Journal of the Indian Society of Soil science* **48**(1) : 52-56.
- Raj MF, Patel BK and Shah PG (1999) Adsorption and desorption of pendimethalin, fluchloralin and oxadiazon on soils. *Pestic Res J* **11**(2) : 162-167.

- Raj MF, Shah PG, Patel BK, Patel BA and Patel JA (1999) Dissipation of triazophos from brinjal and okra fruits. *Pesticide Research Journal* **11**(1) : 102-105.
- Raju J and Gupta VK (1991) A simple spectrophotometric determination of endosulfan in river water and soil. *Fresenius Journal of Analytical Chemistry* **339**(6) : 431-433.
- Raju J and Gupta VK (1992) A simple spectrophotometric determination of endosulfan in river water and soil. *Chemia Analytyczna* **37**(2) : 245-248.
- Ranganathan T and Govindan, K (1996) A new insecticidal mixture to combat *Helicoverpa armigera* in cotton. *Pestology* **20**(2) : 23-25.
- Rani S, Madan VK and Kathpal TS (2001) Persistence and dissipation behaviour of triazophos in canal water under Indian climatic conditions. *Ecotoxicology and Environmental Safety-Environment Research*, Section-B, **50**(1) : 82-84.
- Rapparini G and Campagna G (1996). Weed control in direct soon and transplanted tomatoes. *Informatore Agrario* **52**(17) : 83-93.
- Reddy MS, Rao PG and Babu RSH (1999). Integrated weed management in tomatoes (*Lycopersicon esculentum* Mill). *Journal of Research ANGRAU*, **27**(4) : 7-11.
- Reducker S, Uchrin CG and Wimnet G (1998). *Bull Envt Cont Toxic* **41** : 633.
- Rouchaud J, Gustin F and Wauter A (1994) Soil biodegradation and leaf transfer of insecticide Imidacloprid applied in seed dressing in sugar beet crops. *Bulletin of Environments Contamination and Toxicology* **53**(3) : 344-350.
- Rouchaud J, Gustin F and Wauters A (1996) Imidacloprid insecticide soil metabolism in sugarbeet field crops. *Bull Environ Contam Toxicol* **56** : 29-36.
- Sannino A, Bandini M and Bolzon L (1999) Multiresidue determination of 10 fungicide in processed fruits and vegetables by capillary gas chromatography after gel permeation chromatography. *Journal of AOAC International* **82**(5) : 1229-1238.

- Sarkar MA, Roy S, Kole RK, Chowdhury A (2001) Persistence and metabolism of imidacloprid in different soils of West Bengal. *Pest Management Science* **57**(7) : 598-602.
- Sato T, Kohnosu S and Hartwig JF (1987) Adsorption of butachlor to soils. *J Agric Food Chem* **35** : 397-402.
- Sato M, Shimokawa S and Sakaguchi M (2001) A simple extraction and clean up method for multiresidue analysis of pesticides in fruits and vegetables. *Journal of Pesticide Science* **26**(3) : 253-256.
- Savage KE and Jordan TN (1978) Persistence of three dinitroaniline herbicides as affected by delayed incorporation. *Abstracts Weed Sci. Soc. Am.* 83.
- Savage KE and Jordan TN (1980) Persistence of three dinitroaniline on the soil surface. *Weed Sci* **28** : 105-110.
- Scholz K and Spiteller M (1992) Influence of ground cover on degradation of ^{14}C imidacloprid in soil. *Proc Brighton Crop Prot Conf –Pests and Dis* Nov 23-26. pp. 883-888.
- Seguera ZLM and Abakerli RB (1999) Pesticide residues in tomato fruits. *Pesquisa Agropecuaria Brasileira* **34**(3) : 473-480.
- Sharma D, Mohapatra S, Ahuja AK, Awasthi MD (1998) Persistence pattern of triazophos and lindane residues on okra and brinjal. In : *Advances in IPM for Horticultural Crops*. Proceedings of the first National Symposium on Pest Management in Horticultural crops : environmental implications and thrusts (Eds. Reddy PP and Kumar NKK). Bangalore, India 15-17 October, 1997, 252-256.
- Sheikh BA and Tikoo PK (1998) Dissipation of endosulfan and quinalphos on cauliflower crop. *Pest Management and Economic Zoology* **6**(1) : 31-35.
- Shetty PK, Mitra J, Murthy NBK, Namitha KK, Savitha KN and Raghu K (2000) Biodegradation of cyclodiene insecticide by *Mucor-hylospora* MTCC 1384. *Current Science* **79** (9) : 1381-1383.

- Singh A and Chawla RP (1978). Residue implications of insecticides spray schedule in vineyards. In "Pesticide Residue in Environment in India". (Eds.) CA Edwards, GK Veeresh and HR Krueger UAS Tech Series No. 32, p 166-170.
- Singh I P and Mukerjee U (1994). Dissipation of endosulfan in cowpea following foliar applications. *Pestic Res J* **6** (2) : 153-156.
- Singh P and Thapliyal PN (1987) *Indian Phytopathology* **40** : 88.
- Singh S, Dureja P, Kumar S and Jain MC (1999) Persistence of alpha, beta isomers of endosulfan and endosulfan sulfate in diverse soils of India as influence by flowing. *Journal of Environmental Science and Health Part-B-Pesticides Food Contaminations and Agricultural Wastes*. **34**(6) : 965-974.
- Singh SB and Kulshretha G (1991) Microbial degradation of pendimethalin. *Journal of Environmental Science and Health-Part B Pesticides, Food Contaminants and Agricultural Wastes* **26**(3) : 309-34.
- Singh SV and Kavadia VS (1988) Determination of endosulfan and carbaryl residues in /on brinjal fruits. *Indian J Ent* **50**(4) : 437-440.
- Singh SV, Kumar R, Katiyar RR and Upadhyay D (1993) Degradation of endosulfan residues on sponge gourd and radish. *Indian Journal of Entomology* **55**(1) : 103-105.
- Singh SV, Kumar R, Ram S and Pandey ND (1991) Assessment of endosulfan residues on spring tomato. *Indian J Ent* **53**(1) : 99-100
- Sinha S, Aggarwal R and Gopal M (2002) *In vitro* detoxification of β -cyfluthrin-A synthetic pyrethroid by two fungal antagonists. *Pestic Res J* **14**(2) : 319-322.
- Smith AE, Andrew JA and Tara CM (1995) Field persistence studies with emulsifiable concentrate and granular formulations of the herbicide pendimethalin in Saskatchewan. *J Agric Food Chem* **43** : 2988-2991.

- Soloway SB, Henry AC, Kollmeyer WD, Padgett WM, Powell JE, Roman SA, Tiemann CH, Corey RA and Horne CA (1978) Nitromethylene insecticides. In : Giessbuhler H, Brook GT and Kearney C (eds.) *Advances in Pesticide Science*, part 2, Pergamon Press, pp. 206-227.
- Stahnke GK, Shea PJ, Tupy DR, Stougaard RN and Shearman RC (1991) Pendimethalin dissipation in Kentucky blue grass turf. *Weed Science* **39**(1) : 97-103.
- Stan JH and Linkerhagner M (1993) Capillary gas chromatography atomic emission detection : a useful instrumental method in pesticide residue analysis of plant food stuffs. *Journal of High Resolution Chromatography* **16**(9) : 539-548.
- Sukul P and Handa SK (1984). Persistence and metabolism of endosulfan on chickpea. *Indian J Agric Sci* **54**(11) : 925-930.
- Sundraraj R, Chowhan S and Punikar SD (1998) Field evaluation of two neem formulation alone and in combination with chloropyriphos against *Rhodia defoliator* and *Patialus tecomena*. *Pestology* **22**(6) : 29-32.
- Suzuki S and Oda M (1977) Simultaneous determination of chlorothalonil (TPN), captan and diazinon and apples. *Bulletin of the Agricultural Chemicals Inspection Station* **17** : 43-45.
- Takagi K and Wada H (1990) A long-term change in biodegradation of a fungicide (Chlorothalonil : TPN) in upland, soils. *Translocations 14th International Congress of Soil Science*, Kyoto, Japan, August, 1990. **3** : 196-201.
- Tanwar RS and Handa SK (1998) Persistence, translocation and metabolism of endosulfan residue on pigeonpea (*Cajanus cajan* L. Mill sp.) *Pestic Res J* **10**(1) : 73-79.
- Tillman RW, Siegel MR and Long JW (1973) *Pestic Biochem Physiol* **3** : 160.
- Tomizawa M and Yamamoto I (1993) Structure activity relationship of nicotinoids and Imidacloprid analysis. Nihon Noyaku Gakkaishi. *J Pestic Sci* **18** : 91-108

- Tomlin C (ed) (1997) *The Pesticide Manual*, 11th Edition, BCPC, Farnham, Surrey, UK p. 707.
- Tondreau RE, Pasarela NR, Boughton PJ and Wyckoff JC (1977a) Presented at 90th Annual Meeting of the Assoc. Off Anal Chem
- Tripathi B, Kumar PG, Rengarajan T, Chandurkar PS and Vijayalakshmi (1993). Pendimethalin, metochlor residues in soil and their effect on soil microflora, weed spectrum and green peas yield. *Proc In Symp*, Indian Soc Weed Sci, Hisar, Nov. 18-20, 1993. Vol II : 98-100.
- Turner NJ and Battershell RD (1969) *Contrib Boyce Thomson Inst* **24** : 139
- Turner NJ and Battershell RD (1970) *Contribution Thomson Inst.* **24** : 203.
- Uesugi Y (1989) Use of pesticide mixture in Japan-Fungicides. *Japan Pesticide Information* **55**:6-10.
- Uygun U (1997). Determination of organophosphorus pesticide residue in carrot using gel permeation chromatography. *Journal of Liquid Chromatography and related technologies* **20**(5) : 771-777.
- Valverde A, Pihlstrom T and Anderson A (1997) Analysis of pyrachfos in crops by the Swedish National Food Administration Gas Chromatographic multiresidues methodology. *Analytica Chimica Acta* **338**(1-2) : 63-68.
- Valverde GA, Fernandez Alba AR, Contreras M and Aguera A (1996) Supercritical fluid extraction of pesticides from vegetables using anhydrous magnesium sulphate for sample preparation. *J Agric Food Chem* **44**(7) : 1780-1784.
- Valverde GA, Gonzalez PE, Aguilera DRA, Urena AMD and Camacho Ferre F (1993) Determination and degradation study of chlorothalonil residues in cucumber, peppers and cherry tomatoes. *Analytica Chimica Acta* **276**(1) : 15-23.

- Valverde Garcia A and Fernandez Alba AR (1995) Extraction of Methamidophos residues from vegetables with supercritical fluid carbon dioxide. *Journal of AOAC International* **78**(3) : 867-873.
- van der Pas LJT, Matser AM, Boesten JJTI and Leistra M (1999). Behaviour of metamitron and hydroxy chlorothalonil in low-humic sandy soils. *Pesticide Science* **55** : 923-934.
- Vargyas LD, Walls GE, Bramstedt WR and Eilrich GL (2000) Simultaneous determination of chlorothalonil and hexachlorobenzene in technical and formulated materials by capillary gas chromatography : collaborative study. *Journal of AOAC International*. **83**(5) : 1047-1052.
- Ververde GA, Gonzalez M, Vil-Lafranca-Sanchez, Ray FB and Garcia AR (1988). *Soil Sci Soc Am J* **52** : 1571.
- Verma S (1975) Determination of the residues of phorate and endosulfan during the development of moong and arhar plants. *Entomologists Newsletter* **5**(1) : 7-8.
- Vincet PG and Sisler HD (1968) *Physiol. Plant* **21** : 1249
- Wahid PA and Sethunathan N (1978) Sorption-desorption of parathion in soils. *J Agric Food Chem* **26** : 101-105.
- Walker A and Bond W (1977) Persistence of the herbicide AC 92553 N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine in soils. *Pestic Sci* **8** : 359-365.
- Walunj AR, Pawar SA, Hole UB and Darekar KS (1997) Evaluation of spark 36 EC against diamond back moth (*Plutella xylostella*) on cabbage. *Pestology* **21**(7) : 33-35.
- Westwood, F, Bean KM, Dewar AM, Bromilow RH and Chamberlain K (1998) Movement and persistence of ¹⁴C-imidacloprid in sugarbeet plants following application to pelleted sugarbeet seed. *Pesticide Science* **52**(2) : 97-103.

- Yamamoto I, Yabuta G, Tomizawa M, Saito T, Miyamoto T and Kagabu S (1995) Molecular mechanism for selective toxicity of nicotinoid and neonicotinoids Nihon Noyaku Gakkaishi. *J Pestic Sci* **20** : 33-40.
- Yeung JM and Newsome WH (1995) Determination of chlorothalonil in produce by enzymeimmunoassay. *Bulletin of Environmental Contamination and Toxicology* **54**(3) : 444-450.
- Zheng W, Liu HJ and Liu WP (2000) Influence of pesticide Imidacloprid on catalase activity in soil. *China Environmental Science* **20**(6) : 524-527.
- Zimdahl RL, Catizone P and Butcher AC (1984) Degradation of Pendimethalin in soil. *Weed Sci.* **32**(3) : 408-412.
- Zimdahl RL, Crammer BK and Stroup WW (1994) Use of empirical equations to describe dissipation of metribuzin and pendimethalin. *Weed Science* **42** (2): 241-248.
- Zulalian J (1990). *Journal of Agricultural and Food Chemistry* **38** : 1743-1754.
- Zulalian J, Haugwitz MI and Blinn RC (1976). Presented at 172nd Natl. Meet, Am Chem Soc Pest 091.

ANNEXURE

Annexure – I

Meteorological observation during field experiment

	Temp (Max)	Temp (Min)	RH % (Morn.)	RH % (Eve.)	Sunshine	Rainfall (mm)
1.10.01-07.10.01	32.7	22.9	88	51	6.1	18.2
8.10.01-14.10.01	34.3	17.3	74	40	7.1	0
15.10.01-21.10.01	33.2	15.2	76	26	6.7	0
22.10.01-28.10.01	33.7	17.7	83	28	5.9	0
29.10.01-4.11.01	34.4	16.9	72	29	6.5	0
5.11.01-11.11.01	29.3	12.7	77	32	6.3	0.5
12.11.01-18.11.01	28.6	10.4	82	29	5.7	0
19.11.01-25.11.01	27.6	9.2	76	26	6.4	12
26.11.01-02.12.01	25.7	8.8	77	29	5.3	0
3.12.01-9.12.01	26.1	8.5	79	27	6.4	0
10.12.01-16.12.01	22.6	9.8	88	50	4.5	0
17.12.01-23.12.01	21.8	7.3	91	39	5.6	0
24.12.01-31.12.01	22.8	6.3	109	59	3.5	0
1.1.02-7.01.02	19.3	3	93	48	3.1	0
8.1.02-14.01.02	21.3	6.7	88	46	4.6	0
15.01.02-21.01.02	18.2	8.9	87	67	1.6	5.6
22.01.02-28.01.02	19.7	6.9	83	52	5.2	0
29.01.02-4.2.02	20.3	3.7	85	34	6.2	0
5.2.02-11.2.02	20.8	6.8	83	54	5.9	8.4
12.2.02-18.2.02	21.4	8.5	90	48	4.3	4.8
19.2.02-25.2.02	27.2	11.7	75	38	4.6	1
26.2.02-4.3.02	23.9	11.8	82	55	5.5	0
5.3.02-11.3.02	27	12	72	32	9	0
12.3.02-18.3.02	29.1	12	72	28	8.8	0
19.3.02-25.3.02	33.3	13.4	72	30	9.1	0
26.3.02-1.4.02	33.9	16.9	61	25	9.4	3
2.4.02-8.4.02	35.9	17.7	52	28.5	7.1	0
9.4.02-15.4.02	36.5	19	53	26.8	8.6	0
16.4.02-22.4.02	40.6	21.9	42.8	20.2	8.6	0
23.4.02-29.4.02	40	24.2	57	25.8	6.5	0
30.4.02-6.5.02	39.4	22.8	55.8	31.5	7.5	0
7.5.02-13.5.02	43	28	44	21	7.6	0
14.5.02-20.5.02	36.9	28.1	50	26	4.2	0
21.5.02-27.5.02	41	25.3	50	35	5.5	17.2
28.5.02-3.6.02	34.4	21.4	72	58	6.2	97.6