# STUDIES ON THE FATE AND BEHAVIOUR OF FLUBENDIAMIDE IN TROPICAL ENVIRONMENT

*A Thesis*
*Submitted to the*
*Bidhan Chandra Krishi Viswavidyalaya*
*in partial fulfilment of the requirements for the Award of the Degree of*
*Doctor of Philosophy (Agriculture)*
*in*
**AGRICULTURAL CHEMICALS**

*By*
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## *Certificate*

This is to certify that the work recorded in the thesis entitled **"STUDIES ON THE FATE AND BEHAVIOUR OF FLUBENDIAMIDE IN TROPICAL ENVIRONMENT"** submitted by **Mr. M.Paramasivam**, in partial fulfilment of the requirements for award of the Degree of Doctor of Philosophy (Agriculture) in Agricultural Chemicals, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, is a faithful and bonafide research worker and carried out his research work under my personal supervision and guidance.

The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received from various sources during the course of this investigation have been duly acknowledged.

Hemanta Banerjee
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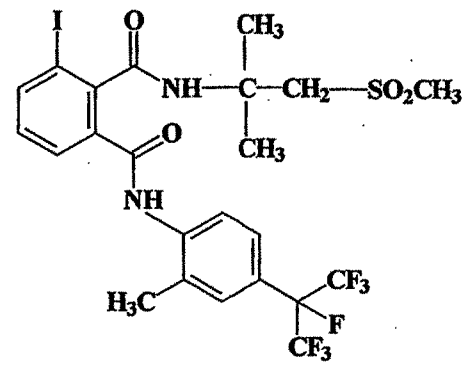
# Preface

The use of synthetic pesticides in modern agriculture is inevitable and will continue to play a key role in securing sustainable agriculture into the foreseeable future as it continues to contribute to improved crop yields knowing fully aware of the fact that pesticides are inherently toxic chemicals and are bound to have residues after use on crop produce, soil, water or air. However, their continuous use may lead to build up of toxic residues on crop produce, which may exert adverse effect on human health in addition to disturbing ecosystem and thus become a burning issue and integral criteria related to food safety. The presence of residues above the permissible level is also a major bottleneck in the acceptance of food commodities by the importing countries. The peoples are becoming more interested in the aesthetic and sustainable value of the quality and safety of their food. Hence, it is important to know the status of pesticide residues to ensure the safety to the consumers.

The complicated problem of pest and pest control is causing a lot of rethinking among the scientist's all over the world. Resistance has often been a problem for insecticides and efforts are therefore needed to search for new mode of action and consequently many new insecticides are coming up with new mode of action. Very recently, ryanodine receptor mode of action compound flubendiamide was discovered by Nihon Nohyaku (Tohnishi *et al.*, 2005; Hamaguchi & Hirooka, 2007) and developed jointly with Bayer Crop Science (Nishimatsu *et al.*, 2005). It is a novel insecticide belongs to a new chemical class named benzenedicarboxamide or phthalic acid diamide, Its unique chemical structure is a combination of a phthaloyl moiety, an aromatic amide moiety and an aliphatic amide moiety (Hirooka *et al.*, 2007; Tsubata *et al.*, 2007) which characterizes **flubendiamide (I) as totally new.**

Cereals, pulses, vegetables and other agricultural commodities are cultivated in West Bengal tropical climatic condition which favors proliferation of pest problem and thereby needs crop protective chemicals. The consumption and cultivation of vegetables and pulses have become more popular during the past decades. The vegetables cabbage, tomato and the pulse pigeon pea are susceptible to lepidopteran pest attack (Moudgal *et al.*, 2003; Gupta *et al.*, 2007) throughout the season and extensive pesticide applications

are done by the farmers. The flubendiamide has an excellent fast-acting and residual activity against broad spectrum of lepidopterous insect pests (Tohnishi *et al.*, 2005; Shane *et al.*, 2006; Palrang & Hand 2007). However, for any new pesticide to be registered in India for use, data requirements on the residue




Flubendiamide (I)

are to be generated in crops claimed for use through supervised field trial at different geographical regions following good agricultural practices (GAP) as per regulatory provisions made in the Insecticides Act 1968. Literature survey revealed very little information of flubendiamide in context to residue data in different agricultural crops and environmental samples. It is pertinent to mention that research recommendations on chemical control of pests are considered incomplete if data on toxic residues of the parent molecule as well as their metabolites / degradation products are not provided. Thus, the present investigator took an attempt to generate the residue data of flubendiamide and its metabolite in cabbage, tomato, pigeon pea, soil and water under West Bengal agro climatic (tropical) condition as because these data are very much pertinent in predicting not only the waiting period for safe consumption of agricultural raw commodities but also useful in fixation of MRL value from the view point of national context. Moreover, photochemical reactions are enhanced by the solar energy which is prevailing at West Bengal and plays a key role in environmental degradation or detoxification of various anthropogenic chemicals. This work form the subject matter of the thesis entitled **"Studies on the Fate and Behaviour of Flubendiamide in Tropical Environment"** consists of two part and their salient findings are presented below:

**PART-I:** Fate and behaviour of flubendiamide in crops, soil and water

The analytical method for residue analysis in cabbage head, tomato and pigeon pea (grain, shell and straw) was developed by using new emerging technique QuEChERS. This method involves primary secondary amine (PSA) as cleaning agent. The amount of PSA was standardized and found that 10 mg was enough for clean up in tomato fruit & pigeon pea grain, 25 mg for cabbage head and pigeon pea shell, and 50 mg for pigeon pea straw. The residue method was validated by conducting recovery experiments with different substrate under study. By using this technique, the limit of quantification (0.01 mg/kg) in all substrates was achieved. The individual recovery rates for determination of flubendiamide and its metabolite des-iodo flubendiamide in plant matrices ranged from 87.26 - 97.30% and 88.45-98.25% respectively with RSD typically less than 5%. Thus, the method complies with all guideline requirements for determination flubendiamide and its metabolite residue in different plant matrices.

The supervised field trial was conducted with flubendiamide 20% WG on cabbage head @ 12.5 ($T_1$) and 25 g a.i $ha^{-1}$ ($T_2$). First order reaction kinetics was observed and the half life values in cabbage head were found to be 3.4-3.6 days irrespective of dose. The initial deposit of total residue was found to be 0.16 ppm and 0.31 ppm in case of $T_1$ and $T_2$ respectively which further dissipated to 12.5% ($T_1$) and 9.68% ($T_2$) on day 1 and steady reduction of residue occurred with increment of time. The residue became non detectable below 0.01 ppm on day 10 and 15 at $T_1$ and $T_2$ respectively. Further scrutiny of the residue data revealed that in $T_1$ the flubendiamide dissipated to 50% on day 3 of which 77.5% was accounted for by the formation of des-iodo flubendiamide metabolite and similarly in $T_2$ it was 22.6% on day 1 of which 70.0% metabolite was formed from this dissipated amount. Thus, des-iodo flubendiamide is the major metabolite formed via the process of deiodination in cabbage head. Based on LOQ value of 0.01 ppm, the suggested safe waiting period found to be 14.54 and 16.79 days at $T_1$ and $T_2$ respectively. **Thus, from the residue point of view, consumption of cabbage head is safe after 14 days of application of flubendiamide 20% WG @ 12.5 g a.i $ha^{-1}$.**

In case of tomato, flubendiamide 20% WG was sprayed twice at an interval of 15 days @ 50 ($T_1$) and 100 ($T_2$) g a.i $ha^{-1}$. The results indicated that initial deposit of flubendiamide was found to be 0.27 ($T_1$) and 0.46 ($T_2$) ppm respectively with corresponding reduction of 43-44 % irrespective of treatment on first day. The calculated

half life ranged from 1.64-1.98 days irrespective of the doses. Furthermore, from the total reportable residue data, it was revealed that flubendiamide dissipated to 44.4 % on day 1 of which 81.66 % was accounted for by the formation of des-iodo flubendiamide metabolite ($T_1$) and in $T_2$, dissipation was 43.5 % on day 1 of which 92.5 % metabolite was formed from this dissipated amount. Thus, des-iodo flubendiamide is the major metabolite formed via the process of deiodination in tomato fruits. Based on LOQ (0.01 mg/kg), the suggested safe waiting period calculated was found to be 7.7 and 10.6 days irrespective of $T_1$ and $T_2$. **Based on the residue data, it was concluded that after application of flubendiamide 20% WG at recommended dose (50 g a.i. / ha) during fruiting stage, the tomato fruit may be plucked at 7 days for safe consumption.**

No flubendiamide and des-iodo flubendiamide residue could be detected at harvest (42 days after last spray) in pigeon pea grain, shell and straw @ 50 and 100 g a.i $ha^{-1}$. **Thus, flubendiamide 20% WG can suitably fit in plant protection practices.**

Harvest soil samples (cabbage, tomato and pigeon pea) collected after last spray of flubendiamide did not show the presence of either flubendiamide or des-iodo metabolite at detection limit of 0.01 ppm which indicated that **carry over damage to rotation crop will not occur.**

Laboratory dissipation study of flubendiamide at a concentration of 5 ($T_1$) and 10 ($T_2$) ppm was conducted in three types of soil (New alluvial, Red & lateritic and Coastal) for a period of 150 days. The half-life values ($t_{1/2}$) of all the soil irrespective of doses were calculated and found to be 44.26 – 59.02 (coastal soil), 41.23 – 51.90 (red and lateritic soil) and 37.16 – 44.92 (new alluvial soil) days. First order reaction kinetics followed in all the three soils. The persistence of flubendiamide was in the order of coastal soil > red and lateritic soil > new alluvial soil. Des-iodo flubendiamide was also quantified with varying amount in three soil types and persisted upto 90 days. **Thus, the present study concludes that persistence of flubendiamide is more in coastal than neutral to acidic soil and coupled with its low water solubility gives an indication of minimal contamination of ground water.**

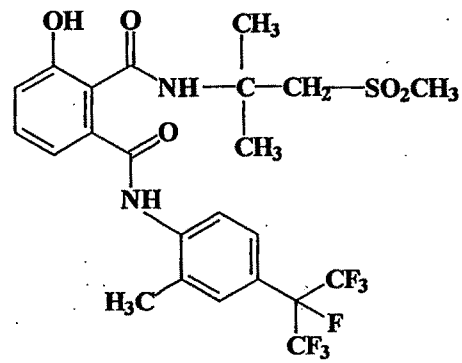
A laboratory study on the persistence and dissipation behaviour in water with three different pH (4.0, 7.0 and 9.2) was carried out for a period of 120 days at a concentration level of 1 ($T_1$) and 2 ($T_2$) ppm. Flubendiamide dissipation in water at all the pH under study followed the first order reaction kinetics. The half-life ($T_{½}$) value at pH -

4.0, 9.2 and 7.0 for $T_1$ was found to be 107.5, 115.78 and 81.36 days whereas for $T_2$ it was 111.5, 125.42 and 83.6 days. The persistence was in the order of pH 9.2 > pH 4.0 > pH 7.0. **Thus, the present study indicated that flubendiamide molecule is hydrolytically stable in acidic, neutral and alkaline medium.**

**PART–II:** Photolytic degradation of flubendiamide in aqueous acetonitrile, methanol and isopropanol under UV and sunlight.

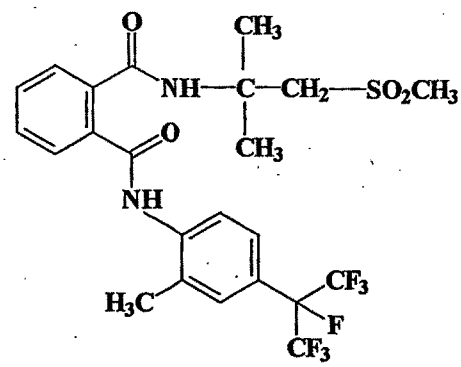
The photolytic degradation was carried out with flubendiamide analytical and its formulation (10 ppm) in aqueous acetonitrile, methanol and isopropanol under UV and sunlight. The photolytic half life value of analytical grade flubendiamide under sunlight was found to be 55.75, 73.42 and 94.01 hours and that of formulation was 79.22, 158.43 and 150.51 hours while in UV light, for analytical grade it was 3.17, 12.04 and 36.26 hours and that of formulation was 7.60, 21.81 and 55.74 hours in aqueous acetonitrile, methanol and isopropanol respectively. The overall photokinetic studies under UV and sunlight showed that flubendiamide (analytical and formulation) undergoes faster photodegradation in aqueous acetonitrile followed by aqueous methanol and aqueous isopropanol. Furthermore, faster degradation occurred in the analytical grade than the formulation. The probable reason for slower rate of degradation of formulation may be attributed to the consumption of hydroxyl radicals (OH) by surfactants and the formation of a substrate-surfactant complex, resulting in the unavailability of flubendiamide to OH attack. Flubendiamide degradation rate was faster under UV light as compared to sunlight. Moreover, aqueous acetonitrile irradiated under sunlight led to the formation of maximum number of photo products and subjected to analysis by Q-TOF micro mass spectrometer for characterization. Four photo metabolites were able to characterize and designated as $F_1$, $F_2$, $F_3$ and $F_4$.

The MS-MS spectrum of the photo metabolite $F_1$ showed the molecular ion peak at m/z value of 572.26 ($M^+$) with some diagonistic peaks at m/z – 1 value of 556, 281. The molecular ion peak may lose an OH· to form a cation (m/z = 556) which may further fragment to form a positively charged substituted phthalimide species (m/z = 281) and a neutral molecule flubendiamide aniline (mol. wt 275). Based on mass fragmentation the structure of $F_1$ appeared to be 3-hydroxy flubendiamide.



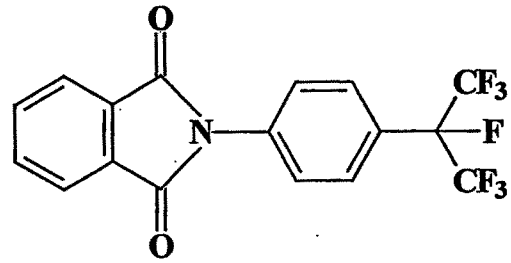

**3-hydroxy flubendiamide ($F_1$)**

The radical ion peak of $F_2$ corresponding to the m/z – 23 values of 556.26 when subjected to MS-MS produced characteristic peaks at m/z 281, 147 and 134. $F_2$ undergoes rearrangement along with the elimination of the aniline moiety to produce a substituted phthalimide species with m/z 281 (100%) which further undergoes cleavage to produce a peak at m/z = 147 corresponding to $M^+$ of phthalimide and at m/z = 134 corresponding to the sulfone moiety. The probable structure of $F_2$ could be assigned as des-iodo flubendiamide.



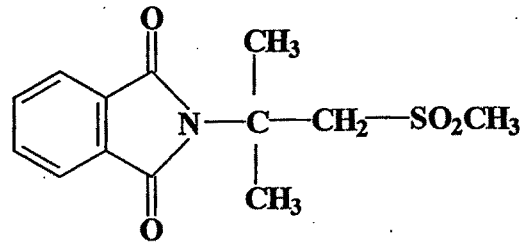

**des-iodo flubendiamide ($F_2$)**

The MS-MS spectrum of the photo metabolite $F_3$ showed $M^+$ at m/z = 390 is suspected to be a phthalimide derivative. The cation formed from it loses a carbon monoxide molecule to form a cation with m/z = 362 which finally loses a fluorine atom to give a peak at m/z = 343. The probable structure of $F_3$ could be assigned as N-[4'-{1,2,2,2-tetrafluoro-(1-trifluoromehtyl) ethyl}-phenyl]-phthalimide.



N-[4'-{1,2,2,2-tetrafluoro-(1-trifluoromehtyl) ethyl}-phenyl]-phthalimide **($F_3$)**

The MS-MS spectrum of the photo metabolite $F_4$ showed the molecular ion peak at m/z – 1 value of 281. The peak at m/z = 281 may be due to another phthalimide derivative. The radical ion of which loses the phthalimide part to form a cation with m/z = 135 or it may otherwise fragment to give a cationic species having m/z value of 147. The probable structure of $F_4$ could be assigned as N'(2-mesyl-1,1-dimethylethyl)phthalimide.



**N'(2-mesyl-1,1-dimethylethyl)phthalimide ($F_4$)**

The plausible pathways for the formation of four photo products ($F_1$, $F_2$, $F_3$ and $F_4$) from flubendiamide were also ascertained and the main photo transformation pathway involves viz. dehalogenation, hydrolysis and elimination. The photo products $F_1$, $F_2$, $F_3$ and $F_4$ have been reported for the first time.

# Abbreviation & Acronyms

| | |
|---|---|
| % | : Percentage |
| λ | : Lamda |
| @ | : at the rate of |
| ® | : Registered trademark |
| °C | : Centigrade temperature scale |
| < | : less than |
| ≤ | : less than or equal |
| > | : greater than |
| ≥ | : greater than or equal |
| μ | : micron |
| μg | : microgram |
| μL | : microLitre |
| μm | : micrometer |
| AFT | : Anodic fenton treatment |
| a.i. | : active ingredient |
| ATP | : Adenosine Tri-Phosphate |
| BCF | : Bioconcentration Factor |
| BDL | : Below Detectable Limit |
| BDS | : Base Deactivated Silica |
| bw | : body weight |
| C-18 | : Octadecylsilane |
| Ca | : Calcium |
| CAS | : Chemical Abstracts Service |
| CAS RN | : Chemical Abstracts Service Registry Number |
| CEC | : Cation Exchange Capacity |
| CIPAC | : Collaborative International Pesticides Analytical Council |
| cm | : centimeter |
| CMC | : Critical micelle concentration |
| $C_{mol}$ | : centimole |

| | |
|---|---|
| d | : days |
| DAT | : Days after Treatment |
| ds/m | : decisiemens/metre |
| d-SPE | : Dispersive Solid Phase Extraction |
| DT | : Degradation time |
| d.wt.s. | : dry weight soil |
| e.g. | : exempli gratia (for example) |
| EC | : Emulsifiable Concentrate |
| EC | : Electrical Conductivity |
| $EC_{50}$ | : Effective Concentration |
| ECD | : Electron Capture Detector |
| $ErC_{50}$ | : Effective Concentration for algae growth rate |
| ESI | : Electron Spray Ionization |
| et al | : et alii (and others) |
| FPD | : Flame Photometric Detector |
| g | : gram |
| g/mol | : grams per mole |
| GCB | : Graphatised Carbon Black |
| GC | : Gas Chromatography |
| GLP | : Good Laboratory Practice |
| GPS – GIS | : Global Positioning System – Geographic Information System |
| h/hr | : hour |
| HAc | : acetic acid |
| HDPE | : High Density Polyethylene |
| HPLC | : High Performance Liquid Chromatography |
| i.d. | : internal diameter |
| IPM | : Integrated Pest Management |
| IRAC | : Insecticide Resistance Classification |
| IRM | : Insecticide Resistance Management |
| ISO | : International Standards Organization |
| IUPAC | : International Union of Pure and Applied Chemistry |
| kcal/mol | : kilo calories per mole |
| kg | : kilogram |
| Koc | : Organic-carbon sorption constant |

| | |
|---|---|
| $K_{OW}$ | : octanol-water partition coefficient |
| LC | : Liquid Chromatography |
| $LC_{50}$ | : Lethal Concentration |
| $LD_{50}$ | : Lethal Dosage |
| L | : Litre |
| LOD | : Limit of Detection |
| LOQ | : Limit of Quantification |
| $LR_{50}$ | : Lethal body residue |
| max | : maximum |
| MCPBA | : *m*-chloroperbenzoic acid |
| MeCN | : Acetonitrile |
| mg | : milligram |
| $MgSO_4$ | : Magnesium Sulphate |
| min | : minute |
| min | : minimum |
| mL | : milliliter |
| mm | : millimeter |
| MRL | : Maximum Residue Levels |
| MS/MS | : Mass Spectroscopy/ Mass Spectroscopy |
| MW | : Molecular Weight |
| $Na_2SO_4$ | : Sodium Sulphate |
| NaAc | : Sodium acetate |
| NaCl | : Sodium Chloride |
| ng | : nanogram |
| nm | : nanometer |
| $NaHCO_3$ | : Sodium bi-carbonate |
| NOAEC | : no-observed adverse effect concentration |
| NOAEL | : no-observed adverse effect level |
| NOEAEC | : no-observed ecological adverse effect concentration |
| NOEL | : no-observed effect level |
| NPD | : Nitrogen Phosphorous Detector |
| OC | : Organic carbon |
| Op | : Organo phosphorus |
| pH | : p (otential of) H (ydrogen) |

| | |
|---|---|
| PHI | : Pre-Harvest Interval |
| ppm | : parts per million |
| PSA | : Primary Secondary Amine |
| Q-TOF | : Quadrupole-time- of flight |
| QuEChERS | : Quick, Easy, Cheap, Effective, Rugged, and Safe |
| RBD | : Randomized Block Design |
| Rf | : Recovery factor |
| rpm | : Revolutions per minute |
| RP | : Reverse Phase |
| RSD | : Relative Standard Deviation |
| RyR | : Ryanodine Receptors |
| SC | : Suspension Concentrate |
| SD | : Standard Deviation |
| S/N | : Signal - to - Noise |
| SPE | : Solid Phase Extraction |
| sq.m | : square meter |
| $T_{1/2}$ | : half life |
| TX | : Triton X |
| TOC | : Total organic carbon |
| US EPA | : United States of Environmental Protection Agency |
| UV | : Ultra Violet |
| v/v | : volume by volume |
| WG or WDG | : Water Dispersible Granules |
| $r^2$ | : Correlation Co-efficient |
| WHC | : Water Holding Capacity |

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# PART-I

*Fate and behaviour of flubendiamide in crops, soil and water*

# Chapter 1

# Introduction

# Introduction

The great success stories began in agriculture with the cultivation of grains and the ability to feed humans and animals. The significant contributions made by the different sections of agricultural scientists (chemists, plant and soil scientists, breeders, weed scientists, plant pathologists, entomologists, synthetic fertilizers, pest management technology) allowed the agricultural enterprise to produce the food and fiber to feed and clothe today's 6.0 billion people and the opportunities are greater than ever for our genius in the development of new technologies to be used in feeding and clothing the projected 9.0 billion people on this planet by 2050 (Abernathy, 2002). Today's society now takes for granted that a stable food supply and clothing will always be available at a modest cost. The presence of residues above the permissible level is also a major bottleneck in the acceptance of food commodities by the importing countries. The peoples are becoming more interested in the aesthetic and sustainable value of the quality and safety of their food. Hence, it is important to know the status of pesticide residues to ensure the safety to the consumers.

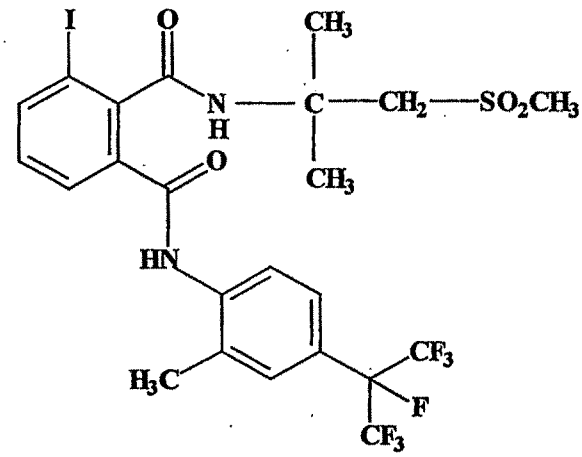
Crop protection is questioned as never before though these crop protection chemicals did a significant contribution over the past four decades in reducing mortality and increasing the availability of fresh, healthy food. The chemicals will remain a vital component of crop protection into the foreseeable future. They will continue to play a key role in securing sustainable agriculture, as it continues to contribute to improved crop yields. Resistance has often been a problem for insecticides and is one of the most important reasons why insecticides with new mode of action have been desired and consequently many new insecticides are coming up with new mode of action. For example, the neonicotinoids and strobilurins both introduced in the last one and half decade, continue to stimulate the market, suggesting that a focus on new mode of action remains an excellent recipe for success. However, new modes of action are not enough to ensure successful products but also to provide cost-effective benefits at today's crop prices. Care of the environment has always been important in agriculture, and the regulatory framework to encourage sustainable agriculture is taking shape. This is overlaid with regulatory initiatives to remove some older, less environmentally friendly compounds from sale.

Very recently, ryanodine receptor mode of action compound flubendiamide was discovered by Nihon Nohyaku (Tohnishi *et al.*, 2005; Hamaguchi & Hirooka, 2007) and developed jointly with Bayer Crop Science (Nishimatsu *et al.*, 2005). It is a novel insecticide belongs to a new chemical class named benzenedicarboxamide or phthalic acid diamide, Its unique chemical structure is characterized by a phthaloyl moiety, an aromatic amide moiety and an aliphatic amide moiety (Hirooka *et al.*, 2007; Tsubata *et al.*, 2007).

It was reported that an iodine atom at the 3-position was the best substituent for the phthaloyl moiety, although compounds having an iodine atom are very rare among commercial agrochemicals. The introduction of heptafluoroisopropyl group is very novel since it has never been reported as a substituent in the chemical structure of commercial pesticides so far and seldom used in pesticide research. Moreover, the introduction of a hetero atom especially a sulfur atom onto the alkyl side chain markedly increased the insecticidal activity. This sulfonylalkylamine moiety is also novel as an amine in pesticide chemistry. Flubendiamide has these unique substituents as essential parts of the structure in three adjacent positions on the benzene ring, which characterizes the chemical structure of **flubendiamide (I)** as **totally new**. It has an excellent fast-acting and residual activity against a broad spectrum of lepidopterous insect pests, such as *Helicoverpa* spp., *Heliothis* spp., *Spodoptera* spp., *Plutella* spp., *Pseudoplusia* spp., *Trichoplusia* spp., and *Agrotis* spp., including resistant strains of them as well (Tohnishi *et al.*, 2005; Shane *et al.*, 2006; Palrang & Hand 2007). Furthermore, flubendiamide (20% WDG) showed no phytotoxicity to vegetables, tea and top fruits at recommended doses.

Besides rice, cultivation of vegetables, pulses and many other crops is a common practice in West Bengal. The consumption and cultivation of vegetables and pulses have become more popular during the past decades. India secures the second position in vegetable and fruit production with consumption of 13% of total pesticides (Lal, 1996). Tropical climatic condition favors proliferation of pest problem throughout the season and thereby needs crop protective chemicals. In West Bengal, cabbage and tomato is widely cultivated and has the potentiality for export. The crop pigeon pea is also cultivated and has a very high nutritional value. These crops are susceptible to lepidopteran pest attack (Moudgal *et al.*, 2003; Gupta *et al.*, 2007) and many chemicals are used for controlling them. As flubendiamide fit well into insecticide resistance

management (IRM) programs and integrated pest management (IPM) programs (Ohkawa *et al.*, 2007) it may also be recommended for use in these crops.




**Flubendiamide (I)**

It is obviously true that for any new pesticide to be registered in India for use, data requirements on the residues are to be generated in crops claimed for use through supervised field trial at different geographical regions following good agricultural practices (GAP) as per regulatory provisions made in the Insecticides Act 1968. Literature survey revealed very little information of flubendiamide in context to residue data in different agricultural crops and environmental samples. It is pertinent to mention that research recommendations on chemical control of pests are considered incomplete if data on toxic residues of the parent molecule as well as their metabolites / degradation products are not provided. In addition, all the pesticides do have their toxic action for a certain period to kill the target organism and after this period it is also desirable that the molecule be deactivated through various transformation processes to some non-toxic products and photochemical reactions play a key role in environmental degradation or detoxification of various anthropogenic chemicals. However, no detailed systematic study on flubendiamide in cabbage, tomato, pigeon pea and its environmental fate has so far been made in West Bengal. Moreover, the residue data are very much pertinent not only in predicting the waiting period for safe consumption of agricultural commodities but also useful in fixation of MRL value from the view point of national context. Thus, an attempt has been made by the present candidate to examine the persistence and dissipation behaviour of flubendiamide along with the nature of transformation products formed in various components of the environment *viz.* plant, soil and water. These works form the

subject matter of the thesis entitled **"Studies on the Fate and Behaviour of Flubendiamide in Tropical Environment"** with the following objectives:

**PART-I:** Fate and behaviour of flubendiamide in crops, soil and water

1. Method standardization of flubendiamide/metabolite in different crops, soil and water.
2. Persistence and dissipation behaviour of flubendiamide in cabbage
3. Persistence and dissipation behaviour of flubendiamide in tomato
4. Harvest time residues of flubendiamide in pigeon pea grain, shell and straw
5. Studies on the persistence and dissipation behaviour of flubendiamide in three different soils of West Bengal (New Alluvial, Red & Lateritic and Coastal),
6. Studies on the persistence and dissipation behaviour of flubendiamide in water at three different pH (4.0, 7.0 and 9.2)

**PART–II:** Photolytic degradation of flubendiamide in aqueous acetonitrile, methanol and isopropanol under UV and sunlight.

1. Studies on the photo kinetics of flubendiamide using various aqueous organic solvents in presence of sunlight and UV light.
2. Characterization of photo products on the basis of Q-TOF micro mass spectral data.
3. To portray a plausible mechanism for understanding the photo transformation products of flubendiamide.

Chapter 2

# Review of Literature

# Review of Literature

The review of literature pertaining to flubendiamide in the present study is discussed under the following heads:

2.1 General information

2.2 Chemistry of Flubendiamide

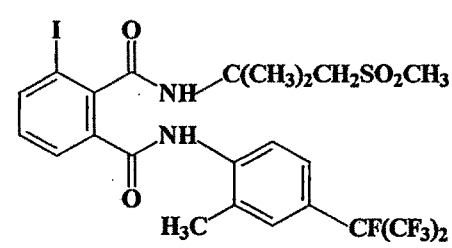
2.3 Biological profiles and features

2.4 Field study and Bio-efficacy

2.5 Metabolism in plant

2.6 Residue analytical method

**2.1 General Information**

| | |
|---|---|
| Pesticide Type | Insecticide |
| Chemical Group | Benzenedicarboxamides or Phthalic acid diamides |
| Common Name | Flubendiamide (BSI, pa ISO) |
| Chemical Name (IUPAC) | 3-iodo-*N*'-(2-mesyl-1,1-dimethylethyl)-*N*-{4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]-o tolyl}phthalamide |
| Chemical Name (CAS) | $N^2$-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-$N^1$-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl) ethyl] phenyl]-1,2-benzenedicarboxamide |
| Code Number | NNI-0001 |
| CAS Registry Number | 272451-65-7 |
| CIPAC Number | 788 |
| US EPA Chemical code | 027602 |
| Chemical Formula | $C_{23}H_{22}F_7IN_2O_4S$ |
| Structure |   |

| | |
|---|---|
| Molecular Mass (g $mol^{-1}$) | 682.39 |
| Trade name | Phoenix, Takumi, Fame, Fenos, Synapse, Amoli and Belt |
| Formulation | 20% WDG; 24% WDG; 480 SC |
| Insecticide Resistance (IRAC) Classification | Ryanodine receptor modulator |
| Mode of Action | Exhibits larvicidal activity as an orally ingested toxicant by targeting and disrupting the $Ca^{2+}$ balance |

### 2.1.1 Physico-chemical properties

| | | |
|---|---|---|
| Appearance | | Colourless crystals |
| Form | | White crystalline powder |
| Odour | | No characteristic odour |
| Melting Point (°C) | | 217.5 - 220.7 |
| Density (at 20.8 °C) (g/cm³) | | 1.659 |
| Solubility in water (at 20 °C) (g $L^{-1}$) | | $29.9 \times 10^{-6}$ |
| Solubility in Organic solvents (at19.8°C) (g $L^{-1}$) | *n*-Heptane | $83.5 \times 10^{-5}$ |
| | *p*-Xylene | 0.488 |
| | 1,2-Dichloroethane | 8.12 |
| | Acetone | 102 |
| | Methanol | 26.0 |
| | Ethyl acetate | 29.4 |
| Partition coefficient in octanol/water at 24.9 °C | | Log $P_{ow}$ = 4.20 |
| Koc - Organic-carbon sorption constant (mL $g^{-1}$) | | 2197 |
| Thermal stability | | Stable at ambient temperature under air |

(Source: Tsubata *et al.*, 2007)

### 2.1.2 Ecotoxicological properties

| Property | | Value (Interpretation) |
|---|---|---|
| Bio-concentration factor (BCF) | | 66 (Low potential) |
| Bioaccumulation potential | | Low |
| Mammals - Acute oral $LD_{50}$ (mg $kg^{-1}$) | | 2000 (Moderate) |
| Mammals - Short term NOEL | (mg $kg^{-1}$) | 18 (High) |
| | (ppm diet) | 200 |
| Birds - Acute $LD_{50}$ (mg $kg^{-1}$) | | 2000 (Moderate) |
| Fish - Acute $LC_{50}$ (mg $L^{-1}$) | | 0.06 (High) |
| Fish - Chronic 21day NOEC (mg $L^{-1}$) | | 0.06 |
| Aquatic invertebrates - Acute $EC_{50}$ (mg $L^{-1}$) | | 0.06 (High) |
| Aquatic invertebrates - Chronic 21day NOEC (mg $L^{-1}$) | | 0.033 |
| Aquatic plants - $EC_{50}$ (mg $L^{-1}$) | | 0.0546 (Moderate) |
| Algae - Acute $EC_{50}$ (mg $L^{-1}$) | | 0.069 (Moderate) |
| Honeybees - $LD_{50}$ (µg $bee^{-1}$) | | 200 (High) |
| Earthworms - Acute $LC_{50}$ (mg $kg^{-1}$) | | 1000 (Moderate) |
| Other arthropod ($LR_{50}$ g $ha^{-1}$) | | 675 (Harmless at 1 kg $ha^{-1}$) |
| Soil micro-organisms | | Nitrogen and Carbon mineralization: No significant effect |
| Mesocosm study data-NOEAEC mg $L^{-1}$ | | 0.053 |

(Source:http://sitem.herts.ac.uk/aeru/footprint/en/)

### 2.1.3 Mammalian Toxicology

**Oral:** Acute oral $LD_{50}$ for male and female rats >2000 mg $kg^{-1}$.

**Skin and eye**: Acute percutaneous $LD_{50}$ for male and female rats >2000 mg $kg^{-1}$. Slight eye irritant; not a skin irritant.

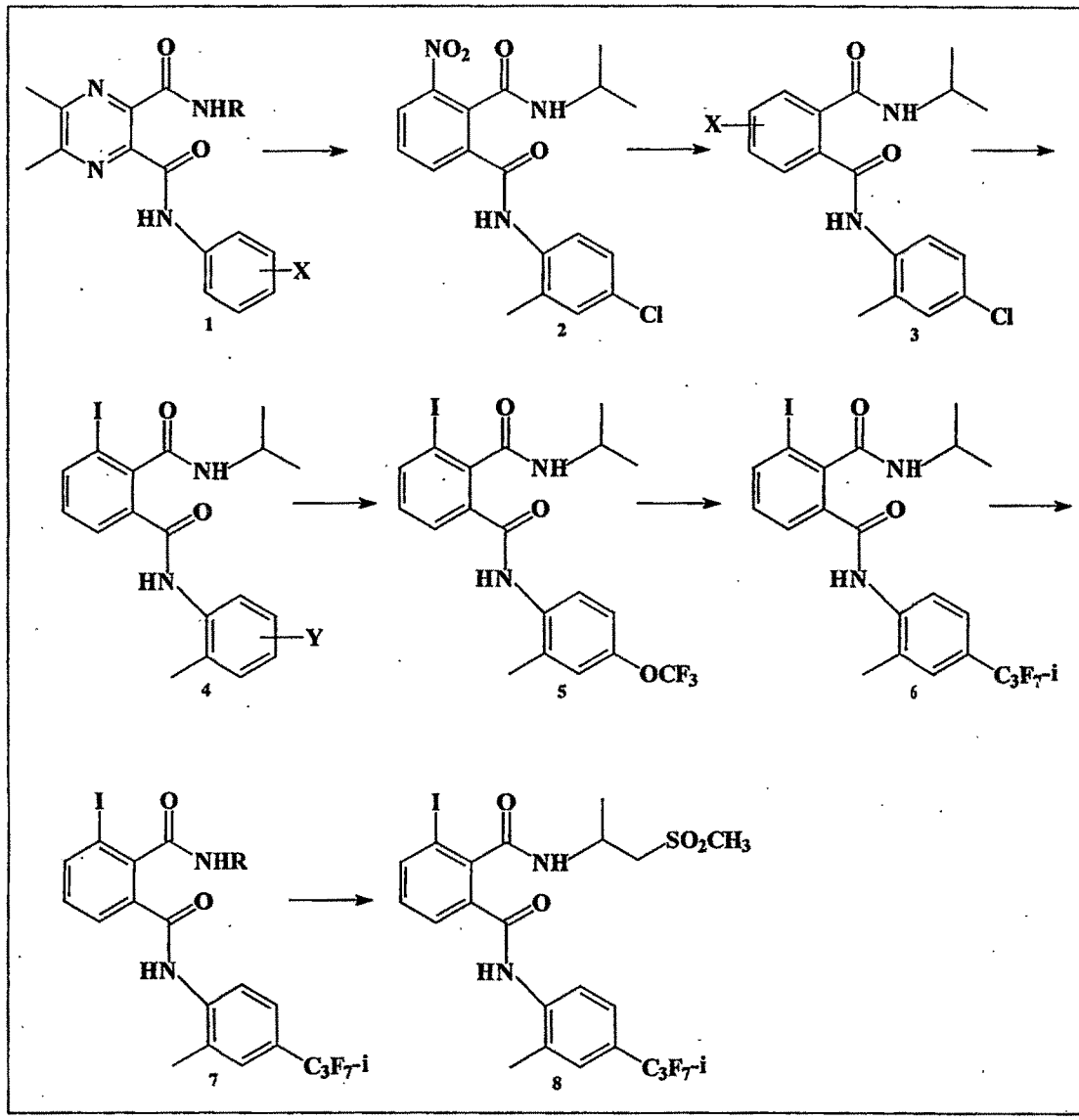
## 2.2 Chemistry of Flubendiamide

### 2.2.1 Discovery

In 1993, during a synthetic research program on pyrazinedicarboxamide herbicides 1, the benzenedicarboxamide analogue 2 having a nitro group at the 3-position was found to show an insecticidal activity against lepidopteran insects (Scheme 1). Although the level of activity of the lead compound was low, both the novelty of the chemical structure and intriguing insecticidal symptoms were enough to prompt the researchers to make further efforts on the insecticide research. Optimization of the substituent X at the phthaloyl moiety of compound 3 led to the result that a compound with a halogen atom substituted at the 3-position showed good activity, with iodine providing the best.

The substituent Y at the aniline moiety of compound 4 was investigated next. Compounds having a lipophilic substituent at the 4-position such as 5 were highly active. Although it was very challenging to introduce a heptafluoroisopropyl group into crop protection chemistry, a synthetic chemist dared to synthesize such an unusual perfluoroalkyl- substituted anilide derivative to improve the activity. The result was beyond his expectation and compound 6 showed a very high activity.

To improve the activity even further the substituent R at the aliphatic amide moiety of compound 7 was investigated. The introduction of some kind of heteroatom or functional group into this moiety increased the activity. Finally, the fine tuning of the substituent led to the synthesis of Flubendiamide 8 in 1998 (Tohnishi *et al.*, 2000).




Scheme 1: Discovery of flubendiamide

### 2.2.2 Synthesis

The synthetic pathway of flubendiamide (Figure 1) was reported by Tohnishi *et al.*, (2005), and Lei & Jiaanbeng (2006). 3-Iodo phthalic anhydride 4 was prepared from commercially available 3-nitrophthalic acid according to methods described in the literature. Phthalamic acid 6 was obtained by the reaction of phthalic anhydride 4 with thioalkylamine 5 with high regioselectivity. Phthalamic acid 6 was treated with trifluoroacetic anhydride to give isoimide 7, which was reacted with aniline derivative 8 to afford diamide 9 as described previously. Flubendiamide was obtained by the oxidation of diamide 9 with *m*-chloroperbenzoic acid (MCPBA) or hydrogen peroxide.

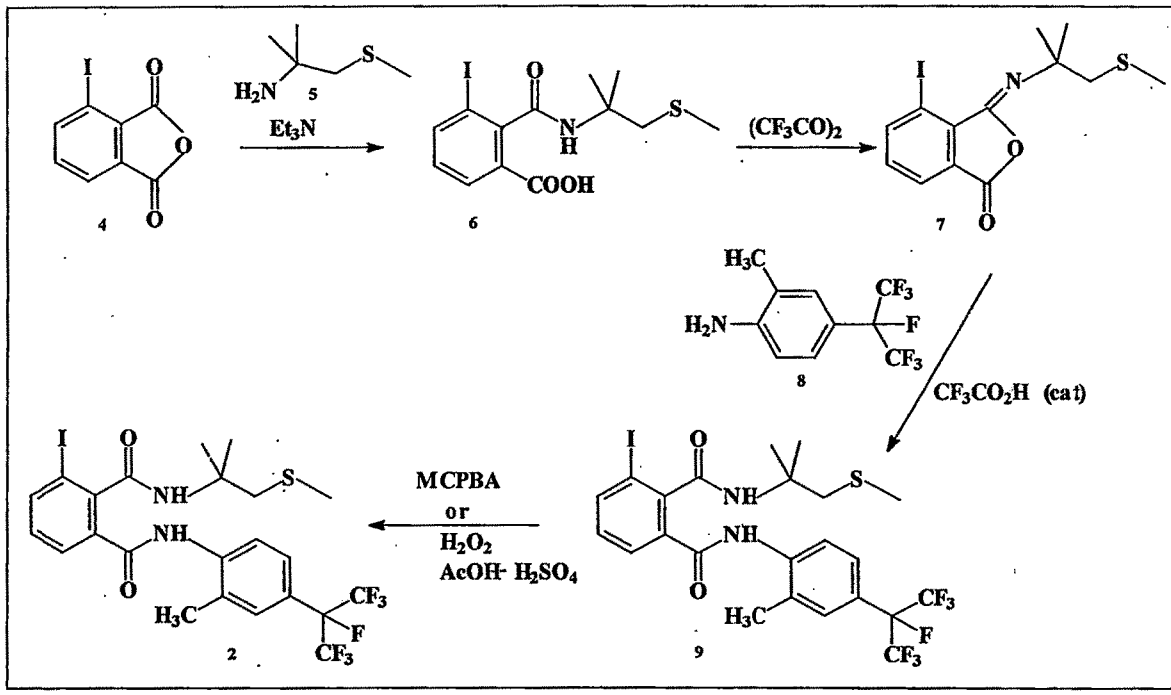



Figure 1: Synthetic pathway of flubendimaide

### 2.2.3 Structure–Activity Relationship

Flubendiamide (Figure 2) is a novel insecticide belongs to a new chemical class named benzenedicarboxamide or phthalic acid diamide, discovered by Nihon Nohyaku (Tohnishi *et al.*, 2005; Hamaguchi & Hirooka, 2007) and developed jointly with Bayer Crop Science (Nishimatsu *et al.*, 2005; Shane *et al.*, 2006). Its unique chemical structure is characterized by a three-part chemical structure (Figure 3): (1) a phthaloyl moiety (X), (2) an aromatic amide moiety (Y) and (3) an aliphatic amide moiety ($R_1$, $R_2$) (Hirooka *et al.*, 2007; Seo *et al.*, 2006; Tsubata *et al.*, 2007).

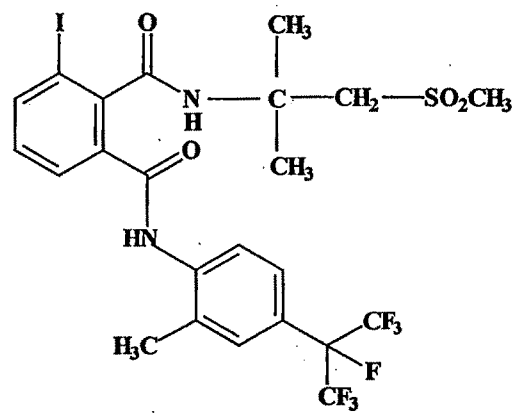


Figure 2: Structure of flubendiamide

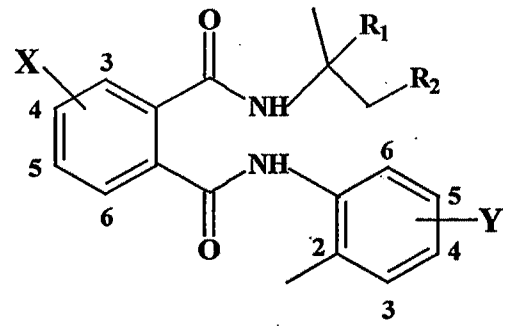



Figure 3: Three part chemical structure of flubendiamide

It was reported that an iodine atom at the 3-position was the best substituent for the phthaloyl moiety, although compounds having an iodine atom are very rare among commercial agrochemicals. The introduction of heptafluoroisopropyl group is very novel since it has never been reported as a substituent in the chemical structure of commercial pesticides so far and seldom used in pesticide research. Moreover, the introduction of a hetero atom especially a sulfur atom onto the alkyl side chain markedly increased the insecticidal activity. This sulfonylalkylamine moiety is also novel as an amine in pesticide chemistry. Flubendiamide has these unique substituents as essential parts of the structure in three adjacent positions on the benzene ring, which characterizes the chemical structure of flubendiamide as totally new.

### 2.2.4 Mode of action

Eisberg (2005) reported that flubendiamide affects the calcium signalling crucial for movement that is controlled by the receptor. The new insecticides works by first paralyzing and eventually killing the pest. The tests revealed high activity against the tobacco budworm which is responsible for damaging cotton, tobacco, and other crops.

Masaki *et al.*, (2006) reported that the flubendiamide, is a novel activator of ryanodine-sensitive calcium release channels (ryanodine receptors, RyRs), and is known to stabilize insect RyRs in an open state in a species-specific manner and to desensitize the calcium dependence of channel activity. In their study, using flubendiamide as an experimental tool, examined an impact of functional modulation of RyR on $Ca^{2+}$ pump. Strikingly, flubendiamide induced a fourfold stimulation of the $Ca^{2+}$-pump activity ($EC_{50}$ = 11 nM) of an insect that resequesters $Ca^{2+}$ to intracellular stores, a greater increase than with the classical RyR modulators, ryanodine and caffeine. This prominent stimulation, which implies tight functional coupling of $Ca^{2+}$ release with $Ca^{2+}$ pump, resulted in a marginal net

increase in the outer vesicular calcium concentration despite robust $Ca^{2+}$ release from the intracellular stores by flubendiamide. Further analysis suggested that luminal $Ca^{2+}$ is an important mediator for the functional co-ordination of RyRs and $Ca^{2+}$ pumps. However, kinetic factors for $Ca^{2+}$ pumps, including adenosine tri phosphate (ATP) and cytoplasmic $Ca^{2+}$, failed to affect the $Ca^{2+}$-pump stimulation by flubendiamide. Therefore, to conclude that the stimulation of $Ca^{2+}$ pump by flubendiamide is mediated by the decrease in luminal calcium, which may induce calcium dissociation from the luminal $Ca^{2+}$ binding site on the $Ca^{2+}$ pump. This mechanism should play an essential role in precise control of intracellular $Ca^{2+}$ homeostasis.

Ebbinghaus *et al.*, (2006) showed that phthalic acid diamides activate ryanodine-sensitive intracellular calcium release channels (ryanodine receptors, RyR) in insects. With $Ca^{2+}$ measurements, proved that flubendiamide and related compounds induced ryanodine-sensitive cytosolic calcium transients that were independent of the extracellular calcium concentration in isolated neurons from the pest insect *Heliothis virescens* as well as in transfected CHO cells expressing the ryanodine receptor from *Drosophila melanogaster*. Binding studies on microtome membranes from *Heliothis* flight muscles revealed that flubendiamide and related compounds interacted with a site distinct from the ryanodine binding site and disrupted the calcium regulation of ryanodine binding by an allosteric mechanism. This novel insecticide mode of action seems to be restricted to specific RyR subtypes because the phthalic acid diamides reported here had almost no effect on mammalian type 1 ryanodine receptors.

Shane *et al.*, (2006) reported that flubendiamide is a new novel biochemical mode of action and exhibits excellent larvicidal activity as an orally ingested toxicant by targeting and disrupting the $Ca^{2+}$ balance.

Lummen *et al.*, (2007) observed that phthalic acid diamides increase the ryanodine binding affinity while not changing the number of high-affinity binding sites. Furthermore, flubendiamide interferes with the calcium regulation of ryanodine binding. The flubendiamide binding site has been found to be distinct from the ryanodine binding site. In addition, flubendiamide binding itself is regulated by the calcium concentration.

Ohkawa *et al.*, (2007) reported that the flubendiamide is most effective on larvae followed by adults, but it has no ovicidal activity. In the course of extensive research on mode of action of flubendiamide, it was determined that flubendiamide was a ryanodine receptor modulator. Flubendiamide fixes the Ca-channel of insect ryanodine receptors

(RyR) in the open state, and subsequently induces calcium release from the membrane vesicle. In parallel, the RyR activation by flubendiamide induces the stimulation of the Ca-pump via functional connection between these two components. It is suggested that the effect of flubendiamide on intracellular calcium regulation is essential for the insecticidal activity. Furthermore, flubendiamide shows very little effect on the mammalian RyR isoform. This comparative study concluded that flubendiamide specifically activates insect RyR. By the binding assay using $^{3}$H-flubendiamide, it was confirmed that the binding site was specific to insect RyR and its binding site was different from those of other RyR modulators such as ryanodine. Finally, it is known that the binding site of ryanodine is located at a pore region of the RyR. Finally, it was concluded that the selective action of flubendiamide is due to the specificity of the binding site.

## 2.3 Biological profiles and features

### 2.3.1. Birds and mammals

Hall (2007) reported that the flubendiamide is practically non-toxic to birds (bobwhite quail or mallard duck, Table 1) on an acute basis (acute $LD_{50}$ > 2,000 mg a.i./kg body weight). In the mallard reproduction study some low level effects were observed in 14-day hatchling and egg shell quality at the highest level, resulting in an NOAEC of 289 ppm. However all predicted environmental exposures are well below any of the observed effect levels, thus indicating no risk for birds. The acute oral toxicity of flubendiamide to wild mammals was assessed using the rat as a surrogate animal. The rat $LD_{50}$ was > 2000 mg a.i./kg bw (Table 1), with no effects observed in the limit dose test; therefore the compound poses no acute risk to wild mammals. Under environmentally realistic exposure scenarios for a chronic assessment the potential exposure of flubendiamide to wild mammals is below the level that would produce the effect, thus indicating a high safety margin for wild mammals.

Table 1: Ecotoxicologically relevant endpoints for birds and mammals

| Test species | Test regime | Endpoint | |
|---|---|---|---|
| Birds | | | |
| Bobwhite quail | a.i., acute oral, 14 d | $LD_{50}$ | > 2,000 mg a.i./kg bw |
| Bobwhite quail | SC 480, acute oral, 14d | $LD_{50}$ | > 2,000 mg form/kg bw (>796 mg a.i./kg bw) |
| Bobwhite quail | a.i., acute dietary, 5 d dietary | $LC_{50}$ | > 5,000 mg a.i./kg diet |
| Mallard duck | a.i., acute dietary, 5 d dietary | $LC_{50}$ | > 4,535 mg a.i./kg diet |
| Bobwhite quail | a.i., chronic dietary, 23 w (reproduction) | NOAEC | 1,059 mg a.i./kg diet<br>78 mg a.i./kg bw × day |
| Mallard duck | a.i., chronic dietary, 20 w (reproduction) | NOAEC | 289 mg a.i./kg diet |
| Mammals | | | |
| Rat | a.i., acute, oral | $LD_{50}$ | > 2,000 mg a.i./kg bw |
| Rat | a.i., chronic, 1-generation, dietary | NOAEL | 200 ppm<br>18 mg a.i./kg bw × day |
| Mouse | a.i., chronic, 28-day, dietary | NOEL | 1000 mg a.i./kg bw × day |

### 2.3.2 Fish, aquatic invertebrates, and aquatic plants

Hall (2007) mentioned that the flubendiamide had no acute effect at the limits of water solubility on freshwater fish (Table 2). A fish bioaccumulation test was conducted based on log $K_{OW}$ = 4.2 and indicated that flubendiamide poses no risk to fish. The low bioconcentration factor of 66, demonstrates that flubendiamide poses no risk to birds or mammals from secondary poisonings.

Table 2: Aquatic ecotoxicological endpoints and its primary metabolite

| Test species | Test regime | Endpoint | |
|---|---|---|---|
| Rainbow trout | a.i., acute, static, 96 h | $LC_{50}$ | >60.0 µg a.i./L |
| Bluegill sunfish | a.i., acute, static, 96 h | $LC_{50}$ | >67.7 µg a.i./L |
| Fathead minnow | a.i., acute, static, 96 h | $LC_{50}$ | >66.5 µg a.i./L |
| Common carp | a.i., acute, static, 96 h | $LC_{50}$ | >84.7 µg a.i./L |
| Rainbow trout | SC 480, acute, static,96h | $LC_{50}$ | > 250,000 µg form/L<br>> 100,000 µg a.i./L |
| Bluegill sunfish | SC 480, acute, static, 96h | $LC_{50}$ | > 250.000 µg form/L<br>> 100,000 µg a.i./L |
| Fathead minnow | a.i., chronic early life stage, flow-through, 35d | NOAEC | ≥ 60.2 µg a.i./L |
| Fathead minnow | a.i., chronic fish life cycle, flow through, 260d | NOAEC | ≥ 49.0 µg a.i./L |
| Bluegill sunfish | a.i., bioaccumulation, flow-through, 42 d | BCF | 73 parent, whole fish<br>66 parent, , whole fish, normalized to 6 % lipid content |
| Bluegill sunfish | NNI-0001-des-iodo bioaccumulation, flow-through, 42 d | BCF | 12.6 metabolite, whole fish<br>10.2 metabolite, whole fish, normalized to 6 % lipid content |

Aquatic invertebrates were more sensitive to flubendiamide than the vertebrates or plants. Flubendiamide had no acute effects on *Daphnia magna* ($EC_{50}$ > 60 µg a.i./L, Table 3). A higher tier mesocosm test was conducted with flubendiamide SC 480 to assess the potential effects of flubendiamide on aquatic systems under more environmentally realistic conditions. Direct and indirect effects of the application of flubendiamide SC 480 to the chemical and physical parameters of the pond water were not observed at any test concentration. Also no effects on the coverage of the ponds and the biomass of macrophytes and filamentous algae were observed at any treatment level. No direct toxic effects were observed on the phyto plankton. At the end of the study, no

zoo plankton taxon showed significant differences in abundance compared to controls. *Daphnia longi spina* was the most sensitive zooplankton taxon with effects observed at 2.3 µg a.i./L during the first 7 days after application and at 5.3 µg a.i./L until 4 weeks after application. After 5 weeks, only the highest treatment level (12.0 µg a.i./L) showed significantly lower abundances.

Table 3: Aquatic invertebrates and plants

| Test species | Test regime | Endpoint | |
|---|---|---|---|
| Waterflea | a.i., acute, static, 48 h | $EC_{50}$ | > 60.0 µg a.i./L |
| Waterflea | SC 480, acute, static, 48 h | $EC_{50}$ | 6.5 µg form/L<br>2.6 µg a.i./L |
| Waterflea | WG 24, acute, static, 48 h | $EC_{50}$ | 6.3 µg form/L<br>1.48 µg a.i./L |
| Waterflea | NNI-0001-des-iodo, acute, static, 48 h | $EC_{50}$ | 881 µg p.m./L<br>p.m. = pure metabolite |
| Waterflea (with different algae densities) | SC 480, acute, static, 48 h | $EC_{50}$ | 10.8 µg form/L (4.2 µg a.i./L) with no algae present<br>> 32 µg form/L (12.5 µg a.i./L) with $10^6$ algal cell/mL |
| Waterflea | a.i., chronic, semi-static, 21d | NOAEC | 33.3 µg a.i./L |
| Waterflea | SC 480, chronic, semi-static, 21 d | NOAEC | 1.0 µg form/L<br>0.4 µg a.i./L |
| Midge | SC 480, acute, static, 24 h | $LC_{50}$ | 5.91 mg form/L<br>2.35 mg a.i./L |
| Midge | WG 24, acute, static, 24 h | $LC_{50}$ | 0.825 mg form/L<br>0.198 mg a.i./L |
| Midge | a.i., chronic, static, spiked water, 28 d | $EC_{50}$<br>$EC_{15}$<br>NOAEC | 59 µg a.i./L<br>45 µg a.i./L<br>≥ 40 µg a.i./L |
| Midge | NNI-0001-des-iodo,chronic, static, spiked water, 28 d | $EC_{50}$<br>$EC_{15}$<br>NOAEC | 18.6 µg p.m./L<br>9 µg p.m./L<br>4 µg p.m./L |

| Test species | Test regime | Endpoint | |
|---|---|---|---|
| Green algae | a.i., chronic, static, 96 h | $ErC_{50}$ | > 69.3 µg a.i./L |
| Green algae | SC 480, chronic, static, 96 h | $ErC_{50}$ | > 251 µg form/L |
| Duckweed | a.i., chronic, static, 7 d | $ErC_{50}$ | > 54.6 µg a.i./L |
| Mesocosm, outdoor ponds | a.i.,chronic,1 application at 0.4 to 12.3 µg a.i./ha, 16 w | NOEAEC | 5.3 µg a.i./L |

### 2.3.3. Marine organisms

Hall (2007) reported that flubendiamide has no acute or chronic risk for marine/estuarine organisms.

### 2.3.4. Soil organisms and Terrestrial plants

Hall (2007) reported that the formulated flubendiamide products (SC 480 and WG 24) and the primary metabolite NNI-0001-des-iodo has no acute toxicity to earthworms. It poses minimal risk for non-target terrestrial plants (Table 4). Overall, it has a highly favourable ecological profile and can be used effectively in an IPM program.

Table 4: Ecotoxicological endpoints of flubendiamide for terrestrial plants

| Test species | Test regime | Endpoint | |
|---|---|---|---|
| Soil organism | | | |
| Earthworm (*Eisenia fetida*) | SC 480, acute, 14 d | $LC_{50}$ | > 1,000 mg/kg d.wt.s equal to > 388 mg a.i./kg d.wt.s. |
| Earthworm (*Eisenia fetida*) | NNI-0001-des-iodo acute, 14 d | $LC_{50}$ | LC50 > 1,000 mg Metabolite / kg d.wt.s. |
| Terrestrial plants | | | |
| Corn, onion, grass and oat, turnip, lettuce, cucumber, cabbage, soybean, and tomato | SC 480 seedling emergence and vegetative vigor, 21-d | $EC_{25}$<br>NOAEC<br><br>$EC_{25}$<br>NOAEC | Seedling emergence<br>>534 g a.i./ha<br>534 g a.i./ha<br>Vegetative vigor<br>>534 g a.i./ha<br>>534 g a.i./ha |

| Test species | Test regime | Endpoint | |
|---|---|---|---|
| Corn, oats, cucumber, oilseed rape, soybean and sunflower | 24 WG vegetative vigor screening assay, non-GLP, 21-d | | No effects > 25 % observed on any species for any of the endpoints up to180 g a.i./ha. |
| Corn, oats, cucumber, oilseed rape, soybean and, sunflower | WG 24 180 g a.i./ha seedling emergence screening assay, non-GLP14-d. | | No effects > 25 % observed for any endpoint up to180 g a.i./ha with the exception of sunflower percent emergence. |
| Sunflower | WG 24, seedling emergence, GLP | $EC_{25}$ NOAEC | >180 g a.i./ha 180 g a.i./ha |

Shane *et al.*, (2006) reported that flubendiamide has a favorable ecological, ecotoxicological and environmental profile with low acute mammalian toxicity and no genotoxic, mutagenic or oncogenic properties noted.

Ohkawa *et al.*, (2007) reported that the insecticidal spectrum of flubendiamide is broad among Lepidoptera pests in agriculture (Table 5). Against the resistant strain of diamond back moth, flubendiamide provided the same level of activity as against the susceptible strain. This result indicates that flubendiamide will be useful for insecticide resistance management (IRM) programs. It is also very safe for natural enemies and consequently fit well into integrated pest management (IPM) programs. Field evaluation on vegetables, top fruits, and cotton revealed that it is effective in controlling the major lepidopterous pests at the recommended dose and its efficacy was better than those of standard insecticides. Furthermore, flubendiamide (20% WDG) showed no phytotoxicity to vegetables, tea and top fruits at recommended doses.

Table 5: Insecticidal spectrum of flubendiamide

| Scientific name | Common name | Tested stage | DAT | $EC_{50}$ (mg a.i./L) |
|---|---|---|---|---|
| *Plutella xylostella* | Diamond back Moth | L3 | 4 | 0.004 |
| | (Resistant strain)* | L3 | 4 | 0.002 |
| *Spodoptera litura* | Common cutworm | L3 | 4 | 0.19 |
| *Helicoverpa armigera* | Cotton bollworm | L3 | 4 | 0.24 |
| *Agrotis segetum* | Turnip moth | L2-3 | 7 | 0.18 |
| *Autographa nigrisgna* | Beet semi- looper | L3 | 4 | 0.02 |
| *Pieris rapae crucivora* | Common cabbage worm | L2-3 | 4 | 0.03 |
| *Adoxophyes honmai* | Smaller tea tortrix | L3 | 5 | 0.38 |
| *Homona magnanima* | Oriental tea tortrix | L4 | 5 | 0.58 |
| *Hellula undalis* | Cabbage webworm | L3 | 5 | 0.01 |
| *Chilo suppressalis* | Rice stem borer | L3 | 7 | 0.01 |
| *Diaphania indica* | Cotton caterpillar | L3 | 3 | 0.02 |
| *Sitophilus zeamais* | Maize weevil | A | 4 | >1000 |
| *Nilaparvata lugens* | Brown rice planthopper | 1.3 | 4 | >1000 |
| *Myzus percicae* | Green peach aphid | All | 7 | >1000 |
| *Pseudococcus comstocki* | Comstock mealy bug | L1 | 7 | >1000 |
| *Tetranychus urticae* | Two spotted spider mite | All | 4 | >1000 |

L2, L3, A: second, third and Adult, DAT: Days after treatment,*resistant strains to pyrethroids, BPUs, Ops and Carbamates.

## 2.4 Field Study and Bio-efficacy

Javaregowda (2005) conducted a field experiment in upland paddy to evaluate the bioefficacy of flubendiamide 20 WDG (RIL-038) against Rice stem borer, *Scirpophaga incertulas* (Wlk.) at Agricultural Research Station, Sirsi, Karnataka during summer 2004. The results revealed that Flubendiamide 20WDG at 25 g a.i./ha is promising for the effective management of Rice stem borer.

Javaregowda and Naik (2005) conducted a field study in upland paddy with flubendiamide 20 WDG (RIL-038) against Paddy pests and their natural enemies at

Agricultural Research Station, Sirsi, Karnataka, during kharif 2003 and summer 2004 season. The results revealed that it is an effective chemical for the management of paddy stem borer, leaf folder and safe for the natural enemies.

Tomar *et al.*, (2005) conducted two years of field experiments to evaluate the bioefficacy of flubendiamide 20 WDG (RIL-038) at 12.5, 25, 50, 60 and 100 g a.i./ha, lamda-cyhalothrin 5% EC at 40 g a.i./ha, spinosad 45% SC at 75 g a.i./ha and indoxacarb 14.5% SC at 75 g a.i./ha against cotton bollworms during kharif 2003/04 and 2004/05 in Khandwa, Madhya Pradesh, India. Flubendiamide 20 WDG at all doses significantly reduced bollworm complex infestation over untreated control and also registered higher seed cotton yield during both years. Flubendiamide 20 WDG at 50 g a.i./ha was found to be highly effective in minimizing the bollworm damage and increasing the yield of seed cotton. Based on the results, it was suggested that flubendiamide 20 WDG at 50 g a. i./ha could be considered as the optimum dose for controlling cotton bollworms.

Tohnishi *et al.*, (2005) reported that flubendiamide (proposed ISO name, internal code NNI-0001) is a novel class insecticide, which acts through a new biochemical mode of action in lepidopterous insect pests. After foliar application, flubendiamide has excellent fast-acting and residual activity against a broad spectrum of lepidopterous insect pests, such as *Helicoverpa* spp., *Spodoptera* spp., *Plutella* spp. and *Agrotis* spp., including resistant strains of them as well. Flubendiamide has a very low toxicity against beneficial arthropods. Flubendiamide will be an essential tool for the control of important lepidopterous insect pests and be compatible with IPM and insect resistance management programs.

Tohnishi *et al.*, (2005) found that flubendiamide was highly active against the nine important lepidopteran pests with $EC_{50}$ values of between 0.004 & 0.58 mg $L^{-1}$ (Table 6).

Table 6: Efficacy of flubendiamide against Lepidopteran insect pests

| *Scientific name* | Host plant | Instar | $EC_{50}$ (mg a.i. $L^{-1}$) |
|---|---|---|---|
| *Plutella xylostella* (Linnaeus) | Cabbage leaf | III | 0.004 |
| *Spodoptera litura* (Fabricius) | Cabbage leaf | III | 0.19 |
| *Autographa nigrisigna* (Walker) | Cabbage leaf | III | 0.02 |
| *Agrotis segetum* (Dennis) | Cabbage leaf | II-III | 0.18 |
| *Pieris rapae crucivora* (Linnaeus) | Cabbage leaf | II-III | 0.03 |
| *Hellula undalis* (Fabricius) | Cabbage leaf | III | 0.01 |
| *Chilo suppressalis* (Walker) | Rice seedling | III | < 0.01 |
| *Adoxophyes honmai* (Yasuda) | Tea leaf | IV | 0.38 |
| *Homona magnanima* (Diakonoff) | Tea leaf | IV | 0.58 |

Lakshminarayana and Rajasri (2006) observed that flubendiamide @ 50 g ai/ha was effective against American bollworm (*Helicoverpa armigera*). No phytotoxicity symptoms were observed with Flubendiamide to the cotton plant even at higher dose of 100 g a.i./ha.

Shane *et al.*, (2006) observed that flubendiamide has a rapid cessation of feeding and extended residual control, providing superior plant protection against a broad-range of economically important lepidopteran pests, including *Helicoverpa* spp., *Heliothis* spp., *Spodoptera* spp., *Plutella* spp., *Pseudoplusia* spp., *Trichoplusia* spp., and *Agrotis* spp. As a new mode of action, flubendiamide exhibits no cross-resistance to conventional chemistries. They also found that it will fit in Integrated Pest (IPM) and Insecticide Resistance Management (IRM) programs in a variety of crops because of the many favorable characteristics, including selective activity against a broad range of lepidopteran pests, a new mode of action, safety to pollinators/beneficials, a favorable environmental and ecological (low toxicity) profile, short REI/PHI, and low use rates for less environmental loading.

Palrang and Hand (2007) reported that flubendiamide causes rapid cessation of feeding and extended residual control, providing superior plant protection against a broad-range of economically important lepidopteran pests, including *Helicoverpa* spp., *Heliothis* spp., *Spodoptera* spp., *Plutella* spp., *Pseudoplusia* spp., *Trichoplusia* spp., and *Agrotis* spp.

Hirooka *et al.*, (2007) evaluated the dose rates of Phoenix WG (20 % flubendiamide) and found that it is effective in controlling lepidopterous pests on vegetables and tea @100 mg a.i./L and 50 mg a.i./L on pome fruit at Japan . In other countries the dose rates are 50 g a.i./ha on vegetables and cotton and 25 g a.i./ha on rice. Registrations have been achieved in Japan (Phoenix) and Thailand (Takumi). Further registrations are expected in India, China, Korea and Taiwan until 2008.

White *et al.*, (2007) reported that BELT® and SYNAPSE® are two new products containing the active ingredient, flubendiamide, for broad-spectrum Lepidoptera pest control on perennial and annual crops. Flubendiamide is the first member of a new chemical class, the phthalic acid diamides with a novel mode of action; disruption of cellular calcium balance. Upon ingestion, flubendiamide produces a rapid cessation of insect feeding, muscle contraction and paralysis. As a new mode of action, flubendiamide exhibits no cross-resistance to current commercial insecticides. Formulated commercial products, BELT and SYNAPSE provide excellent pest knock down, extended residual control and superior plant protection. Studies have shown that BELT and SYNAPSE have favorable toxicological, eco-toxicological and environmental fate profiles. BELT and SYNAPSE should have an excellent fit in Integrated Pest Management (IPM) and Insecticide Resistance Management (IRM) programs due to their new mode of action, low use rates, selective activity against Lepidoptera pests and safety to beneficial organisms including pollinators.

## 2.5 Metabolism in plants

### 2.5.1 Flubendiamide behaviour in plants

The metabolic behaviour of flubendiamide in plants (cabbage, tomato, apple and corn) was studied by Justus *et al.*, (2007) (Figure 4). They reported that NNI-0001-benzylalcohol and NNI-0001-benzoic acid were formed by stepwise oxidation of the methyl group at the aniline ring of flubendiamide. The NNI-0001-des-iodo and NNI-0001-3-OH were formed by des-iodination followed by hydroxylation at the phthalic acid ring of flubendiamide. The NNI-0001-des-anilino and NNI-0001-3-iodo-phthalic acid were detectable as label-specific metabolites of the phthalic acid ring label. Moreover, they also studied the metabolic behaviour in target plants and in confined rotational crops. Two additional label-specific metabolites of the phthalic acid ring label were detectable in the confined rotational crop [spring wheat (small grain), Swiss chard (leafy vegetable) and turnips (root vegetable)] from three consecutive rotations (Figure 5).

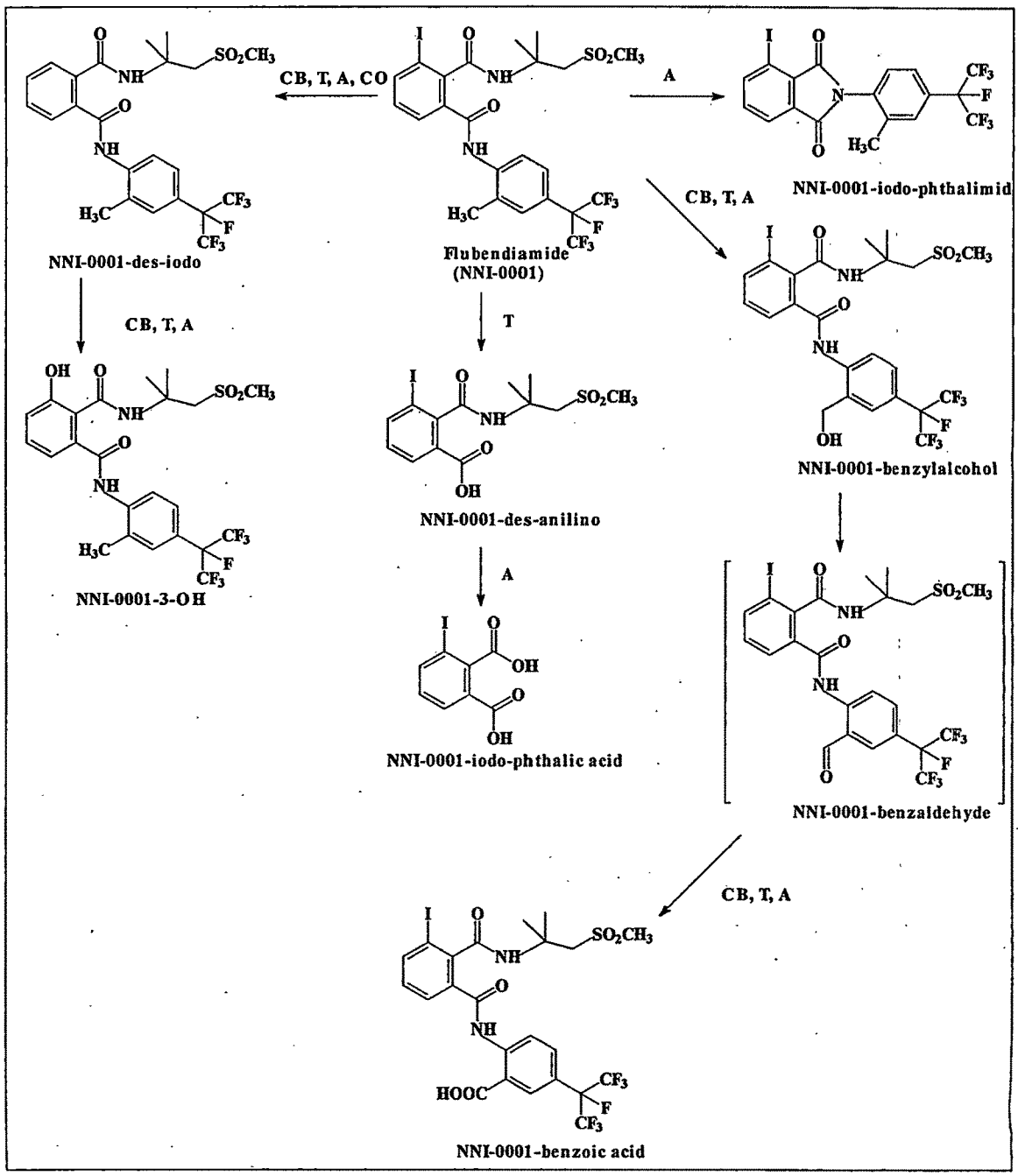



Figure 4: Metabolic pathways of flubendiamide in plants [cabbage, tomato, apple, corn)

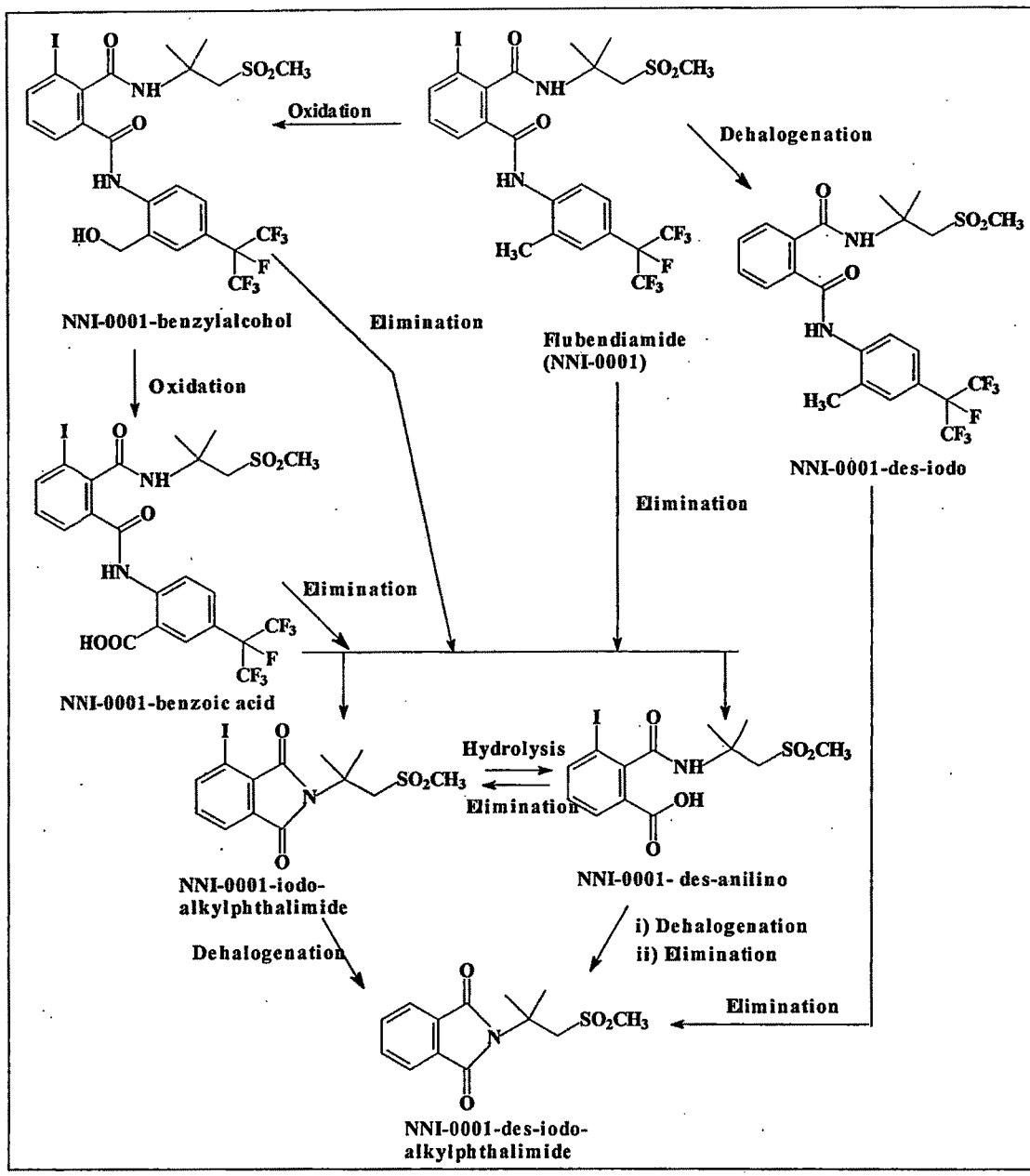



Figure 5: Metabolic pathway of flubendiamide in confined rotational crops

## 2.6 Residue analytical method

Anastassiades *et al.*, (2002) presented that streamlined and effective extraction and cleanup approach for the analysis of diverse pesticide residues in fruits and vegetables has been developed. We call the novel approach "QuEChERS" (pronounced like "catchers"), which stands for quick, easy, cheap, effective, rugged, and safe. The procedure first involves an extraction by vortexing 10 g chopped food sample with 10 mL acetonitrile (MeCN) for 1 min in a 40 mL Teflon centrifuge tube. Then, 4 g anhydrous $MgSO_4$ and 1 g NaCl are added to the extract which is vortexed another minute. This procedure and combination of salts forces a wide range of pesticides to partition into the upper MeCN phase. In the method development, NMR was used to measure the amount of water in the upper phase, which was correlated with pesticide recoveries to optimize the amount and type of salts to add to the initial extract. In actuality, the amount of MeCN in the lower aqueous layer was more important than the amount of water in the upper layer to achieving high recoveries. $MgSO_4$ gave the most complete liquid-liquid phase separation among the several salts evaluated, thus yielded the highest pesticide recoveries, particularly for the most polar GC-amenable ones, methamidophos, acephate and omethoate. Triphenylphosphate (TPP) internal standard is added at this point in the method to provide more valid and precise results. A 1 mL aliquot of the upper phase is then placed into a 2 mL mini-centrifuge tube and removal of residual water and cleanup are performed simultaneously using a simple procedure that we call "dispersive-SPE." In this case, dispersive-SPE entails vortexing 25 mg primary secondary amine (PSA) sorbent plus 150 mg anhydrous $MgSO_4$ with the 1 mL extract for 30 s. The PSA does not retain the pesticides, but it effectively removes many polar interfering compounds from the food extracts such as organic acids and sugars. The extracts are then centrifuged at high speed for 1 min and the extract is placed into an autosampler vial for analysis. The only piece of "glassware" needing to be cleaned for re-use is the 40 mL Teflon centrifuge tube. GC/MS is utilized to perform quantitative and confirmatory analysis of GC-amenable pesticides. Recoveries mostly above 90% and repeatabilities typically less than 5% have been achieved in different fruits and vegetables for many fortified pesticides tested including very polar and basic compounds such as methamidophos, acephate, omethoate, imazalil and thiabendazole. Further experiments are being conducted to transfer the QuEChERS approach to regulatory laboratories for routine analysis and initial results are very promising. Using this method for a 10 g fruit or vegetable sample, a single chemist can

prepare a batch of 6 previously chopped samples in approximately 20 min with $1 of materials per sample. Detection limits depend on injection volumes, type of instrument, separation conditions and other factors, but if needed the sample: MeCN ratio can be increased by two to further concentrate the final extract without a solvent evaporation step.

Schenck *et al.*, (2002) reported that water-miscible solvents, such as acetone and acetonitrile, effectively extract both polar and nonpolar pesticide residues from nonfatty foods. The addition of sodium chloride to the resulting acetonitrile-water or acetone-water extract (salting out) results in the separation of the water from the organic solvent. However, the organic solvent layer (pesticide extract) still contains some residual water, which can adversely affect separation procedures that follow, such as solid-phase extraction and/or gas chromatography. Drying agents, such as sodium sulfate or magnesium sulfate, are used to remove the water from the organic extracts. In the present study, we used nuclear magnetic resonance spectroscopy to study the composition of the phases resulting from salting out and to compare the effectiveness of sodium sulfate and magnesium sulfate as drying agents. The study showed that considerable amounts of water remained in the organic phase after phase separation. Sodium sulfate was a relatively ineffective drying agent, removing little or no residual water from the organic solvent. Magnesium sulfate proved to be a much more effective drying agent.

Anastassiades *et al.*, (2003) carried out a simple, fast, and inexpensive method for the determination of pesticide residues in fruits and vegetables which involve initial single-phase extraction of 10 g sample with 10 mL acetonitrile followed by liquid–liquid partitioning formed by addition of 4 g anhydrous $MgSO_4$ plus 1 g NaCl. Removal of residual water and cleanup are performed simultaneously by using a rapid procedure called dispersive solid-phase extraction (dispersive-SPE), in which 150 mg anhydrous $MgSO_4$ and 25 mg primary secondary amine (PSA) sorbent are simply mixed with 1 mL acetonitrile extract. The dispersive-SPE with PSA effectively removes many polar matrix components, such as organic acids, certain polar pigments, and sugars, to some extent from the food extracts. Recoveries between 85 and 101% (mostly >95%) and repeatabilities typically < 5% have been achieved for a wide range of fortified pesticides, including very polar and basic compounds such as methamidophos, acephate, dimethoate, imazalil, and thiabendazole.

Schenck and Hobbs (2004) performed analysis on 11 fruits and vegetable samples of peach, green beans, snow peas, pepper, cabbage and apple containing incurred pesticide (including azinphos-methyl, carbaryl, dimethoate, methamidophos, phosmet, chlorpyrifos, omethoate and endosulfan) residues using QuEChERS method. The method entails extracting the pesticide residues from 10 g of sample by vortex mixing with 10 ml of acetonitrile. Water is removed from the extract by salting out with sodium chloride and magnesium sulfate, and a subsequent cleanup of the acetonitrile extract is performed by vortexing an aliquot of the extract with a small quantity of solid phase extraction sorbent.

Okihashi *et al.*, (2005) established for the determination of 180 pesticide residues in fruits and vegetables. The procedure involved extraction with acetonitrile, followed by a salting-out step with anhydrous $MgSO_4$ and NaCl. Removal of sediment and water was performed simultaneously by centrifugation. Co-extractives were removed with a double-layered SPE column, and graphitized carbon black and primary secondary amine (GCB/PSA) solid phase extraction cleanup cartridge. The eluate was determined by GC/FPD and GC/MS without further cleanup. Recovery data were obtained by fortifying 9 matrices at 0.05–0.1 mg/g. Recoveries of 180 pesticides were mainly 70–110% and the relative standard deviation (RSD) was below 25%. Limits of detection ranged between 0.01 and 0.05 mg/g for tested pesticides.

Lehotay *et al.*, (2005) validation experiments were conducted of a simple, fast, and inexpensive method for the determination of 229 pesticides fortified at 10–100 ng/g in lettuce and orange matrixes. The method is known as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for pesticide residues in foods. The procedure involved the extraction of a 15 g sample with 15 mL acetonitrile, followed by a liquid–liquid partitioning step performed by adding 6 g anhydrous $MgSO_4$ plus 1.5 g NaCl. After centrifugation, the extract was decanted into a tube containing 300 mg primary secondary amine (PSA) sorbent plus 1.8 g anhydrous $MgSO_4$, which constituted a cleanup procedure called dispersive solid-phase extraction (dispersive SPE). After a second shaking and centrifugation step, the acetonitrile extract was transferred to autosampler vials for concurrent analysis by gas chromatography/mass spectrometry with an ion trap instrument and liquid chromatography/tandem mass spectrometry with a triple quadrupole instrument using electrospray ionization. Each analytical method was designed to analyze 144 pesticides, with 59 targeted by both instruments. Recoveries for all but 11 of the analytes in at least one of the matrixes were between 70–120% (90–

110% for 206 pesticides), and repeatabilities typically <10% were achieved for a wide range of fortified pesticides, including methamidophos, spinosad, imidacloprid, and imazalil. Dispersive SPE with PSA retained carboxylic acids (e.g., daminozide), and <50% recoveries were obtained for asulam, pyridate, dicofol, thiram, and chlorothalonil. Many actual samples and proficiency test samples were analyzed by the method, and the results compared favorably with those from traditional methods.

Lehotay *et al.*, (2005) modified that entails the use of buffering during extraction was made to further improve results for certain problematic pesticides (e.g., folpet, dichlofluanid, chlorothalonil, and pymetrozine) in a simple, fast, and inexpensive method for the determination of pesticides in produce. The method, known as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for pesticide residues in foods, now involves the extraction of the sample with acetonitrile (MeCN) containing 1% acetic acid (HAc) and simultaneous liquid–liquid partitioning formed by adding anhydrous $MgSO_4$ plus sodium acetate (NaAc). The extraction method is carried out by shaking a centrifuge tube which contains 1 mL of 1 % HAc in MeCN plus 0.4 g anhydrous $MgSO_4$ and 0.1 g anhydrous NaAc per g sample. The tube is then centrifuged, and a portion of the extract is transferred to a tube containing 50 mg primary secondary amine sorbent plus 150 mg anhydrous $MgSO_4$/mL of extract. After a mixing and centrifugation step, the extract is transferred to autosampler vials for concurrent analysis by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry. Independent of the original sample pH, the use of buffering during the extraction yields pH < 4 in the MeCN extract and >5 in the water phase, which increases recoveries of both acid- and base-sensitive pesticides. The method was evaluated for 32 diverse pesticides in different matrixes, and typical percent recoveries were 95 ± 10, even for some problematic pesticides. Optional solvent exchange to toluene prior to GC/MS analysis was also evaluated, showing equally good results with the benefit of lower detection limits, but at the cost of more time, material, labor, and expense.

Schenck *et al.*, (2005) reported that current methods used for the analysis of pesticide residues in foods are labor intensive and consume large volumes of solvent. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) a procedure which entails extracting the pesticides by vortexing with a 1% acetic acid/acetonitrile mix, followed by salting out with sodium acetate and magnesium sulfate, has been reported. Initially a dispersive SPE cleanup (vortexing with primary-secondary amine [PSA] SPE sorbent and

$MgSO_4$) was used. They modified the method in order to obtain a better cleanup. Two cleanups were evaluated: 1) dispersive SPE with both PSA and graphitized carbon black (GCB) sorbents, and 2) SPE column cleanup using PSA, aminopropyl, or GCB/PSA SPE columns. Recovery data was obtained for >330 pesticide residues, using gas chromatography and/or liquid chromatography with mass spectrometric and/or element specific detection. Recoveries were >80% for most of the compounds tested. When compounds were analyzed by two or more of the laboratories, recoveries were similar, even when different cleanups and detection systems were used. Using the QuEChERS method resulted in a 66-92% reduction in solvent usage and a 56-87% reduction in cost, compared to methods currently being used in our laboratories.

Anastassiades *et al.*, (2006) described that QuEChERS method is basically entails the extraction of 10 g samples with 10 mL acetonitrile followed by the addition of salts that induce a phase separation. Cleanup of the raw acetonitrile extract is performed using the 'Dispersive-SPE" approach employing PSA (primary secondary amine) sorbent. Due to its great simplicity and cost effectiveness and the fact that the final extract can be directly employed for GC- and LC-MS (/MS)-analyses, the QuEChERS method has been meanwhile readily accepted by many pesticide residue analysts around the world. Some modifications to the original QuEChERS method had to be introduced to ensure more efficient extraction of pH dependent compounds (e.g. phenoxyalcanoic acids) at high pH values, to minimize degradation of base and acid labile pesticides, and to expand the spectrum of matrices covered. Buffering with citrate salts has been introduced in the first extraction/partitioning step to adjust the pH to 5-5.5 (as a compromise value), where most acid and base labile pesticides are sufficiently stabilized and acidic pesticides still provide high recoveries. Following PSA cleanup the extract becomes alkaline. Thus, to improve stability of base-labile compounds in the final extract, a small amount of formic acid is added. Acidic pesticides are directly analyzed from the raw extract before PSA cleanup since these types of compounds tend to bind on PSA. When dealing with matrices having a high load of chlorophylls, the cleanup procedure is improved by adding GCB to the PSA at amounts, where some residual chlorophyll still remains in the final extract.

Shane *et al.*, (2006) carried out an experiment with flubendiamide and reported that it is hydrolytically stable, relatively immobile in soils, practically non-detectable in key rotated crops, mobile in the xylem following penetration into plant tissue, and exhibits strong rainfast characteristics due to the unique chemical properties.

Aysal *et al.*, (2007) modified the QuEChERS method by using ethyl acetate rather than acetonitrile for extraction in the determination of multiple pesticide residues in fruits and vegetables. Ethyl acetate is better suited than acetonitrile for gas chromatographic (GC) analysis with electron capture detection (ECD) and nitrogen-phosphorus detection (NPD). The method entailed extraction of 30 g chopped sample plus 5 g $NaHCO_3$ and 30 g anhydrous $Na_2SO_4$ with 60 mL ethyl acetate using a probe blender. After a centrifugation step, removal of residual water and cleanup were performed using dispersive solid-phase extraction (dispersive-SPE) with $MgSO_4$ and primary secondary amine (PSA) sorbent. The method was validated for 22 pesticides in tomato, apple and frozen green bean matrices spiked at 0.05, 0.5, and 5 mg/kg. Recoveries averaged 93 % for all three commodities over the validation range with a relative standard deviation of 10 % (n = 1182). Lower recoveries of dichlorvos were obtained with the method and iprodione determination was compromised in the green beans by an interfering peak. Typical limits of detection were 0.005-0.01 mg/kg with the method.

Billian (2007) described the method of residue analysis of flubendiamide (NNI-0001) and its metabolite A-1 in plant material like citrus (fruit), head cabbage (head), wheat (grain), cotton (oil), bean (bean with pod), tomato (fruit). NNI-0001 and A-1 residues are extracted from plant materials using microwave and clean-up using Chromabond XTRTM cartridges. The samples are subjected to HLPC-MS/MS analysis. The individual recovery rates for determination of NNI-0001 and A-1 in plant matrices ranged from 69 to 103 % with mean recoveries per analyte and sample material between 78 and 100 % (RSDs of up to 8.8 %). The intra-laboratory repeatability (n = 5) is given for different plant matrices at 0.01 mg/kg (LOQ).

Battu *et al.*, (2008) standardized the estimation of residues of flubendiamide and its metabolite desiodo flubendiamide in various substrates comprising cabbage, tomato, pigeon pea grain, pigeon pea straw, pigeon pea shell, chilli, and soil. The samples were extracted with acetonitrile, diluted with brine solution, and partitioned into chloroform, dried over anhydrous sodium sulfate, and treated with 500 mg of activated charcoal powder. Final clear extracts were concentrated under vacuum and reconstituted into HPLC grade acetonitrile, and residues were estimated using HPLC equipped with a UV detector at 230 $\lambda$ and a $C_{18}$ column. Acetonitrile/water (60:40, v/v) at 1 mL/min was used as mobile phase. Both flubendiamide and desiodo flubendiamide presented distinct peaks at retention times of 11.07 and 7.99 min, respectively. Consistent recoveries ranging from

85 to 99% for both compounds were observed when samples were spiked at 0.10 and 0.20 mg/kg levels. The limit of quantification of the method was worked out to be 0.01 mg/kg.

Gopal and Mishra (2008) developed a method for residue analysis of flubendiamide in rice (*Oryza sativa*), which includes improved extraction, cleanup and determination of flubendiamide in rice seeds, husk and straw by using LC with UV detection. Safety evaluation of this insecticide in rice has been carried out after applying its soluble concentrate (SC) formulation at recommended dose (30 g a.i. $ha^{-1}$) and double of the recommended dose (60 g a.i. $ha^{-1}$) on rice crop. MRL of flubendiamide on rice grain can be proposed as 0.2 mg $kg^{-1}$.

Caboni *et al.*, (2008) reported an analytical method for the simultaneous determination of the two diamide insecticides [chlorantraniliprole (CAP) and flubendiamide (FLU)] on fruits and vegetables by liquid chromatography–electrospray tandem mass spectrometry operated in the positive and negative ionization switching mode. The two diamides were extracted with acetonitrile and separated on a Zorbax Column Eclipse XDB C8 (4.6 mm × 150 mm i.d., 3 μm) by isocratic elution with a mobile phase consisting of acetonitrile and water with 0.1% formic acid pumped at a flow rate of 0.4 mL/min. The diamides were selectively detected by multiple reaction monitoring for transitions of proton adduct precursor ions simultaneously: positive *m/z* 484.3→285 for CAP, *m/z* 445.5→169 for internal standard, and negative *m/z* 681.4→253 for FLU. For CAP calibration in the positive mode was linear over a working range of 2 to 1000 μg/L with $r > 0.992$. The limit of detection (LOD) and limit of quantification (LOQ) for CAP were 0.8 and 1.6 μg/kg, respectively. For FLU in the negative mode the corresponding values were 1–1000 μg/L for linear working range, with $r > 0.996$ and 0.4 and 0.8 μg/L for LOD and LOQ, respectively. Moreover, the presence of interfering compounds in the fruit and vegetable extracts was found to be minimal. Due to the linear behavior of the MS detector response for the two analytes, it was concluded that the multiple reaction transitions of molecular ions in the ion-switching mode can be used for analytical purposes, that is, for identification and quantification of diamides in fruit and vegetable extracts at trace levels.

Chapter 3

# Material and Methods

# Material and Methods

The materials used and methods employed for the present investigation of flubendiamide has been divided into four sections and are summarized in this chapter.

Section A - Method standardization of flubendiamide and des-iodo flubendiamide in crops, soil and water.

Section B - Supervised field trials of flubendiamide in cabbage, tomato and pigeon pea

Section C- Persistence and dissipation study of flubendiamide in three types of soil under laboratory condition

Section D -Persistence and dissipation study of flubendiamide in water at different pH under laboratory condition

**SECTION - A**

**3.1 Method standardization of flubendiamide and des-iodo flubendiamide**

**3.1.1 Matrices**

The substrates considered for the present study are listed below

i) Cabbage head, ii) Tomato fruit, iii) Pigeon pea grain, iv) Pigeon pea shell, v) Pigeon pea straw, vi) Soil, and vii) Water.

**3.1.2 Chemicals and reagents**

1. Acetonitrile (J. T. Baker)
2. Water (HPLC Grade Ranbaxy)
3. Hexane (J. T. Baker)
4. Ethyl Acetate (J. T. Baker)
5. Dichloromethane (Ranbaxy )
6. Anhydrous Sodium Sulphate (E. Merck)
7. Anhydrous Magnesium Sulphate (Rankem)
8. Bondesil-Primary Secondary Amine (PSA) 40μm ,Varian
9. Sodium Chloride (Rankem-AR Grade)
10. Whatman filter paper 42 No.
11. Solvent mixture: Acetonitrile / Water (6/4,v/v)

12. Buffer powder of pH: 4.0, 7.0 and 9.2
13. HPLC column: BDS Hypersil RP C-18, Dimension (mm): 250 x 4, particle size (μ): 5, LOT:7116, SN: 0153487E
14. Analytical standards of flubendiamide and des-iodo flubendiamide
15. Flubendiamide formulation (20 % WG)

### 3.1.3 Equipment and apparatus

1. Conical flasks and beakers (250 mL) of Borosil made
2. Pipettes: 1 mL, 2 mL, 5 mL and 10 mL
3. Micropipettes: 5-50 μL range, 40-200 μL range and 0.5-5 mL range
4. Stainless steel knife
5. Spatula
6. Centrifuge tubes (50mL), High Density Polyethylene (HDPE), Merck
7. Micro centrifuge tubes (2 mL)
8. Non absorbent cotton
9. Volumetric flasks: 25 mL, 50 mL and 100 mL
10. Graduated tubes:10 mL and 25 mL
11. Measuring cylinder: 10 mL, 50 mL, 100 mL, and 250 mL
12. Funnels
13. Pear shaped flask: 500 mL
14. Autosampler vials (1.5 mL) (Agilent Technologies)
15. Remi auto mix blender
16. Rotary vacuum evaporator with temperature controlled water bath (Hahn Shin, Model-HS-2001NS)
17. Top loading balance with sensitivity of 1 mg
18. Analytical Balance (Model: GD603, Sartorius)
19. Cold Centrifuge (Model: Superspin, Plastocraft)
20. pH meter (Elico)
21. Mechanical shaker
22. Soil auger
23. Sieve
24. Separatory funnel
25. Ultrasonic Bath (Trans-O-Sonic)
26. Buchner Funnel

27. Vortex mixer
28. Hamilton syringe (100 μL)
29. High Performance Liquid Chromatography (Model: 1200 series, Agilent Technologies) equipped with UV variable wavelength detector

### 3.1.4 Preparation of the standard solutions

#### 3.1.4.1 Reference standard

Flubendiamide and Des-iodo flubendiamide with purity of 96.7% and 99.3% w/w respectively was supplied by M/s Rallis India Limited, Bangalore.

#### 3.1.4.2 Primary stock solution

The technical grade flubendiamide (96.7% purity) and des-iodo flubendiamide (99.3% purity) was adjusted to 100 mg a.i. and transferred to a 100 mL volumetric flask and the volume was made upto 100 mL with acetonitrile separately. Then the flask was shaken well to get a homogenous solution of 1000 ppm of flubendiamide and des-iodo flubendiamide.

#### 3.1.4.3 Secondary stock solution

Flubendiamide and des-iodo flubendiamide of 10 ppm was prepared individually by transferring 1 mL of stock solution to 100 mL volumetric flask and the volume was made up with acetonitrile.

#### 3.1.4.4 Working standard

From the secondary stock solution, working standards of 0.1 to 1 ppm was prepared by diluting 1 mL of 10 ppm solution with acetonitrile: water (6:4, v/v). The retention time of flubendiamide (Figure 6) and des-iodo flubendiamide (Figure 7) was recorded, maintaining the same instrument condition.

#### 3.1.4.5 Mixture working standard solutions of flubendiamide and des-iodo flubendiamide

Depending upon retention time and instrumental sensitivity of each compound, mixed working standard solutions was prepared at different concentration (1.0, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, 0.005, 0.002 and 0.001 ppm) for analysis in HPLC.

### 3.1.4.6 Standardization of mixture standard solution

The mixed working standard solutions of different concentration were injected into HPLC and retention time of flubendiamide and des-iodo flubendiamide were verified from the records of individual pesticide retention time (Figure 8).

### 3.1.4.7 Storage of standard solutions

All the stock and working standard solutions of flubendiamide and des-iodo flubendiamide was kept under refrigerator and protected from light.

### 3.1.4.8 Instrumental conditions

The Reverse Phase High performance liquid chromatography (HPLC) parameter for determining flubendiamide and des-iodo flubendiamide employed in the present study is shown in table 7.

Table 7: HPLC parameters for flubendiamide and des-iodo flubendiamide

| | | | |
|---|---|---|---|
| 1 | Model | : | Agilent 1200 series |
| 2 | Column | : | BDS, Hypersil C-18, (5μm), 150 mm Long,4.6mm i.d, |
| 3 | Detector | : | UV-Vis variable wavelength |
| 4 | Pump | : | Isocratic |
| 5 | Mobile phase | : | Acetonitrile:Water (60:40,v/v) |
| 6 | Flow rate | : | 1mL/min |
| 7 | Wavelength ($\lambda_{max}$) | : | 210 nm |
| 8 | Injection volume | : | 100μl |
| 9 | Retention time (minutes) | : | Flubendiamide (8.97) & des-iodo flubendiamide (7.08) |

### 3.1.4.9 Linearity Check

The linearity of mixed standard solution of different concentration level (0. 01 – 1.0 ppm) was checked in HPLC (Figure 9-10) in order to determine the linearity range of flubendiamide and des-iodo flubendiamide.

### 3.1.5 Method of analysis

#### 3.1.5.1 Method of analysis in crops

**Principle:** The method involves the initial single phase extraction of plant samples with acetonitrile followed by dispersive solid-phase extraction (dispersive - SPE) clean-up with anhydrous $MgSO_4$ and primary secondary amine (PSA).

#### 3.1.5.2 Sample preparation

The samples of cabbage heads and tomato fruits (1 kg each) were finely chopped and homogenized in a Remi auto mix blender. Pigeon pea straw (1 kg) was cut into small pieces and grinded in a blender. Pigeon pea pod (3 kg) from which grain and shell were separated. Grain was crushed using a mortar and pestle and shell was blended.

#### 3.1.5.3 Extraction/partitioning

Homogenized plant samples (10 g) were taken into 50 mL Teflon centrifuge tube and 10 mL acetonitrile was added with the dispenser and tightened with screw cap. It was shaken vigorously for 1 min by using vortex mixer. To it 4 g anhydrous $MgSO_4$ and 1 g NaCl was added and mixed on a vortex mixer immediately for 1 minute and centrifuged for 10 minutes at 10,000 rpm.

#### 3.1.5.4 Standardization of primary secondary amine (PSA)

For the standardization of PSA, individual cabbage head, tomato fruit, pigeon pea grain, pigeon pea shell and pigeon pea straw (10 g each) were fortified with mixture standard solutions of flubendiamide and des-iodo flubendiamide (0.05 ppm) and extracted as mentioned in step 3.1.5.4. After extraction, varying amounts of PSA [10, 25, 50, 75 and 100 mg, (1 mL aliquot of 1 g sample equivalent)] were added and subjected to dispersive-SPE Clean-up. The co-extractives removal (Figure 11) along with the recovery percentage will determine the standardization of PSA amount (Figure 12-16).

#### 3.1.5.5 Dispersive-SPE Clean-up

From the supernatant aliquot obtained from step 3.1.5.3 one (1) mL aliquot was transferred into 2 mL micro centrifuge tube containing 25 mg PSA sorbent and 150 mg anhydrous $MgSO_4$ followed by vortexing for 30 seconds. Then it was centrifuged for 10 minutes at 10,000 rpm to separate solids from solution. The supernatant extract (0.5 mL)

was concentrated by purging with gentle stream of nitrogen. The final volume made up with acetonitrile: water (6:4, v/v) for HPLC analysis.

### 3.1.6 Method of analysis in soil

**Principle:** Soil samples are extracted with acetonitrile by shaking on a mechanical shaker followed by filtration and partitioning with hexane and dichloromethane. The organic layer was concentrated and ready for HPLC analysis.

#### 3.1.6.1 Collection and preparation

Soil samples (1 kg) were collected randomly from 6-8 places at a depth of 0-15 cm with soil auger. Composites were prepared by removing visible plant debris and stones. Each fraction was thoroughly mixed to achieve homogeneity, air dried, disaggregated manually, passed through a 2-mm sieve, and stored in darkness.

#### 3.1.6.2 Extraction

A representative homogenized soil sample (20 g each) was taken in a conical flask (250 mL) and shaken for half an hour using a mechanical shaker with 100 mL acetonitrile and the extract was filtered through filter paper (Whatman No.42.) mounted on a buchner funnel with 100 mL of acetonitrile. The pooled filtrate was transferred into a 500 mL round bottom flask and concentrated to about 50 mL using a rotary evaporator with a water bath at 40° C.

#### 3.1.6.3 Clean-up

The concentrated acetonitrile extracts obtained from step 3.1.6.2 was transferred quantitatively to a 500 mL separatory funnel. The sample was partitioned thrice with 100 mL hexane (saturated with acetonitrile) and the upper hexane layer was discarded each time. The lower acetonitrile layer was partitioned against dichloromethane (3 x 100 mL) by addition of 4 % saturated NaCl solution. The combined acetonitrile layer was collected into a 500 mL pear shaped flask and concentrated to dryness on a rotary evaporator (40° C). The residue was made up with acetonitrile: water (60:40, v/v) for estimation by HPLC.

### 3.1.7 Method of analysis in water

**Principle:** Extraction with 40 % ethyl acetate in hexane followed by concentration and volume was made up with acetonitrile: water (6:4, v/v) for HPLC analysis.

#### 3.1.7.1 Extraction

The water samples (100 mL) were transferred into a 500 mL separatory funnel

which was made saturated with addition of 50 mL NaCl solution (4%) and were partitioned with 100 mL distilled 40% ethyl acetate in hexane (3x100 mL). The upper organic phase was collected in pear shaped flask by passing over anhydrous $Na_2SO_4$ and evaporated to dryness in rotary vacuum evaporator (40°C). The residues were reconstituted with acetonitrile: water (60:40, v/v) for estimation by HPLC.

### 3.1.8 Limit of quantitation (LOQ) and limit of detection (LOD)

A Two – step approach method as recommended by US EPA was followed for estimating Limit of detection (LOD) and Limit of quantification (LOQ) (Source: Handbook of Residue Analytical Methods for Agrochemicals, Vol.1, Ed. In Chief: Dr. Philip W Lee, Pg. 23).

The LOD and LOQ of flubendiamide and des-iodo flubendiamide in different plant matrices, soil and water samples was established by conducting recovery experiments.

### 3.1.9 Recovery experiment

The LOQ so determined from the above procedure provides a concentration limit above which fairly precise quantitative measurements can be done. The adaptability of the method was carried out upon considering the performance characteristics *viz.* specificity, detection limit (LOD), quantification limit (LOQ), precision, and recovery along with cost efficiency and time factor. Recovery studies were carried out in order to establish the reliability of the analytical method and to know the efficiency of extraction and clean up step for the present study by fortifying flubendiamide and des-iodo flubendiamide with different plant matrices, soil and water samples at the level of LOQ, and 10 times of LOQ irrespective of substrate in triplicate along with untreated control samples. Fortified samples were left to stand for 1 hour before extraction to allow the pesticides absorption onto the matrix. The recovery experiment was carried out with plant matrices, soil and water samples as described in 3.1.5, 3.1.6 and 3.1.7 of section - A respectively and the results are shown in table 9-13.

### 3.1.10 Identification and quantification of analyte

#### 3.1.10.1 Identification

The working mixture standard solution of analytical grade pesticides comprising of flubendiamide and des-iodo flubendiamide was used as an external standard, for which the retention time was recorded. Then 100 µL of each cleaned up test samples were

injected into the HPLC. The residues were identified by comparing the retention time of sample peaks with that of mixture of analytical standard.

**3.1.10.2 Quantification**

The amount of residues in ppm ($\mu g\ g^{-1}$) was calculated as follows:

$$\text{Residues in ppm} = \frac{A_1 \times V_1 \times C}{A_2 \times W \times V_2} \times R_f$$

Where,

$A_1$ = Area of sample in the Chromatogram

$A_2$ = Area of standard in the Chromatogram

$V_1$ = Total volume of sample in mL

$V_2$ = Injected volume of the sample in μL

C = Concentration of analytical standard in ppm (μg/mL)

W = Weight of the sample in gm or mL

$R_f$ = Recovery factor

SECTION- B

## 3.2 Supervised field trials of flubendiamide in cabbage, tomato and pigeon pea

### 3.2.1 Details of supervised field trial on cabbage

| Application data | |
|---|---|
| Year of study | 2006 |
| Location of field trial | Basantpur, Nadia, West Bengal |
| Date of Crop planting/sowing | 07.07.2006 |
| Transplanting Date | 05.08.2006 |
| Crop | Cabbage |
| Variety | KK Cross |
| Insecticide | Flubendiamide |
| Formulation used | 20 % WG |
| Description of the plot plan/crop layout/cropping system | RBD with 3 replications |
| Plot Size | 30sq.m. for each replication |
| Number of plants per plot | 100 |
| Number of plots per treatment | Three |
| Method of application and equipment | Foliar Spraying and knapsack sprayer |
| No. of application | Two |
| Date of application | 1st Spray - 05.10.06 (60 DAT) |
| | 2nd Spray - 15.10.06 (70 DAT) |
| Intervals between two application | 10 days |
| Growth stage at last treatment | Head formation |

| Application details | | | |
|---|---|---|---|
| Dose Rate | | | |
| $T_1$ | 62.5 g product/ha (12.5 g a.i.$ha^{-1}$) | | |
| $T_2$ | 125 g product/ha (25.0 g a.i.$ha^{-1}$) | | |
| $T_0$ | Untreated control | | |
| Spray volume | 500 liter water $ha^{-1}$ | | |
| **Sampling data** | | | |
| Date of sampling with time | Substrate | Date | Time |
| | Cabbage head | 15.10.06 | 11 a.m. |
| | | 16.10.06 | 9 a.m. |
| | | 18.10.06 | 9 a.m. |
| | | 20.10.06 | 9 a.m. |
| | | 22.10.06 | 9 a.m. |
| | | 25.10.06 | 9 a.m. |
| | | 30.10.06 | 9 a.m. |
| | Soil | 30.10.06 | 9 a.m. |
| No. of samples taken / test/ treatment | Three | | |
| Sample weight and preparation | 1.0 kg | | |
| Interval between last application and sampling | 0 hrs (2 hours after second spraying), 1, 3, 5, 7, 10, and 15 days after the last ($2^{nd}$) spray in cabbage head. Soil sampling on $15^{th}$ day after the $2^{nd}$ spray | | |
| Other pesticides applied to trial plot with relevant information | Nil | | |
| Storage condition before analysis | Extracted immediately after sampling and analyzed after processing | | |

#### 3.2.1.1 Weather conditions during the period of study

| | |
|---|---|
| Av. Min. Temperature (°C) | : 25.0 |
| Av. Max. Temperature (°C) | : 32.6 |
| % Relative humidity (max) | : 98.0 |
| % Relative humidity (min) | : 78.6 |
| Total Rainfall (mm) | : 09.8 |
| Av. Relative humidity (%) | : 88.3 |

#### 3.2.1.2 Method of Analysis

The method of analysis in cabbage head and soil are described in 3.1.5 and 3.1.6 of Section – A respectively.

#### 3.2.1.3 Recovery experiment

The recovery experiment was carried out as described in 3.1.9 of section-A

#### 3.2.1.4 Interpretation of Residue Data

##### 3.2.1.4.1 Computation of Regression Equation

The dissipation of pesticides should follow a first order kinetics i.e. the residue should dissipate linearly, and the concentration versus time equation will be

$y = mx + C$ ..... (i)

Where y = log of residues in ppm

C = exposed initial deposit in ppm

x = time in days

m = slope of regression line

The equation can be expressed in the simple regression equation form as:

$\log R = \log K_2 - (\log K_1)\, T$ ..... (ii)

Where, log R = logarithm of residue in ppm at any time T (in hours or days)

$\log K_2$ = logarithm of initial residue in ppm

$\log K_1$ = slope of the straight line from the regression equation, i.e., the rate of dissipation of residue with time

T = Time (in hours/days).

#### 3.2.1.4.2 Computation of Residual Half Life ($RL_{50}$ or $T_{1/2}$)

Residual half life ($RL_{50}$ or $T_{1/2}$) can be defined as the time required for the pesticide residue to come down to half of its initial concentration. The half-life can be calculated from equation (iii) by putting log ($K_2$) instead of log R and the equation will be

$$RL_{50} \text{ or } t_{1/2} = \frac{\text{Log } 2}{\text{Log } K_1} \quad \text{..... (iii)}$$

This is analogous to first order half-life.

#### 3.2.1.4.3 Computation of Safe Waiting Period or Pre-Harvest Interval ($T_{MRL}$ or $T_{tol}$)

$T_{MRL}$ is the maximum time (days) required for the residue to reach below the tolerance limit. This is calculated from equation (iv) which is derived from equation (ii) by substituting $T_{MRL}$ for T, log tolerance in ppm for log R followed by the rearrangement to find equation (iv).

$$T_{MRL} = \frac{\text{Log } K_2 - \log(\text{tolerance in ppm})}{\log K_1} \quad \text{(iv)}$$

This is analogous to first order half-life.

### 3.2.2 Details of supervised field trial on tomato

| Application data | |
|---|---|
| Year of study | 2006 |
| Location of field trial | District Seed Farm, Kalyani |
| Date of Crop planting/sowing | 20.08.2006 |
| Transplanting Date | 14.09.2006 |
| Crop | Tomato |
| Variety | ARCH-128 |
| Insecticide | Flubendiamide |
| Formulation used | 20 % WG |
| Description of the plot plan/crop layout/cropping system | RBD with 3 replications |
| Plot Size | 30sq.m. for each replication |

| Application data | | | |
|---|---|---|---|
| Number of plants per plot | 111 | | |
| Number of plots per treatment | Three | | |
| Method of application and equipment | Foliar Spraying & knapsack sprayer | | |
| No. of application | Two | | |
| Date of application | 1st Spray - 07.11.06 (55 DAT) | | |
| | 2nd Spray - 22.11.06 (70 DAT) | | |
| Intervals between two application | 15 days | | |
| Growth stage at last treatment | Fruit formation stage | | |
| **Application details** | | | |
| Dose Rate | | | |
| $T_1$ | 250 g product/ha (50 g a.i. $ha^{-1}$) | | |
| $T_2$ | 500 g product/ha (100 g a.i. $ha^{-1}$) | | |
| $T_0$ | Untreated control | | |
| Spray volume | 500 liter water $ha^{-1}$ | | |
| **Sampling data** | | | |
| Date of sampling with time | **Substrate** | **Date** | **Time** |
| | Tomato fruit | 22.11.06 | 11 a.m. |
| | | 23.11.06 | 9 a.m. |
| | | 25.11.06 | 9 a.m. |
| | | 27.11.06 | 9 a.m. |
| | | 29.11.06 | 9 a.m. |
| | | 02.12.06 | 9 a.m. |
| | Soil | 02.12.06 | 9 a.m. |
| No. of samples taken / test/ treatment | Three | | |
| Sample weight and preparation | 1.0 kg | | |

| Sampling data | |
|---|---|
| Interval between last application and sampling | 0 hrs (2 hours after second spraying), 1, 3, 5, 7, and 10 days after the last ($2^{nd}$) spray in Tomato fruit. Soil sampling on $10^{th}$ day after the $2^{nd}$ spray |
| Other pesticides applied to trial plot with relevant information | Nil |
| Storage condition before analysis | Extracted immediately after sampling & analyzed after processing |

**3.2.2.1 Weather conditions during the period of study**

Av. Min. Temperature (°C) : 23.10

Av. Max. Temperature (°C) : 31.50

% Relative humidity (max) : 98.70

% Relative humidity (min) : 72.40

Total Rainfall (mm) : 06.00

Av. Relative humidity (%) : 85.55

**3.2.2.2 Method of Analysis**

The method of analysis in tomato fruit and soil are described in 3.1.5 and 3.1.6 of Section – A respectively.

**3.2.2.3 Interpretation of Residue Data**

Same as discussed under 3.2.1.4

### 3.2.3 Details of supervised field trial on Pigeon pea

| Application data | |
|---|---|
| Year of study | 2006 |
| Location of field trial | Mondouri Research Farm, BCKV, West Bengal |
| Date of Crop planting/sowing | 24.06.2006 |
| Crop | Pigeon pea |
| Variety | T-17 |
| Insecticide | Flubendiamide |
| Formulation used | 20 % WG |
| Description of the plot plan/crop layout/cropping system | RBD with 3 replications |
| Plot Size | 30sq.m. for each replication |
| Number of plants per plot | 240 plants/plot |
| Number of plots per treatment | Three |
| Method of application and equipment | Foliar Spraying & knapsack sprayer |
| No. of application | Two |
| Date of application | 1st Spray - 18.01.2007 (205 DAS) |
| | 2nd Spray - 02.02.2007 (220 DAS) |
| Intervals between two application | 15 days |
| Growth stage at last treatment | Flowering stage to pod formation stage |

| Application details | | | |
|---|---|---|---|
| Dose Rate | | | |
| $T_1$ | 250 g product/ha (50 g a.i. $ha^{-1}$) | | |
| $T_2$ | 500 g product/ha (100 g a.i. $ha^{-1}$) | | |
| $T_0$ | Untreated control | | |
| Spray volume | 500 liter water $ha^{-1}$ | | |
| **Sampling data** | | | |
| Date of sampling with time | Substrate | Date | Time |
| | Pigeon pea pod and straw | 16.03.07 | 9 a.m. |
| | Soil | 16.03.07 | 9 a.m. |
| No. of samples taken / test/ treatment | Three | | |
| Sample weight and preparation | 3.0 kg pigeon pea pod and 1 kg pigeon pea straw and soil | | |
| Interval between last application and sampling | 42 days after the last (2nd) spray in pigeon pea pod, straw and soil | | |
| Other pesticides applied to trial plot with relevant information | Nil | | |
| Storage condition before analysis | Extracted immediately after sampling & analyzed after processing | | |

**3.2.3 .1 Weather conditions during the period of study (June 2006 to March 2007)**

Av. Min. Temperature (°C) : 20.32

Av. Max. Temperature (°C) : 30.82

% Relative humidity (max) : 97.47

% Relative humidity (min) : 64.51

Total Rainfall (mm) : 04.15

Av. Relative humidity (%) : 80.99

#### 3.2.3.2 Method of Analysis

The method of analysis in pigeon pea and soil are described in 3.1.5 and 3.1.6 of Section – A respectively.

#### 3.2.3.3 Interpretation of Residue Data

Same as discussed under 3.2.1.4

SECTION-C

## 3.3 Persistence and dissipation study of flubendiamide in three types of soil under laboratory condition

### 3.3.1 Collection and preparation of soil

The soil of three different agro-climatic zones of West Bengal: New alluvial zone (Mondouri), Red and lateritic zone (Jhargram) and Coastal zone (Canning) were collected from plough layers (0-15 cm) having no previous history of flubendiamide application. Soil sampling was carried out following the standard methodology (Jackson ML, 1973). Soils were air dried ground and passed through a 2.0 mm sieve and sub-sampled by the usual methods of quartering. Soil texture, pH, organic carbon and CEC were determined following the standard methodology (Black, 1965; Gee & Bauder, 1986; Nelson & Soemmers, 1982; Dewis & Freitals, 1984). The physicochemical properties for different soil are given in table 41.

### 3.3.2 Experimental details of flubendiamide application

Flubendiamide (100 ppm) was prepared by dilution technique from the stock solution (1000 ppm). From it 1 and 2 mL was applied to the processed (air-dried and sieved) soil samples (20g) taken in amber glass bottles which corresponds to 5 ppm ($T_1$) and 10 ppm ($T_2$) respectively along with an untreated control samples. Each treatment was replicated three times. Samples were stirred well and the solvent was allowed to evaporate for 1hour. The bottles were weighed and plugged with non-adsorbent cotton plug and kept at room temperature.

### 3.3.3 Sampling

Three replicates of soil samples for each treatment along with untreated control were taken for analysis at an interval of 0 (1 hour after application), 3, 7, 15, 30, 60, 90, 120 and 150 days after spiking with flubendiamide standard solution.

### 3.3.4 Method of analysis

The method of analysis in soil are described in 3.1.6 of section – A

### 3.3.5 Recovery study

The recovery experiment was carried out with three types of soil as described in 3.1.6 of section – A and the results are shown in table 42-43.

### 3.3.6 Interpretation of Residue Data

Same as discussed under 3.2.1.4

SECTION-D

## 3.4 Persistence and dissipation study of flubendiamide in water at different pH under laboratory condition

### 3.4.1 Preparation of water samples

Water samples of different pH were prepared by using the buffer powder (Rankem) pH = 4.0, pH = 7.0 and pH = 9.2) by dissolving each powder packet in 100 mL distilled water. The pH was verified by pH meter.

### 3.4.2 Experimental details of flubendiamide application

Flubendiamide (100 ppm) was prepared by dilution technique from the stock solution (1000 ppm). From it 1 and 2 mL was applied to 100 mL water samples of varying pH under study taken in amber glass bottles which corresponds to 1 ppm ($T_1$) and 2 ppm ($T_2$) respectively along with an untreated control samples. Each treatment was replicated three times. The bottles were kept at room temperature.

### 3.4.3 Sampling

The water samples of pH 4.0, 7.0 and 9.2 were taken at 0 (1 hr after application), 3, 7, 15, 30, 60, 90 and 120 days intervals for analysis. Control samples were also collected in the same day for every pH and treatments.

### 3.4.4 Method of analysis

The method of analysis in water are described in 3.1.7 of section – A

### 3.4.5 Recovery study

Recovery study was carried out in order to establish the efficiency and reliability of the analytical method employed. Distilled water samples, maintained at pH 4.0, 7.0 and 9.2 levels, were fortified at the level of 0.01, 0.05 and 0.1 ppm with the analytical standard solutions of flubendiamide and were analyzed following the procedure, as described under section extraction and cleanup.

### 3.4.6 Interpretation of Residue Data

Same as discussed under 3.2.1.4

# Chapter 4

# Results and Discussion

# Results and Discussion

The results of method standardization, residue analysis in crops, soil and water form the subject matter of discussion in the present chapter.

**SECTION- A**

**4.1 HPLC standardization and detector linearity of flubendiamide and des-iodo flubendiamide**

Analytical standard of flubendiamide and des-iodo flubendiamide (1ppm each) was injected into HPLC following the operational parameters as mentioned under 3.1.4.8 of section A. The retention time of flubendiamide (8.93 ± 0.1minute) and des-iodo flubendiamide (7.08 ± 0.1minute) was recorded (Figure 6-7). The mixed working standard solution was injected into HPLC and retention time of flubendiamide and des-iodo flubendiamide were verified from the records of individual pesticide retention time (Figure 8). The mixture standard solution of different concentration was also injected to find out detector linearity and it was observed that the detector response of flubendiamide and des-iodo flubendiamide was found to be 0.01-1.0 ppm respectively. An excellent linear correlation between the injected amount of flubendiamide and des-iodo flubendiamide and the detector response of HPLC was observed for standards in solvent with the correlation coefficient of the 1/× weighted linear regression was at 0.99989 (flubendiamide) and 0.99986 for des-iodo flubendiamide (Figure 9-10). The calibration curves were considered valid as correlation coefficient of the regression line were higher than 0.99.

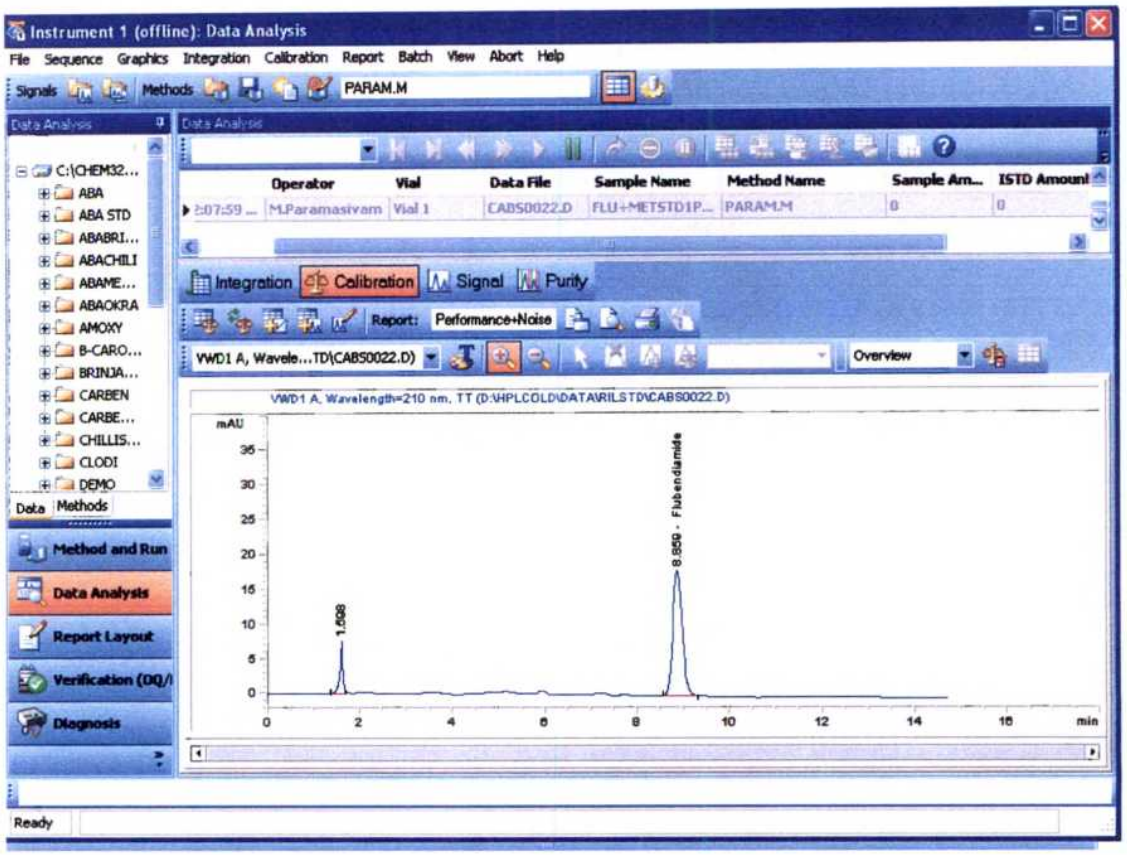



Figure 6: Analytical standard of flubendiamide (1 ppm)

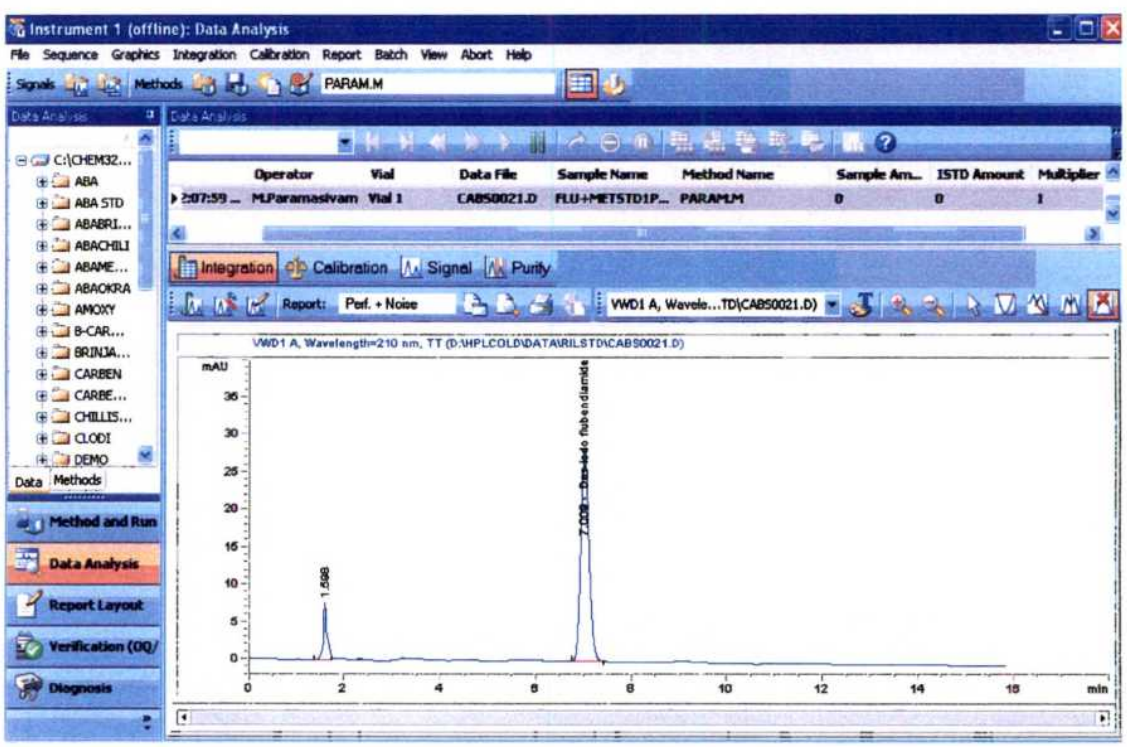



Figure 7: Analytical standard of des-iodo flubendiamide (1 ppm)

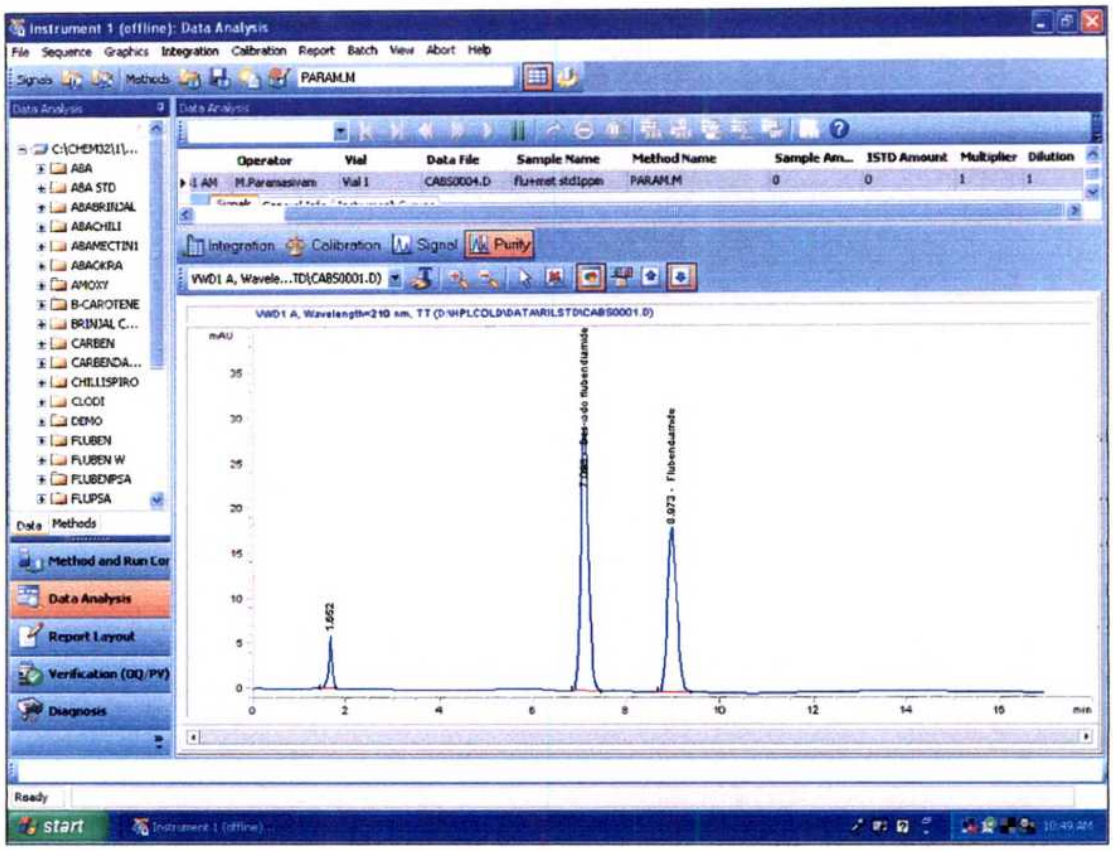



Figure 8: Analytical standard of flubendiamide and des-iodo flubendiamide (1 ppm)

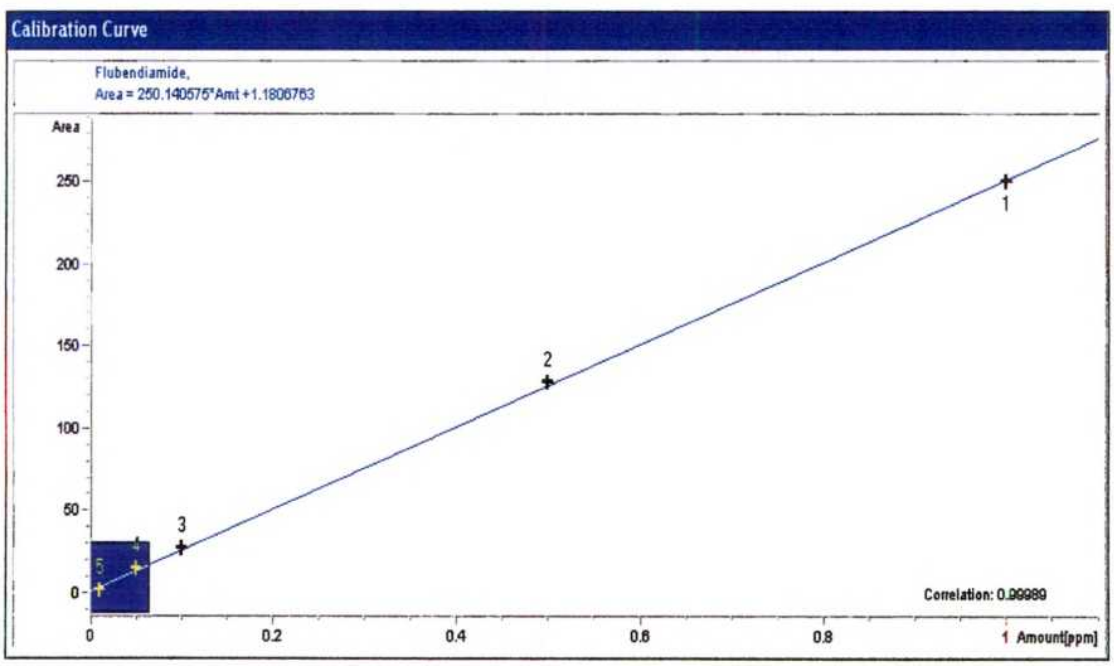



Figure 9: Detector linearity of flubendiamide 0.01-1.0 ppm

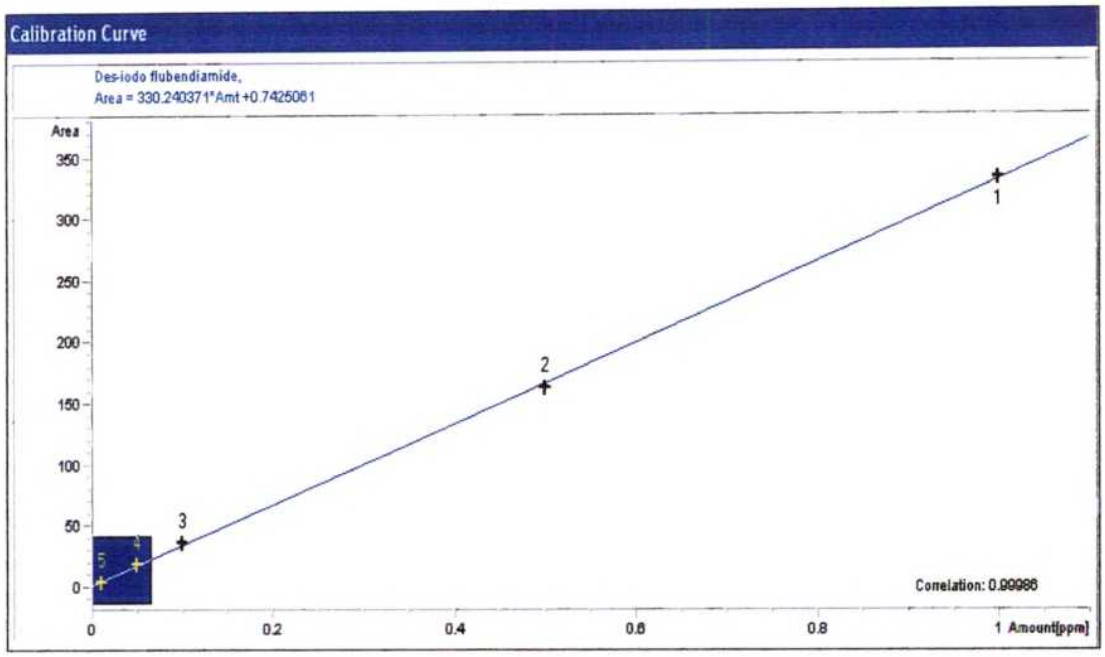



Figure 10: Detector linearity of des-iodo flubendiamide 0.01-1.0 ppm

### 4.1.1 Standardization of primary secondary amine (PSA) amount in crops (Cabbage head, Tomato fruit, Pigeon pea Grain, Pigeon pea shell and Pigeon pea straw)

The method of analysis of flubendiamide and des-iodo flubendiamide in different crops involves the use of PSA for clean-up technique. The co-extractive percentage removal by using varying amounts of PSA and without addition of PSA in different crops is depicted in figure 11. It was observed that without addition of PSA in clean-up step the co-extractive percentage removal ranged from 6.7- 25.6%. The figure 11 further revealed that the co-extractives removal in cabbage head, pigeon pea shell and pigeon pea straw was in the order of 48.65, 40.43 and 30.20 % respectively with the addition of 10 mg PSA, but in case of tomato fruits and pigeon pea grain, it was 86.88 and 84.57 % respectively. Further scrutiny indicated that co-extractives removal ranged from 81.67 – 83.78 % in cabbage head, 91.49 – 95.66 % in tomato fruits, 85.06 – 85.21 % in pigeon pea grain and 82.22 – 87.19 % in pigeon pea shell with the addition of 25 – 100 mg PSA. However, in case of pigeon pea straw the removal percentage was only 55.21 with 25 mg PSA while increasing PSA amount (50 – 100 mg) the co-extractives was removed ~ 82.00 %.

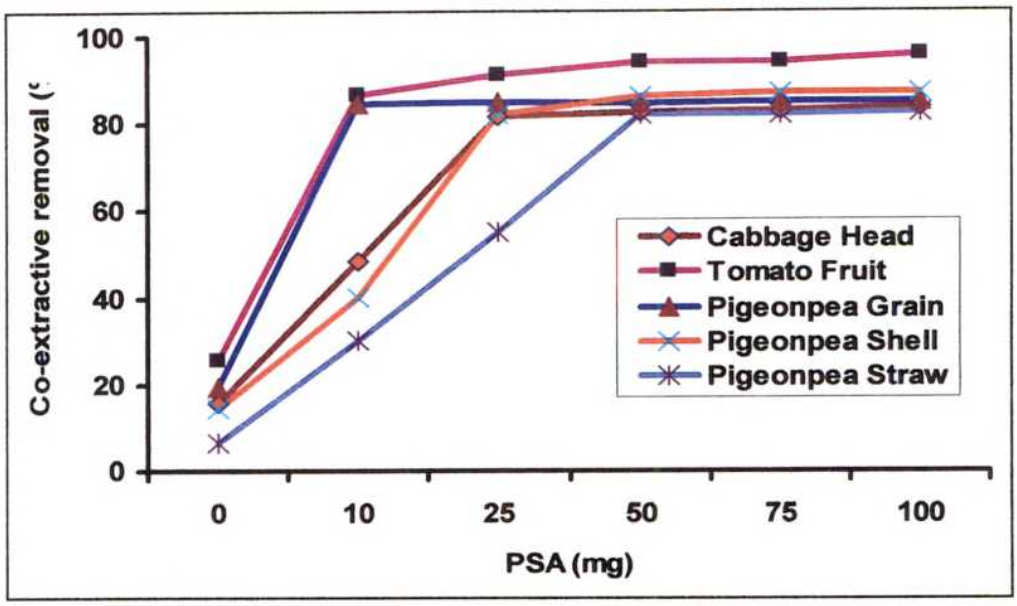



Figure11: Cleanup capabilities of PSA in different crops

The recovery percentage of flubendiamide and des-iodo flubendiamide remained more or less same in cabbage heads (Figure 12) and pigeon pea shell (Figure 15) with the addition of 25-100 mg PSA, in tomato fruits (Figure 13) and pigeon pea grain (Figure 14) it was from 10 – 100 mg and 50-100 mg for pigeon pea straw (Figure 16). From the results of co-extractive removal and recovery percentage in different plant substrates it was further revealed that PSA does not bind flubendiamide and des-iodo flubendiamide. Keeping in view of the fact that as there was no significant variation in the recovery percentage with 10 – 100 mg PSA in case of tomato fruit and pigeon pea grain, 25-100 mg PSA for cabbage heads and pigeon pea shell and 50-100 mg for pigeon pea straw and considering the cost of PSA, co-extractive removal and recovery percentage, the amount of PSA was standardized (Table 8) and adopted for the final method of analysis (1 mL aliquot of 1 g sample equivalent) in tomato fruit and pigeon pea grain (10 mg), cabbage head and pigeon pea shell (25 mg) and pigeon pea straw (50 mg). It was also observed that there were no co-extractive peaks in the zone of flubendiamide and des-iodo flubendiamide which might have accounted for the higher recovery percentage. Although the PSA removed several interfering matrix (polar matrices and fatty acids) components in the HPLC analysis because PSA has higher capacity due to the presence of the secondary as well as primary amine (Anastassiades *et al.*, 2003; Okihashi *et al.*, 2005). The PSA did not affect recoveries because the exposure time as long as 30 minutes did not lead to any losses of the analytes tested.

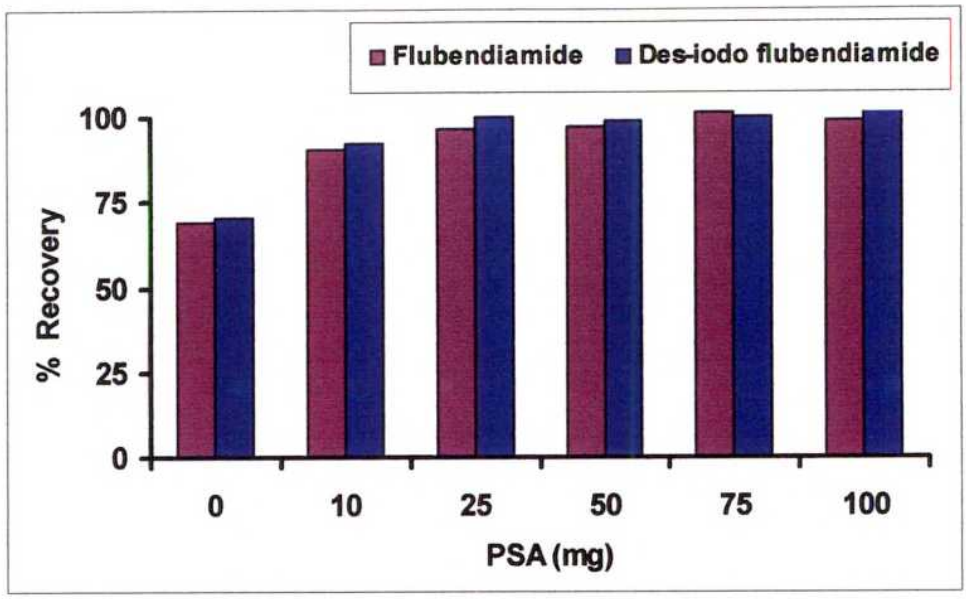



Figure 12: Recovery (%) of flubendiamide and des-iodo flubendiamide in cabbage head at 0.05ppm level

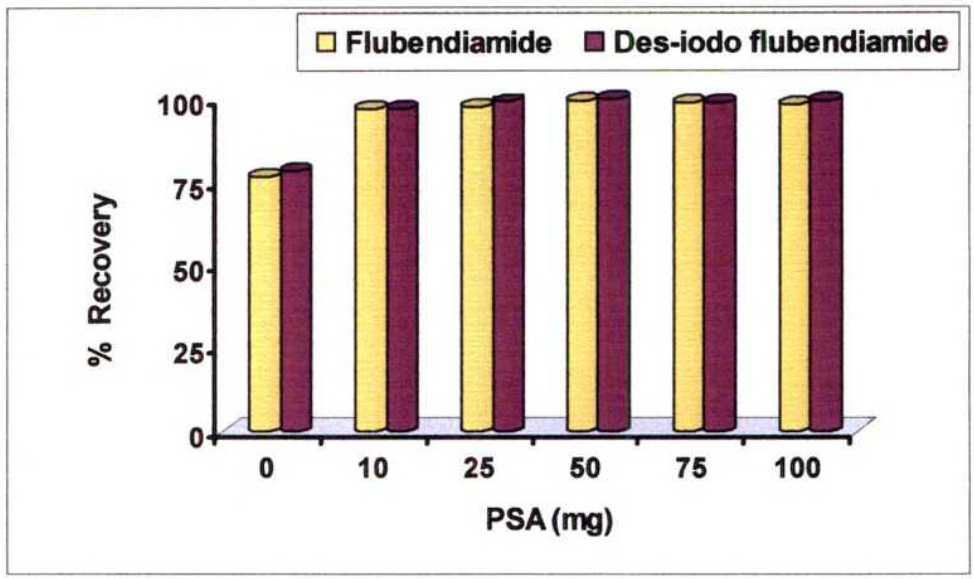



Figure 13: Recovery percentage of flubendiamide and des-iodo flubendiamide in tomato fruit at 0.05ppm level

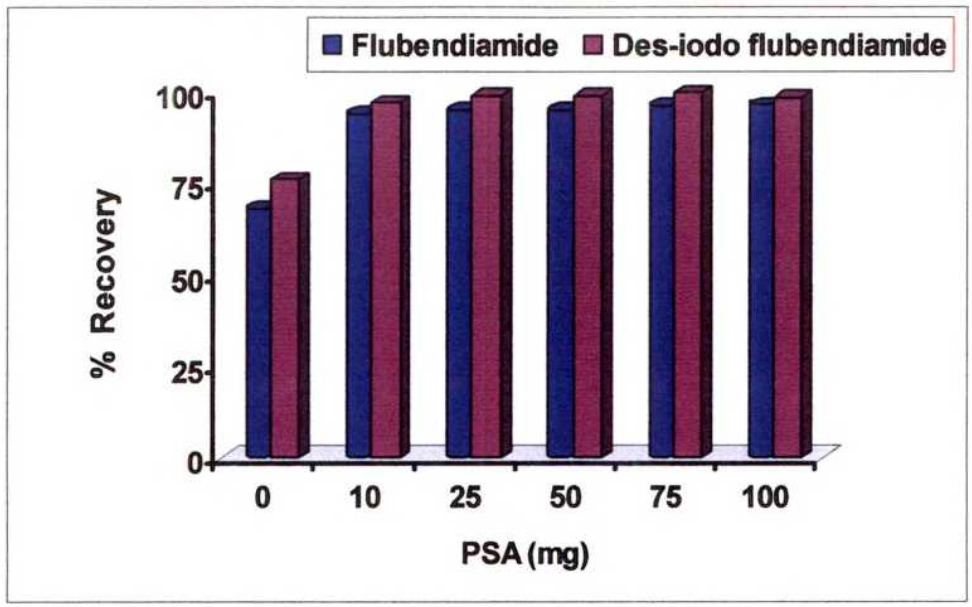



Figure 14: Recovery percentage of flubendiamide and des-iodo flubendiamide in pigeon pea grain at 0.05ppm level

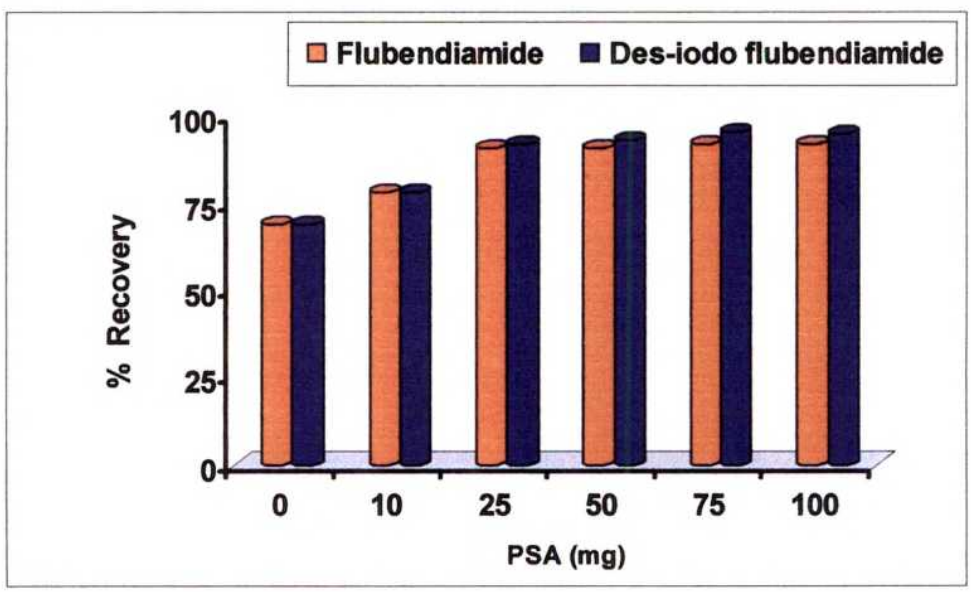



Figure 15: Recovery percentage of flubendiamide and des-iodo flubendiamide in pigeon pea shell at 0.05ppm level

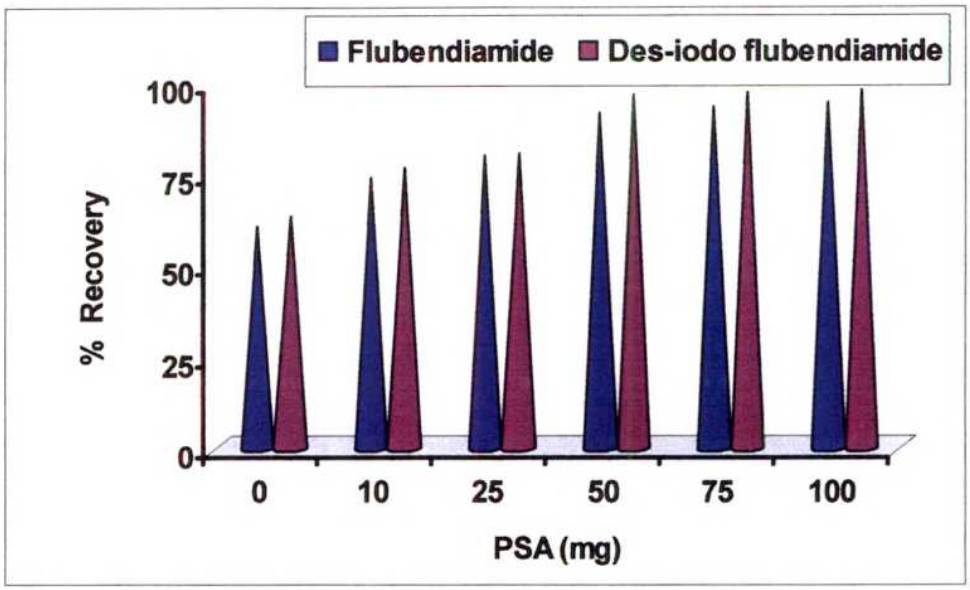



Figure 16: Recovery percentage of flubendiamide and des-iodo flubendiamide in pigeon pea straw at 0.05ppm level

Table 8: Co-extractive removal and recovery percentage of flubendiamide and des-iodo flubendiamide with standardized amount of PSA in different plant matrices

| Plant matrices | PSA (mg) | Co-extractives removal (%) | Flubendiamide recovery (%) | Des-iodo flubendiamide recovery (%) |
|---|---|---|---|---|
| Cabbage Head | 25 | 81.67 | 96.65 | 98.25 |
| Tomato Fruit | 10 | 86.88 | 97.30 | 97.14 |
| Pigeon pea Grain | 10 | 84.57 | 94.38 | 97.26 |
| Pigeon pea Shell | 25 | 82.21 | 90.94 | 92.26 |
| Pigeon pea Straw | 50 | 81.99 | 87.26 | 88.45 |

### 4.1.2 Limit of detection (LOD), limit of quantification (LOQ) and Recovery experiment

Following the standard US-EPA method and considering the signal to noise ratio 1:3, the limit of detection of flubendiamide and des-iodo flubendiamide in different crops was found to be 0.003 ppm and limit of quantification was 0.01ppm based on recovery percentage (≥ 85%), RSD (≤ 20%) and Signal to noise ratio (1:≥10) which is in well agreement with the reported literature (Billian, 2007; Battu *et al.*, 2008). The method was validated by conducting recovery experiments with different samples at the level of LOQ and ten times of LOQ and presented in table 9-13.

The average recovery of flubendiamide in cabbage heads was found to be 96.65 % and 97.2% at LOQ and ten times of LOQ level with RSD ranged from 0.38 - 1.06 %. The des-iodo flubendiamide recovery was also found to be more than 98% with RSD < 1%. Similarly, in tomato fruits, pigeon pea grain and shell showed recoveries of flubendiamide and des-iodo flubendiamide more than 90 % at LOQ and ten times of LOQ level except pigeon pea straw (87%). As PSA removed polar pigments, fatty acids and sugars which reduced the matrix interferences and no scope for losses of pesticide in the present method might be the probable reason for getting higher recovery percentage. The recovery percentage of flubendiamide carried out in different substrates under study is in the order of tomato fruits > cabbage heads > pigeon pea grain > pigeon pea shell > pigeon pea straw and in case of des-iodo flubendiamide it is in the order of pigeon pea grain > cabbage heads > tomato fruits > pigeon pea shell > pigeon pea straw.

Thus, considering the recovery percentage, RSD and S/N ratio, the present method is suitable for analysis of flubendiamide and des-iodo flubendiamide in cabbage heads, tomato fruits, pigeon pea grain, pigeon pea shell and pigeon pea straw.

Table 9: Recovery (%) of flubendiamide and des-iodo flubendiamide in cabbage head

| Fortification Level (ppm) | Replication | Flubendiamide recovery (%) | Des-iodo flubendiamide recovery (%) |
|---|---|---|---|
| 0.01 | $R_1$ | 96.85 | 98.58 |
| | $R_2$ | 97.55 | 99.50 |
| | $R_3$ | 95.54 | 98.10 |
| | Mean ± S.D | 96.65 ± 1.02 | 98.73 ± 0.71 |
| | RSD (%) | 1.06 | 0.71 |
| 0.1 | $R_1$ | 97.10 | 99.54 |
| | $R_2$ | 97.60 | 100.02 |
| | $R_3$ | 96.89 | 98.90 |
| | Mean ± S.D | 97.20 ± 0.36 | 99.49 ± 0.56 |
| | RSD (%) | 0.38 | 0.56 |

Table 10: Recovery (%) of flubendiamide and des-iodo flubendiamide in tomato fruit

| Fortification Level (ppm) | Replication | Flubendiamide recovery (%) | Des-iodo flubendiamide recovery (%) |
|---|---|---|---|
| 0.01 | $R_1$ | 96.00 | 97.10 |
| | $R_2$ | 98.10 | 97.25 |
| | $R_3$ | 97.90 | 98.05 |
| | Mean ± S.D | 97.33 ± 1.16 | 97.47 ± 0.51 |
| | RSD (%) | 1.19 | 0.52 |
| 0.1 | $R_1$ | 97.10 | 98.05 |
| | $R_2$ | 99.80 | 97.50 |
| | $R_3$ | 98.10 | 98.45 |
| | Mean ± S.D | 98.33 ± 1.37 | 98.00 ± 0.48 |
| | RSD (%) | 1.40 | 0.49 |

Table 11: Recovery (%) of flubendiamide & des-iodo flubendiamide in pigeon pea grain

| Fortification Level (ppm) | Replication | Flubendiamide recovery (%) | Des-iodo flubendiamide recovery (%) |
|---|---|---|---|
| 0.01 | $R_1$ | 94.40 | 98.85 |
| | $R_2$ | 95.15 | 98.60 |
| | $R_3$ | 94.40 | 99.10 |
| | Mean ± S.D | 94.65 ± 0.43 | 98.85 ± 0.25 |
| | RSD (%) | 0.45 | 0.25 |
| 0.1 | $R_1$ | 95.50 | 99.50 |
| | $R_2$ | 94.75 | 99.15 |
| | $R_3$ | 96.50 | 98.75 |
| | Mean ± S.D | 95.58 ± 0.88 | 99.13 ± 0.38 |
| | RSD (%) | 0.92 | 0.38 |

Table 12: Recovery (%) of flubendiamide & des-iodo flubendiamide in pigeon pea shell

| Fortification Level (ppm) | Replication | Flubendiamide recovery (%) | Des-iodo flubendiamide recovery (%) |
|---|---|---|---|
| 0.01 | $R_1$ | 90.65 | 94.45 |
| | $R_2$ | 89.85 | 92.86 |
| | $R_3$ | 91.90 | 89.02 |
| | Mean ± S.D | 90.80 ± 1.03 | 92.11 ± 2.79 |
| | RSD (%) | 1.14 | 3.03 |
| 0.1 | $R_1$ | 91.25 | 95.35 |
| | $R_2$ | 89.50 | 94.64 |
| | $R_3$ | 92.75 | 89.90 |
| | Mean ± S.D | 91.17 ± 1.63 | 93.30 ± 2.96 |
| | RSD (%) | 1.79 | 3.22 |

Table 13: Recovery (%) of flubendiamide & des-iodo flubendiamide in pigeon pea straw

| Fortification Level (ppm) | Replication | Flubendiamide recovery (%) | Des-iodo flubendiamide recovery (%) |
|---|---|---|---|
| 0.01 | $R_1$ | 89.05 | 90.50 |
| | $R_2$ | 86.20 | 88.90 |
| | $R_3$ | 87.24 | 92.60 |
| | Mean ± S.D | 87.50 ± 1.44 | 90.67 ± 1.86 |
| | RSD (%) | 1.58 | 1.93 |
| 0.1 | $R_1$ | 89.50 | 91.45 |
| | $R_2$ | 88.80 | 90.50 |
| | $R_3$ | 88.10 | 89.75 |
| | Mean ± S.D | 88.80 ± 0.70 | 90.57 ± 0.85 |
| | RSD (%) | 0.76 | 0.88 |

## SECTION-B

### 4.2 Supervised field trials of flubendiamide in cabbage, tomato and pigeon pea

### 4.2.1 Recovery experiment of flubendiamide and des-iodo flubendiamide in cabbage head

The recovery results were presented in table 9 and the suitability of the method was discussed under 4.1.2 of section-A.

#### 4.2.1.1 Persistence and dissipation of flubendiamide in cabbage head

The analytical data relating to persistence and dissipation of flubendiamide 20% WG in cabbage head are summarized in table 14-15 & figure 17-18. A thorough scrutiny of the results indicated that initial deposit of flubendiamide was found to be 0.16 ppm and 0.31 ppm at the recommended ($T_1$) and double the recommended dose ($T_2$) respectively with corresponding reduction of 75 % and 77 % on $7^{th}$ day. It was observed that a slow and steady dissipation of flubendiamide occurred throughout the period of study. Residues of flubendiamide dissipated below detectable level of 0.01 ppm on $10^{th}$ day at recommended dose and 0.04 ppm at double the recommended dose which still exceeded the lower limit of quantification level. However, in case of $T_2$ the residue became below detectable level on $15^{th}$ day. Dissipation of flubendiamide residue followed first order reaction kinetics (Figure 19) in both the doses as a straight line was obtained in each case when log values of the residue were plotted against different time intervals. The significant correlation co-efficient ($r^2$=0.97-0.99) indicated statistical conformity of the dissipation data to first order kinetics. The calculated half life value from the regression equation (Table 16) was found to be 3.6 days ($T_1$) and 3.4 days ($T_2$).

Table14: Persistence and dissipation of flubendiamide @ 12.5 g a.i.ha$^{-1}$ in cabbage head

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | 0.15 | 0.17 | 0.16 | 0.16 ± 0.01 | - |
| 1 | 0.14 | 0.15 | 0.13 | 0.14 ± 0.01 | 12.5 |
| 3 | 0.08 | 0.07 | 0.08 | 0.08 ± 0.01 | 50.0 |
| 5 | 0.07 | 0.06 | 0.07 | 0.07 ± 0.01 | 56.3 |
| 7 | 0.03 | 0.04 | 0.04 | 0.04 ± 0.01 | 75.0 |
| 10 | BDL | BDL | BDL | BDL | 100.0 |
| 15 | BDL | BDL | BDL | BDL | 100.0 |

DAT-days after treatment, BDL-below detectable limit, SD- standard deviation

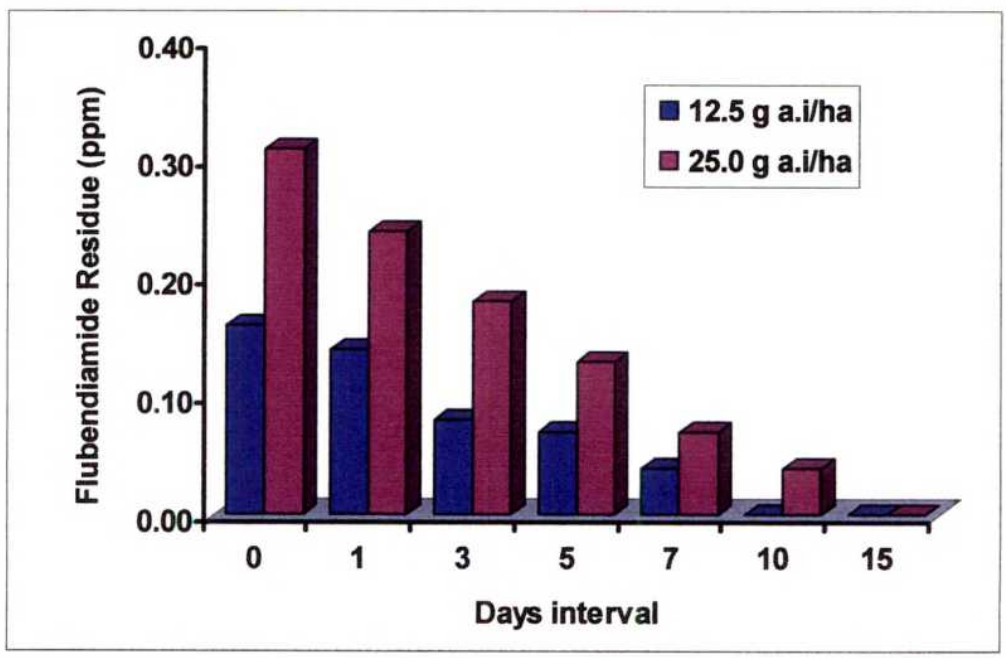



Figure 17: Persistence and dissipation of flubendiamide @12.5 and 25.0 g a.i ha$^{-1}$ in cabbage head

Table15: Persistence and dissipation of flubendiamide @ 25 g a.i.ha$^{-1}$ in cabbage head

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | 0.30 | 0.32 | 0.31 | 0.31 ± 0.01 | - |
| 1 | 0.23 | 0.24 | 0.24 | 0.24 ± 0.01 | 22.6 |
| 3 | 0.18 | 0.17 | 0.19 | 0.18 ± 0.01 | 42.0 |
| 5 | 0.12 | 0.14 | 0.13 | 0.13 ± 0.01 | 58.0 |
| 7 | 0.09 | 0.08 | 0.07 | 0.07 ± 0.01 | 77.0 |
| 10 | 0.04 | 0.03 | 0.05 | 0.04 ± 0.01 | 87.0 |
| 15 | BDL | BDL | BDL | BDL | 100.0 |

Table16: Results of statistical interpretation of dissipation data of flubendiamide in cabbage head

| Dose | Regression equation | $r^2$ | $T_{(1/2)}$ days |
|---|---|---|---|
| $T_1$ (12.5 g a.i.ha$^{-1}$) | y = 2.208-0.0837x | 0.97 | 3.6 |
| $T_2$ (25.0 g a.i.ha$^{-1}$) | y = 2.4991-0.0887x | 0.99 | 3.4 |

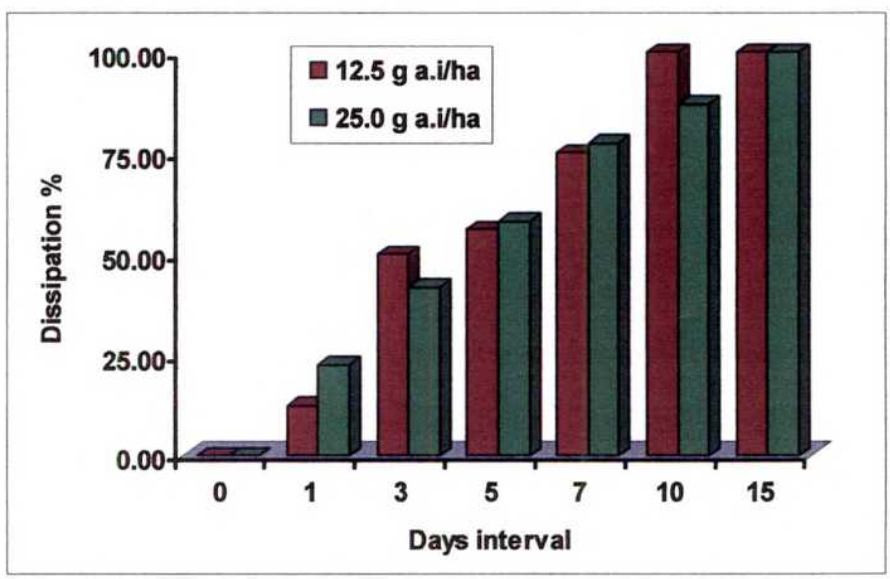



Figure 18: Dissipation percentage of flubendiamide (20% WG) in cabbage head

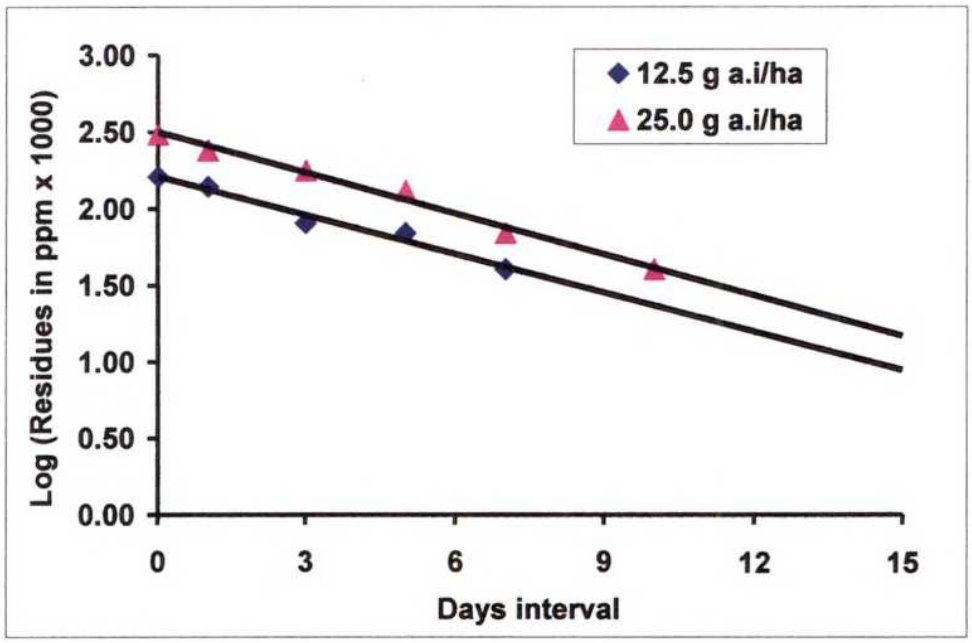



Figure 19: Linear plots of dissipation of flubendiamide (20% WG) in cabbage head

#### 4.2.1.2 Metabolism study of flubendiamide in cabbage head

The persistence and dissipation behaviour of the metabolite des-iodo flubendiamide in cabbage head are presented in table 17-18 and figure 20. From the table it was revealed that in the recommended dose the metabolite des-iodo flubendiamide could not be detected at day 0 and 1 which might be due to insignificant formation of quantifiable amount. The significant amount of metabolite formation was observed on third day in case of $T_1$ and persisted upto $5^{th}$ day whereas in $T_2$ the quantifiable amount metabolite formation started on first day and persisted upto $7^{th}$ day with the maximum amount of 0.08 ppm ($T_2$) on third day after last application. The metabolite residue gradually dissipated to 75 % on $7^{th}$ day ($T_2$). The calculated half life value of des-iodo flubendiamide in $T_2$ was found to be 1.94 days. The persistence and dissipation of des-iodo flubendiamide varied considerably in $T_1$ and $T_2$. It was observed that 60% metabolite dissipated at day 5 in case of $T_1$ and 50% in $T_2$. Upon application of flubendiamide 20% WG at double the recommended dose, it was found that metabolite concentration increased from 0.04 ppm (day 1) to 0.08 ppm (day 3) and thereafter decreased gradually and became BDL on day 10. The formation of des-iodo flubendiamide in cabbage was also reported by Justus *et al.*, 2007.

Table 17: Persistence and dissipation of des-iodo flubendiamide @12.5 g a.i.ha$^{-1}$ in cabbage head

| Days after treatment | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | BDL | BDL | BDL | BDL | - |
| 1 | BDL | BDL | BDL | BDL | - |
| 3 | 0.05 | 0.06 | 0.05 | 0.05 ± 0.01 | - |
| 5 | 0.02 | 0.03 | 0.01 | 0.02 ± 0.01 | 60.00 |
| 7 | BDL | BDL | BDL | BDL | 100.00 |
| 10 | BDL | BDL | BDL | BDL | 100.00 |
| 15 | BDL | BDL | BDL | BDL | 100.00 |

BDL-below detectable limit, SD- standard deviation

Table 18: Persistence and dissipation of des-odo flubendiamide @ 25 g a.i.ha$^{-1}$ in cabbage head

| DAR | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) | Regression equation | $T_{(1/2)}$ days |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | | |
| 0 | BDL | BDL | BDL | BDL | - | y = 2.3683-0.155x | 1.94 |
| 1 | 0.04 | 0.05 | 0.04 | 0.04 ± 0.01 | - | | |
| 3 | 0.09 | 0.08 | 0.07 | 0.08 ± 0.01 | - | | |
| 5 | 0.05 | 0.04 | 0.04 | 0.04 ± 0.01 | 50.00 | | |
| 7 | 0.01 | 0.02 | 0.02 | 0.02 ± 0.01 | 75.00 | | |
| 10 | BDL | BDL | BDL | BDL | 100.00 | | |
| 15 | BDL | BDL | BDL | BDL | 100.00 | $r^2 = 0.99$ | |

BDL-below detectable limit, SD- standard deviation

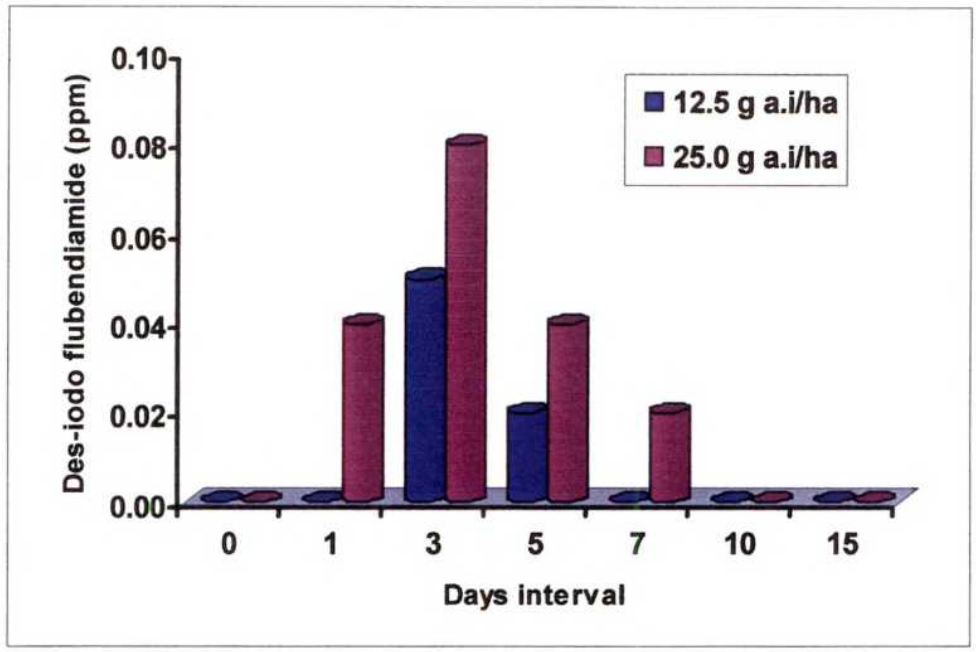



Figure 20: Formation of des iodo flubendiamide in cabbage head following application flubendiamide (20% WG) @ 12.5 and 25.0 g a.i.ha$^{-1}$

The total residue data of flubendiamide along with des-iodo flubendiamide in cabbage head were presented in table 19 – 20. The data revealed that the initial deposit of total residue was found to be 0.16 ppm and 0.31 ppm in case of $T_1$ and $T_2$ respectively which further dissipated to 12.5% ($T_1$) and 9.68% ($T_2$) on day 1 and steady reduction of residue occurred with increment of time. The residue became non detectable below 0.01 ppm on day 10 at the recommended dose and on day 15 at double the recommended dose.

Table 19: Persistence and dissipation of flubendiamide + des-iodo flubendiamide @12.5 g a.i.ha$^{-1}$ in cabbage head

| Days after treatment | Mean Residue (ppm) | | | Dissipation (%) |
|---|---|---|---|---|
| | Flubendiamide (a) | Des-iodo flubendiamide (b) | Total (a+b) | |
| 0 | 0.16 | BDL | 0.16 | - |
| 1 | 0.14 | BDL | 0.14 | 12.50 |
| 3 | 0.08 | 0.05 | 0.13 | 18.75 |
| 5 | 0.07 | 0.02 | 0.09 | 43.75 |
| 7 | 0.04 | BDL | 0.04 | 75.00 |
| 10 | BDL | BDL | BDL | 100.00 |
| 15 | BDL | BDL | BDL | 100.00 |

Table 20: Persistence and dissipation of flubendiamide + des-iodo flubendiamide @ 25 g a.i.ha$^{-1}$ in cabbage head

| Days after treatment | Mean Residue in ppm | | | Dissipation ( %) |
|---|---|---|---|---|
| | Flubendiamide (a) | Des-iodo flubendiamide (b) | Total (a+b) | |
| 0 | 0.31 | BDL | 0.31 | - |
| 1 | 0.24 | 0.04 | 0.28 | 9.68 |
| 3 | 0.18 | 0.08 | 0.26 | 16.13 |
| 5 | 0.13 | 0.04 | 0.17 | 45.16 |
| 7 | 0.07 | 0.02 | 0.09 | 70.97 |
| 10 | 0.04 | BDL | 0.04 | 87.10 |
| 15 | BDL | BDL | BDL | 100.00 |

The amount of des-iodo flubendiamide determined is converted to the equivalent amount of flubendiamide (Table. 21) for reporting purposes. This is readily accomplished by using the following equation:

$$\frac{\mu g\ \text{Des-iodo flubendiamide found}}{\text{Sample } mass(g)} \times 1.23 = \text{ppm flubendiamide}$$

The conversion factor, 1.23 corrects for the difference in molecular weight between flubendiamide and des-iodo flubendiamide.

Table 21: Des-iodo flubendiamide conversion to the equivalent amount of flubendiamide in cabbage head

| Days after treatment | 12.5 g a.i.ha$^{-1}$ | | | 25 g a.i.ha$^{-1}$ | | |
|---|---|---|---|---|---|---|
| | a (ppm) | b (ppm) | c (ppm) | a (ppm) | b (ppm) | c (ppm) |
| 0 | BDL | BDL | 0.16 | BDL | BDL | 0.31 |
| 1 | BDL | BDL | 0.14 | 0.04 | 0.05 | 0.29 |
| 3 | 0.05 | 0.06 | 0.14 | 0.08 | 0.10 | 0.28 |
| 5 | 0.02 | 0.02 | 0.09 | 0.04 | 0.05 | 0.18 |
| 7 | BDL | BDL | 0.04 | 0.02 | 0.02 | 0.09 |
| 10 | BDL | BDL | BDL | BDL | BDL | 0.04 |
| 15 | BDL | BDL | BDL | BDL | BDL | BDL |

a = Des-iodo flubendiamide, b = Equivalent amount of flubendiamide, c = Reportable total flubendiamide residue

The reportable total residue data (Table 21) as well as the residue data presented in table 14 and 15 revealed that initial deposits remained same for both $T_1$ and $T_2$. Further scrutiny of the residue data it was observed that in case of $T_1$ the flubendiamide dissipated to 50% on day 3 of which 77.5% ($\frac{0.062}{0.16-0.08} x100 = 77.5\ \%$) was accounted for by the formation of des-iodo flubendiamide metabolite and similarly in case of $T_2$ the dissipation percentage was 22.6% on day 1 of which 70.0% ($\frac{0.049}{0.31-0.24} x100 = 70.0\ \%$) metabolite was formed from this dissipated amount. Thus, des-iodo flubendiamide is the major metabolite formed via the process of deiodination in cabbage head.

Based on LOQ (0.01 ppm), the suggested safe waiting period found to be 14.54 and 16.79 days at $T_1$ and $T_2$ respectively. Thus, from the residue point of view, consumption of cabbage head is safe after 14 days of application of flubendiamide 20% WG @ 12.5 g a.i $ha^{-1}$.

**4.2.1.3 Persistence of flubendiamide and des-iodo flubendiamide in cabbage cropped soil at harvest**

The physicochemical properties of the cabbage field soil are shown in table 22.

Table 22: Physicochemical properties of cabbage cropped soil

| Soil characteristics | Estimated value |
|---|---|
| pH (Soil:Water,1:2.5) | 6.54 |
| EC (Soil: Water, 1:5)/$dSm^{-1}$ | 0.32 |
| CEC (cmol ($p^+$) /kg) | 22.0 |
| Organic carbon (g/kg) | 6.1 |
| Sand (%) | 13.6 |
| Silt (%) | 64.8 |
| Clay (%) | 21.6 |
| Texture | Silty loam |
| WHC | 28.3 |
| Taxonomic classification (USDA) | Typic Ustochrept |

**4.2.1.3.1 Recovery, limit of detection and limit of quantification**

The method was validated in cabbage cropped soil by conducting recovery experiments at levels ranging from 0.01 – 0.1 ppm for each flubendiamide and des-iodo flubendiamide. The results of recoveries are presented in table 23. Analytical recoveries of flubendiamide and des-iodo flubendiamide were more than 85%.

The limit of detection (LOD) and limit of quantification (LOQ) were statistically determined. The LOD and LOQ were found to be 0.003 ppm and 0.01 ppm for both (flubendiamide and des-iodo flubendiamide).

Table 23: Recovery % of flubendiamide and des-iodo flubendiamide in cabbage cropped soil

| Fortification level (ppm) | Replications | Flubendiamide recovery (%) | Des-iodo flubendiamide recovery (%) |
|---|---|---|---|
| 0.01 | $R_1$ | 87.8 | 84.5 |
| | $R_2$ | 85.9 | 89.3 |
| | $R_3$ | 88.3 | 85.4 |
| | Mean ± S.D | 85.5 ± 2.29 | 86.3 ± 2.68 |
| | RSD (%) | 2.67 | 3.10 |
| 0.10 | $R_1$ | 87.1 | 88.5 |
| | $R_2$ | 80.9 | 92.6 |
| | $R_3$ | 89.7 | 93.9 |
| | Mean ± S.D | 85.1 ± 3.40 | 90.5 ± 3.65 |
| | RSD (%) | 3.99 | 4.03 |

Considering the recovery percentage (85.1 – 90.5%) and RSD (2.29 – 4.03 %), the analytical method was adopted for estimation of flubendiamide and des-iodo flubendiamide residues in soil.

**4.2.1.3.2 Harvest residue of flubendiamide and des-iodo flubendiamide in cabbage cropped soil**

Harvest soil samples collected at 15 days after last spray of flubendiamide did not show the presence of either flubendiamide or des-iodo metabolite at detection limit of 0.01 ppm (Table 24) irrespective of treatments. This indicates that flubendiamide application is quiet safe for other crop in rotation.

Table 24: Harvest residue of flubendiamide and des-iodo flubendiamide in cabbage cropped soil

| Dose | Replicates | Flubendiamide residues (ppm) | Des-iodo flubendiamide residues (ppm) |
|---|---|---|---|
| $T_1$ – 12.5 g a.i.ha$^{-1}$ | $R_1$ | BDL | BDL |
| | $R_2$ | BDL | BDL |
| | $R_3$ | BDL | BDL |
| | Mean ± S.D | BDL | BDL |
| $T_2$ – 25 g a.i.ha$^{-1}$ | $R_1$ | BDL | BDL |
| | $R_2$ | BDL | BDL |
| | $R_3$ | BDL | BDL |
| | Mean ± S.D | BDL | BDL |

BDL-below detectable limit, S.D- standard deviation

### 4.2.2 Recovery experiment of flubendiamide and des-iodo flubendiamide in tomato fruit

The recovery results were presented in table 10 and the suitability of the method was discussed under 4.1.2 of section-A.

#### 4.2.2.1 Persistence and dissipation of flubendiamide in tomato fruit

The analytical data relating to persistence and dissipation of flubendiamide 20% WG in tomato fruit are summarized in table 25-26 & figure 21-22. A thorough scrutiny of the results indicated that initial deposit of flubendiamide was found to be 0.27 ppm and 0.46 ppm at the recommended ($T_1$) and double the recommended dose ($T_2$) respectively with corresponding reduction of 43-44 % irrespective of treatment on first day. It was observed that further reduction of flubendiamide took place more than 80% on fifth day. Residues of flubendiamide dissipated below detectable level of 0.01 ppm on 7$^{th}$ day at recommended dose and double the recommended dose. Dissipation of flubendiamide residue followed first order reaction kinetics (Figure 23) in both the doses as a straight line was obtained in each case when log values of the residue were plotted against different time intervals. The significant correlation co-efficient ($r^2$=0.95-0.99) indicated

statistical conformity of the dissipation data to first order kinetics. The calculated half life value from the regression equation (Table 27) was found to be 1.64 days ($T_1$) and 1.98 days ($T_2$).

Table 25: Persistence and dissipation of flubendiamide @ 50 g a.i.ha$^{-1}$ in tomato fruit

| DAT | Residue in ppm | | | Mean residue | Dissipation |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | (ppm) ± S.D | (%) |
| 0 | 0.24 | 0.29 | 0.27 | 0.27 ± 0.03 | - |
| 1 | 0.12 | 0.17 | 0.15 | 0.15 ± 0.03 | 44.4 |
| 3 | 0.06 | 0.10 | 0.08 | 0.08 ± 0.02 | 70.4 |
| 5 | 0.03 | 0.04 | 0.03 | 0.03 ± 0.01 | 88.9 |
| 7 | BDL | BDL | BDL | BDL | 100.0 |
| 10 | BDL | BDL | BDL | BDL | 100.0 |

DAT-days after treatment, BDL-below detectable limit (0.01ppm), SD- standard deviation

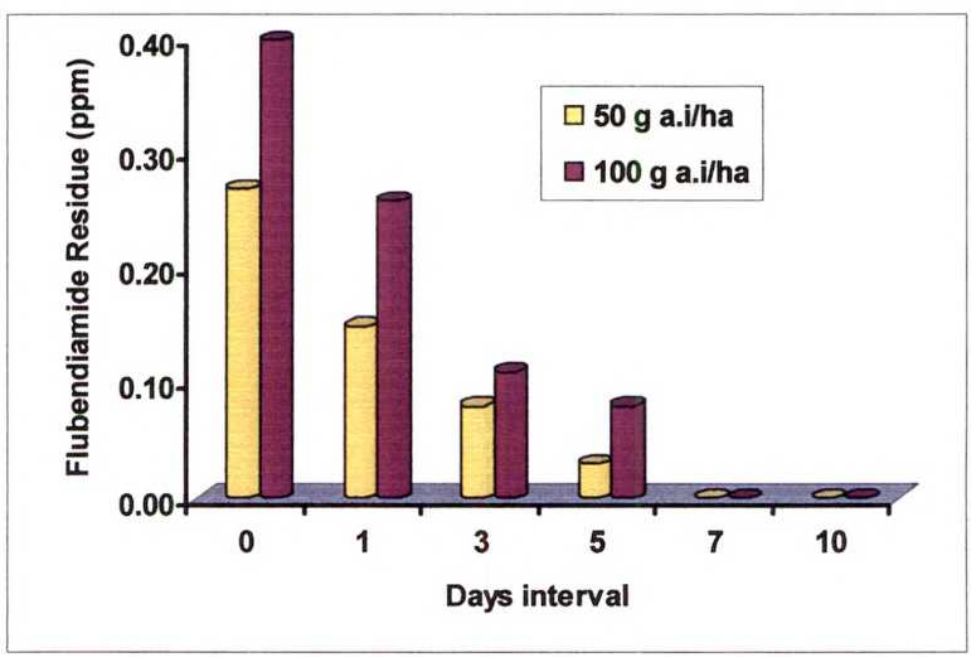



Figure 21: Persistence and dissipation of flubendiamide @ 50 and 100 g a.i.ha$^{-1}$ in tomato fruit

Table 26: Persistence and dissipation of flubendiamide @ 100 g a.i.ha$^{-1}$ in tomato fruit

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | 0.44 | 0.49 | 0.46 | 0.46 ± 0.03 | - |
| 1 | 0.22 | 0.25 | 0.31 | 0.26 ± 0.05 | 43.5 |
| 3 | 0.09 | 0.11 | 0.14 | 0.11 ± 0.03 | 76.1 |
| 5 | 0.07 | 0.10 | 0.08 | 0.08 ± 0.02 | 82.6 |
| 7 | BDL | BDL | BDL | BDL | 100.0 |
| 10 | BDL | BDL | BDL | BDL | 100.0 |

DAT-days after treatment, BDL-below detectable limit (0.01ppm), SD- standard deviation

Table 27: Results of statistical interpretation of dissipation data of flubendiamide in tomato fruit

| Dose | Regression equation | $r^2$ | $T_{(1/2)}$ days |
|---|---|---|---|
| $T_1$ (50 g a.i.ha$^{-1}$) | y = 2.409- 0.1831x | 0.99 | 1.64 |
| $T_2$ (100 g a.i.ha$^{-1}$) | y = 2.5981- 0.1522x | 0.95 | 1.98 |

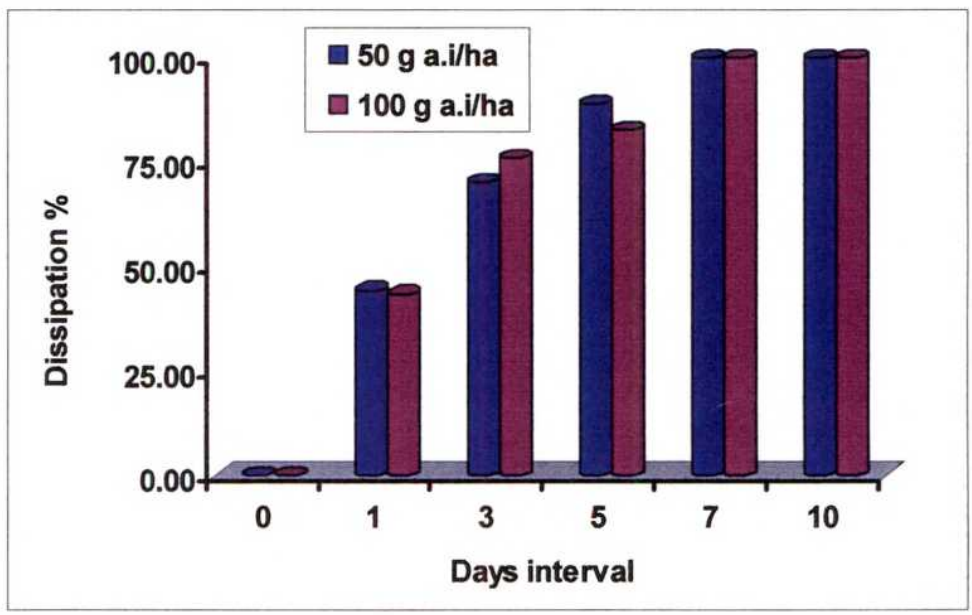



Figure 22: Dissipation percentage of flubendiamide (20% WG) in tomato fruit

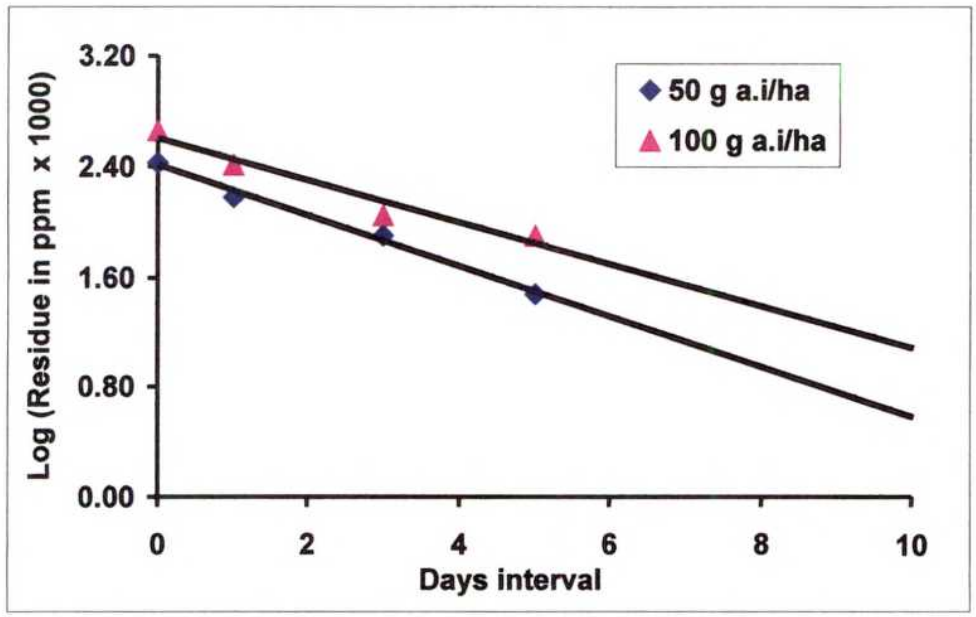



Figure 23: Linear plots of dissipation of flubendiamide (20% WG) in tomato fruit

#### 4.2.2.2 Metabolism study of flubendiamide in tomato fruit

The persistence and dissipation behaviour of the metabolite des-iodo flubendiamide in tomato fruit are presented in table 28-29 and figure 24. From the table it was revealed that in the recommended and double the recommended dose, the metabolite des-iodo flubendiamide could not be detected after two hours of spraying which might be due to insignificant formation of quantifiable amount. The significant amount of metabolite formation was observed on 1$^{st}$ day and persisted upto 3$^{rd}$ day in case of $T_1$ and persisted upto 5$^{th}$ day in $T_2$ with the maximum amount of 0.08 ppm ($T_1$) and 0.15 ppm ($T_2$) on first day after last application. The metabolite residue gradually dissipated to 37.5 – 46.67 % on 3$^{rd}$ day irrespective of treatments. The calculated half life value of des-iodo flubendiamide in $T_2$ was found to be 3.02 days. The residue of the metabolite became non-detectable on 5$^{th}$ day in $T_1$ and on 7$^{th}$ day in $T_2$. The formation of des-iodo flubendiamide in tomato was also reported by Justus *et al.*, 2007.

Table 28: Persistence and dissipation of des-iodo flubendiamide @ 50 g a.i.ha$^{-1}$ in tomato fruit

| Days after treatment | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | BDL | BDL | BDL | BDL | - |
| 1 | 0.08 | 0.07 | 0.10 | 0.08 ± 0.02 | - |
| 3 | 0.06 | 0.04 | 0.05 | 0.05 ± 0.01 | 37.50 |
| 5 | BDL | BDL | BDL | BDL | 100.00 |
| 7 | BDL | BDL | BDL | BDL | 100.00 |
| 10 | BDL | BDL | BDL | BDL | 100.00 |

BDL-below detectable limit (0.01ppm), SD- standard deviation

Table 29: Persistence and dissipation of des-iodo flubendiamide @ 100 g a.i.ha$^{-1}$ in tomato fruit

| Days after treatment | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | BDL | BDL | BDL | BDL | - |
| 1 | 0.18 | 0.12 | 0.15 | 0.15 ± 0.03 | - |
| 3 | 0.08 | 0.10 | 0.07 | 0.08 ± 0.02 | 46.67 |
| 5 | 0.06 | 0.07 | 0.05 | 0.06 ± 0.01 | 60.00 |
| 7 | BDL | BDL | BDL | BDL | 100.00 |
| 10 | BDL | BDL | BDL | BDL | 100.00 |
| Regression equation | | y = 2.2509-0.0995x | | | |
| $T_{(1/2)}$ days | | 3.025 | | | |

BDL-below detectable limit (0.01ppm), SD- standard deviation

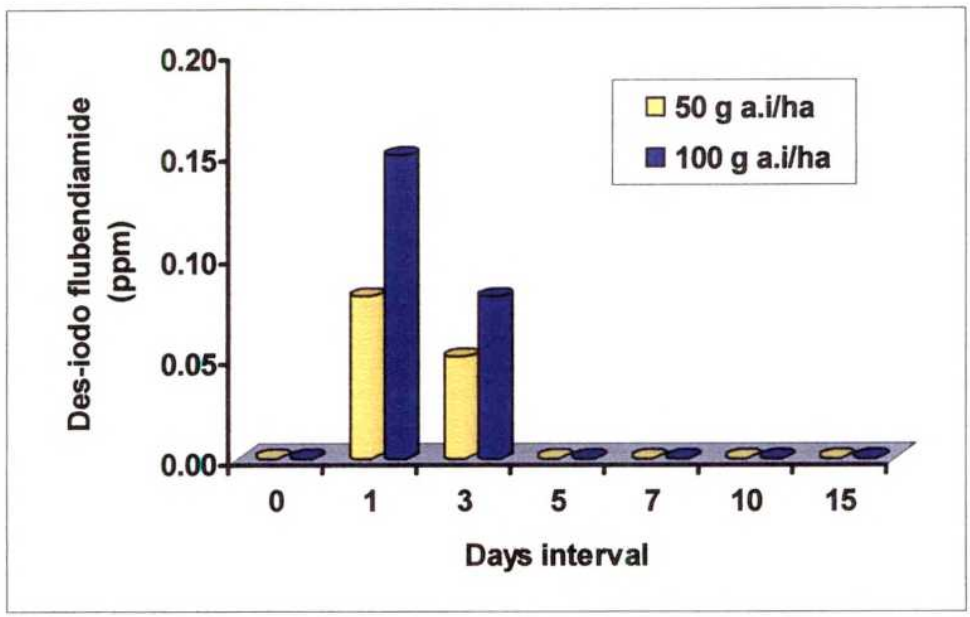



Figure 24: Formation of des-iodo flubendiamide in tomato fruit following application flubendiamide (20% WG) @ 50 and 100 g a.i.ha$^{-1}$

The total residue data of flubendiamide along with des-iodo flubendiamide in tomato fruits are presented in table 30-31. The data revealed that the initial deposit of total residue was found to be 0.27 ppm and 0.46 ppm in case of $T_1$ and $T_2$ respectively which further dissipated to 14.81 % ($T_1$) and 10.87 % ($T_2$) on day 1 and steady reduction of residue occurred with increment of time. The residue became non detectable below 0.01 ppm on day 7$^{th}$ day at the recommended and at double the recommended dose.

Table 30: Persistence & dissipation of flubendiamide + des-iodo @ 50 g a.i.ha$^{-1}$ in tomato

| Days after treatment | Mean Residue in ppm | | | Dissipation (%) |
|---|---|---|---|---|
| | Flubendiamide (a) | Des-iodo flubendiamide (b) | Total (a+b) | |
| 0 | 0.27 | BDL | 0.27 | - |
| 1 | 0.15 | 0.08 | 0.23 | 14.81 |
| 3 | 0.08 | 0.05 | 0.13 | 51.85 |
| 5 | 0.03 | BDL | 0.03 | 88.89 |
| 7 | BDL | BDL | BDL | 100.00 |
| 10 | BDL | BDL | BDL | 100.00 |

BDL-below detectable limit (0.01ppm)

Table 31: Persistence and dissipation of flubendiamide + des-iodo flubendiamide @ 100 g a.i.ha$^{-1}$ in tomato fruit

| Days after treatment | Mean Residue in ppm | | | Dissipation (%) |
|---|---|---|---|---|
| | Flubendiamide (a) | Des-iodo flubendiamide (b) | Total (a+b) | |
| 0 | 0.46 | BDL | 0.46 | - |
| 1 | 0.26 | 0.15 | 0.41 | 10.87 |
| 3 | 0.11 | 0.08 | 0.19 | 58.70 |
| 5 | 0.08 | 0.06 | 0.14 | 69.57 |
| 7 | BDL | BDL | BDL | 100.00 |
| 10 | BDL | BDL | BDL | 100.00 |

BDL-below detectable limit (0.01ppm), SD- standard deviation

The amount of des-iodo flubendiamide determined is converted to the equivalent amount of flubendiamide (Table 32) for reporting purposes. This is readily accomplished by using the equation shown in 4.2.1.2 of section B.

Table 32: Des-iodo flubendiamide conversion to the equivalent amount of flubendiamide in tomato fruit

| Days after treatment | $T_1$ (50 g a.i ha$^{-1}$) | | | $T_2$ (100 g a.i ha$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | a (ppm) | b (ppm) | c (ppm) | a (ppm) | b (ppm) | c (ppm) |
| 0 | BDL | BDL | 0.27 | BDL | BDL | 0.46 |
| 1 | 0.08 | 0.10 | 0.25 | 0.15 | 0.18 | 0.44 |
| 3 | 0.05 | 0.06 | 0.14 | 0.08 | 0.10 | 0.21 |
| 5 | BDL | BDL | 0.03 | 0.06 | 0.07 | 0.15 |
| 7 | BDL | BDL | BDL | BDL | BDL | BDL |
| 10 | BDL | BDL | BDL | BDL | BDL | BDL |

a = Des-iodo flubendiamide, b = Equivalent amount of flubendiamide, c = Reportable total flubendiamide residue, BDL-below detectable limit (0.01ppm), SD- standard deviation

The reportable total residue data (Table 32) as well as the residue data presented in table 25 and 26 revealed that initial deposits remained same for both $T_1$ and $T_2$. Further scrutiny of the residue data it was observed that in case of $T_1$ the flubendiamide dissipated to 44.4 % on day 1 of which 81.66 % ($\frac{0.098}{0.27-0.08} x100 = 81.66\ \%$) was accounted for by the formation of des-iodo flubendiamide metabolite and similarly in case of $T_2$ the dissipation percentage was 43.5 % on day 1 of which 92.5 % ($\frac{0.185}{0.46-0.15} x\ 100 = 92.5\ \%$ ) metabolite was formed from this dissipated amount. Thus, des-iodo flubendiamide is the major metabolite formed via the process of deiodination in tomato fruits.

Based on LOQ (0.01 mg/kg), the suggested safe waiting period found to be 7.7 and 10.6 days irrespective of $T_1$ and $T_2$. Based on the residue data, it was concluded that after application of flubendiamide 20% WG at recommended dose (50 g a.i. / ha) during fruiting stage, the tomato fruit may be plucked at 7 days for safe consumption.

#### 4.2.2.3 Persistence of flubendiamide and des-iodo flubendiamide in tomato cropped soil at harvest

The physicochemical properties of the tomato field soil are shown in table 33.

Table 33: Physicochemical properties of tomato cropped soil

| Soil characteristics | Estimated value |
|---|---|
| pH (Soil:Water,1:2.5) | 6.50 |
| EC (Soil: Water, 1:5)/$dSm^{-1}$ | 0.12 |
| CEC (cmol ($p^+$) /kg) | 18.4 |
| Organic carbon (g/kg) | 6.00 |
| Sand (%) | 41.7 |
| Silt (%) | 19.6 |
| Clay (%) | 38.7 |
| Texture | Clay loam |
| WHC (%) | 42.3 |
| Taxonomic classification (USDA) | Typic Ustochrept |

##### 4.2.2.3.1 Recovery, limit of detection and limit of quantification

The results of recoveries in tomato cropped soil are presented in table 34 and revealed that the method under investigation is suitable for determining residual level of flubendiamide and des-iodo flubendiamide in soil considering the analytical recoveries (86.77-92.18%), LOD (0.003 ppm), LOQ (0.01 ppm) and RSD (1.30 – 2.09 %).

Table 34: Recovery % of flubendiamide and des-iodo flubendiamide in tomato cropped soil

| Fortification level (ppm) | Replications | Flubendiamide recovery (%) | Des-iodo flubendiamide recovery (%) |
|---|---|---|---|
| 0.01 | $R_1$ | 88.80 | 90.30 |
| | $R_2$ | 86.20 | 88.50 |
| | $R_3$ | 85.30 | 88.01 |
| | Mean ± S.D | 86.77 ± 1.82 | 89.02 ± 1.17 |
| | RSD (%) | 2.09 | 1.30 |
| 0.10 | $R_1$ | 92.46 | 94.25 |
| | $R_2$ | 90.25 | 90.50 |
| | $R_3$ | 89.50 | 91.80 |
| | Mean ± S.D | 90.74 ± 1.54 | 92.18± 1.91 |
| | RSD (%) | 1.70 | 2.07 |

**4.2.2.3.2 Harvest residue of flubendiamide and des-iodo flubendiamide in tomato cropped soil**

Harvest soil samples collected at 10 days after last spray of flubendiamide did not show the presence of either flubendiamide or des-iodo metabolite at detection limit of 0.01 ppm (Table 35) irrespective of treatments. This indicates that flubendiamide application is quiet safe for succeeding crop.

Table 35: Harvest residue of flubendiamide and des-iodo flubendiamide in tomato soil

| Dose | Replicates | Flubendiamide residues (ppm) | Des-iodo flubendiamide residues (ppm) |
|---|---|---|---|
| $T_1$ – 50 g a.i. $ha^{-1}$ | $R_1$ | BDL | BDL |
| | $R_2$ | BDL | BDL |
| | $R_3$ | BDL | BDL |
| | Mean ± S.D | BDL | BDL |
| $T_2$ – 100 g a.i. $ha^{-1}$ | $R_1$ | BDL | BDL |
| | $R_2$ | BDL | BDL |
| | $R_3$ | BDL | BDL |
| | Mean ± S.D | BDL | BDL |

BDL-below detectable limit (0.01ppm), S.D- standard deviation

### 4.2.3 Recovery experiment of flubendiamide and des-iodo flubendiamide in pigeon pea grain, shell and straw

The recovery results were presented in table 11-13 and the suitability of the method was discussed under 4.1.2 of section-A.

#### 4.2.3.1 Harvest time residues of flubendiamide and des-iodo flubendiamide in Pigeon pea grain, shell and straw

Data pertaining to the residual level of flubendiamide and its metabolite des-iodo in/on pigeon pea resulting from spray application of flubendiamide 20% WG @ 50 & 100 g a.i.$ha^{-1}$ and are presented in table 36-37.

Residues of flubendiamide in pigeon pea grain, shell and straw samples were not detected at detection limit of 0.01 ppm at harvest time which happens to be 42 days after last application of the insecticide. Gopal and Mishra (2008) also reported that flubendiamide could not be detected at harvested paddy straw and husk. The samples of pigeon pea grain, straw and shell did not show the presence of des-iodo flubendiamide at limit of quantification of 0.01 ppm.

Thus, it may be inferred that spray application of flubendiamide 20% WG on pigeon pea @ 50 & 100 g a.i.$ha^{-1}$ will not pose neither any serious contamination problem nor build up of any residues in food chain.

Table 36: Harvest time residues of flubendiamide in pigeon pea grain, shell and straw

| Substrates | Residues of flubendiamide (ppm) | | | |
|---|---|---|---|---|
| | $T_1$ - 50 g a.i. $ha^{-1}$ | | $T_2$ -100 g a.i. $ha^{-1}$ | |
| | Replicates | Mean ± S.D | Replicates | Mean ± S.D |
| Pigeon pea grain | BDL<br>BDL<br>BDL | BDL | BDL<br>BDL<br>BDL | BDL |
| Pigeon pea shell | BDL<br>BDL<br>BDL | BDL | BDL<br>BDL<br>BDL | BDL |
| Pigeon pea straw | BDL<br>BDL<br>BDL | BDL | BDL<br>BDL<br>BDL | BDL |

BDL-below detectable limit (0.01ppm), SD- standard deviation

Table 37: Harvest time residues of des-iodo flubendiamide in pigeon pea grain, shell and straw

| Substrates | Residues of des-iodo flubendiamide (ppm) | | | |
|---|---|---|---|---|
| | $T_1$ - 50 g a.i. $ha^{-1}$ | | $T_2$ -100 g a.i. $ha^{-1}$ | |
| | Replicates | Mean ± S.D | Replicates | Mean ± S.D |
| Pigeon pea grain | BDL<br>BDL<br>BDL | BDL | BDL<br>BDL<br>BDL | BDL |
| Pigeon pea shell | BDL<br>BDL<br>BDL | BDL | BDL<br>BDL<br>BDL | BDL |
| Pigeon pea straw | BDL<br>BDL<br>BDL | BDL | BDL<br>BDL<br>BDL | BDL |

BDL-below detectable limit (0.01ppm), SD- standard deviation

#### 4.2.3.2 Harvest time residues of flubendiamide and des-iodo flubendiamide in pigeon pea cropped soil

The physicochemical properties of the pigeon pea field soil were analyzed and are shown in table 38.

Table 38: Physicochemical properties of pigeon pea cropped soil

| Soil characteristics | Estimated value |
|---|---|
| pH (Soil:Water,1:2.5) | 6.20 |
| EC (Soil: Water, 1:5)/$dSm^{-1}$ | 0.45 |
| CEC (cmol ($p^+$) /kg) | 28.4 |
| Organic carbon (g/kg) | 6.30 |
| Sand (%) | 25.2 |
| Silt (%) | 30.0 |
| Clay (%) | 44.8 |
| Texture | Clay loam |
| WHC (%) | 58.0 |
| Taxonomic classification (USDA) | Fine Aeric Fluvaquent |

##### 4.2.3.2.1 Recovery, limit of detection and limit of quantification

The method was validated by conducting recovery experiments in pigeon pea cropped soil at levels ranging from 0.01 – 0.1 ppm for each flubendiamide and des-iodo flubendiamide. The results of recoveries are presented in table 39. Analytical recoveries of flubendiamide and des-iodo flubendiamide were found to be in the range of 87-90%.

The LOD and LOQ were found to be 0.003 ppm and 0.01 ppm irrespective of flubendiamide and des-iodo flubendiamide respectively. The RSD varied from 1.37 – 2.46 %. Considering the recovery percentage, RSD and LOQ the analytical method was found to be suitable for residue analysis.

Table 39: Recovery % of flubendiamide and des-iodo flubendiamide in pigeon pea cropped soil

| Fortification level (ppm) | Replications | Flubendiamide recovery (%) | Des-iodo flubendiamide recovery (%) |
|---|---|---|---|
| 0.01 | $R_1$ | 86.50 | 89.75 |
| | $R_2$ | 87.75 | 90.50 |
| | $R_3$ | 89.65 | 88.10 |
| | Mean ± S.D | 87.97 ± 1.59 | 89.45 ± 1.23 |
| | RSD (%) | 1.80 | 1.37 |
| 0.10 | $R_1$ | 92.50 | 89.70 |
| | $R_2$ | 89.30 | 90.40 |
| | $R_3$ | 88.25 | 88.20 |
| | Mean ± S.D | 90.02 ± 2.21 | 89.43 ± 1.12 |
| | RSD (%) | 2.46 | 1.26 |

**4.2.3.2.2 Harvest residue of flubendiamide and des-iodo flubendiamide in pigeon pea cropped soil**

Harvest soil samples collected at 42 days after last spray of flubendiamide did not show the presence of either flubendiamide or des-iodo metabolite at detection limit of 0.01 ppm (Table 40) irrespective of treatments. This indicates that flubendiamide application is quiet safe for other crop in rotation.

Table 40: Harvest residue of flubendiamide and des-iodo flubendiamide in pigeon pea cropped soil

| Dose | Replicates | Flubendiamide residues (ppm) | Des-iodo Flubendiamide residues (ppm) |
|---|---|---|---|
| $T_1$ – 50 g a.i. $ha^{-1}$ | $R_1$ | BDL | BDL |
| | $R_2$ | BDL | BDL |
| | $R_3$ | BDL | BDL |
| | Mean ± S.D | BDL | BDL |
| $T_2$ – 100 g a.i. $ha^{-1}$ | $R_1$ | BDL | BDL |
| | $R_2$ | BDL | BDL |
| | $R_3$ | BDL | BDL |
| | Mean ± S.D | BDL | BDL |

BDL-below detectable limit (0.01ppm), SD- standard deviation

SECTION-C

## 4.3 Persistence and dissipation study of flubendiamide in three types of soil under laboratory condition

### 4.3.1 Physicochemical properties of the soil under study

The physicochemical properties (pH, CEC, EC, OC and textural class) of new alluvial soil, red and lateritic soil, and coastal soil are presented in table 41.

Table 41: Physicochemical properties of three different soils

| Location | Mondouri | Jhargram | Canning |
|---|---|---|---|
| Zone | New Alluvial | Red and Lateritic | Coastal |
| pH (1:2.5) | 6.20 | 5.80 | 7.80 |
| CEC [$C_{mol}$ ($P^+$) $kg^{-1}$] | 13.6 | 14.7 | 14.8 |
| EC (ds/m at 25°C) | 1.40 | 0.80 | 4.60 |
| OC (%) | 0.63 | 0.60 | 0.93 |
| Sand (%) | 20.8 | 45.0 | 41.9 |
| Silt (%) | 56.2 | 28.3 | 23.9 |
| Clay (%) | 23.0 | 26.7 | 35.2 |
| Soil order | Inceptisol | Alfisol | Inceptisol |
| Taxonomic class | Haplaquept | Ustalf | Haplaquept |

### 4.3.2 Analytical method for the estimation of flubendiamide and des-iodo flubendiamide residues

The analytical method adopted for the estimation of flubendiamide and des-iodo flubendiamide residue in soil was by HPLC and the chromatogram of analytical standard mixture was already depicted in figure 8 of section A and the retention time (RT) was found to be 8.93 and 7.08 minutes respectively. The operating conditions for HPLC analysis were stated under 3.1.4.8 of Section A.

### 4.3.3 Results of Recovery experiment in new alluvial soil, red and lateritic soil and coastal soil

The results of recovery experiment of flubendiamide and des-iodo flubendiamide in soils of three different zones of West Bengal are presented in table 42-43. The results revealed that the average recovery percentage of flubendiamide in new alluvial soil was

found to be 87.47 - 88.58% with RSD of 1.49 – 1.61%, in case of red and lateritic soil it was 89.05 – 93.3% with RSD < 2% and in coastal soil it varied from 89.62 – 91.32% with RSD of 0.76 – 1.35% at the fortification level of 0.01 – 0.10 ppm.

Table 42: Recovery results of flubendiamide in three different soils

| Soil Zone | Fortification Level (ppm) | Recovery percentage | | | Mean ± S.D | RSD (%) |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | | |
| New alluvial | 0.01 | 87.90 | 86.02 | 88.5 | 87.47 ± 1.31 | 1.49 |
| | 0.10 | 87.95 | 90.21 | 87.58 | 88.58 ± 1.42 | 1.61 |
| Red and lateritic | 0.01 | 87.40 | 89.25 | 90.50 | 89.05 ± 1.56 | 1.75 |
| | 0.10 | 92.40 | 93.00 | 94.50 | 93.30 ± 1.08 | 1.16 |
| Coastal | 0.01 | 88.90 | 89.70 | 90.25 | 89.62 ± 0.68 | 0.76 |
| | 0.10 | 91.50 | 90.01 | 92.45 | 91.32 ± 1.24 | 1.35 |

Similarly, at the fortification level of 0.01 – 0.10 ppm, the average recovery percentage of des-iodo flubendiamide in new alluvial, red and lateritic and coastal soil was found to be 90.73 – 93.35%, 91.37 – 96.6% and 93.08 – 94.34% respectively. The RSD of three different soils were not more than 2.08%. As the average recovery percentage was more than 87% for all three soils, hence the analytical method was quite satisfactory and adopted for the residue and dissipation study of flubendiamide in soils under laboratory condition.

Table 43: Recovery results of des-iodo flubendiamide in three different soils

| Soil Zone | Fortification Level (ppm) | Recovery percentage | | | Mean ± S.D | RSD (%) |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | | |
| New alluvial | 0.01 | 89.50 | 90.20 | 92.50 | 90.73 ± 1.57 | 1.73 |
| | 0.10 | 93.40 | 92.00 | 94.35 | 93.25 ± 1.18 | 1.27 |
| Red and lateritic | 0.01 | 91.75 | 90.25 | 92.10 | 91.37 ± 0.98 | 1.08 |
| | 0.10 | 96.40 | 98.70 | 94.70 | 96.60 ± 2.01 | 2.08 |
| Coastal | 0.01 | 93.20 | 92.25 | 93.80 | 93.08 ± 0.78 | 0.84 |
| | 0.10 | 95.81 | 93.75 | 93.45 | 94.34 ± 1.28 | 1.36 |

### 4.3.4 Persistence and dissipation behaviour of flubendiamide in new alluvial (Mondouri) soil under laboratory condition

The persistence and dissipation behaviour of flubendiamide in new alluvial soil was carried out in the laboratory condition for a period of 150 days and the analytical data are presented in table 44 – 45. No residue of flubendiamide could be detected in control samples during the study period. The results indicated that the initial deposits (after 2 hours of application) of flubendiamide in mondouri soil at 5 ppm ($T_1$) and 10 ppm ($T_2$) were found to be 4.55 and 9.05 ppm respectively. The residue declined progressively with time in both the doses but at a very slow dissipation rate as evidenced from the data of 3 days with a dissipation percentage of 0.81 - 0.88%. At day seven, the residue was found to be 4.02 ($T_1$) and 8.00 ($T_2$) ppm with a corresponding dissipation of 11.65 and 11.6 % respectively. Moreover, 50% flubendiamide remained in the soil after 30 days in $T_1$ and 60 days in case of $T_2$. Flubendiamide dissipated out 91% at 120 days in case of $T_1$ but 92% dissipation occurred in case of $T_2$ at 150 days (Figure 25). However, no residue could be quantified below the level of quantification of 0.01 ppm in case of $T_1$ at 150 days.

The data on flubendiamide residues in soil at different days was subjected to statistical analysis to give the regression equation and residual half-life ($T_{1/2}$) based on mean residue values (Table 44-45). The half-life value calculated was found to be 37.16 days ($T_1$) and 44.9 days ($T_2$). The dissipation rate followed first order kinetics irrespective of any treatments (Figure 26).

Table 44: Persistence and dissipation of flubendiamide (5 ppm) in new alluvial soil

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) | Regression equation | $T_{(1/2)}$ days |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | | |
| 0 | 4.62 | 4.54 | 4.48 | 4.55 ± 0.07 | - | y = 3.668-0.0081x | 37.16 |
| 3 | 4.45 | 4.49 | 4.6 | 4.51 ± 0.08 | 0.81 | | |
| 7 | 3.98 | 4.02 | 4.06 | 4.02 ± 0.04 | 11.65 | | |
| 15 | 3.42 | 3.75 | 3.5 | 3.56 ± 0.17 | 21.83 | | |
| 30 | 2.41 | 2.25 | 2.28 | 2.31 ± 0.09 | 49.16 | | |
| 60 | 1.72 | 1.68 | 1.81 | 1.74 ± 0.07 | 61.83 | | |
| 90 | 0.95 | 1.12 | 1.16 | 1.08 ± 0.11 | 76.34 | | |
| 120 | 0.43 | 0.39 | 0.41 | 0.41 ± 0.02 | 90.99 | | |
| 150 | BDL | BDL | BDL | BDL | 100.00 | $r^2 = 0.97$ | |

Table 45: Persistence and dissipation of flubendiamide (10 ppm) in new alluvial soil

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) | Regression equation | $T_{(1/2)}$ days |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | | |
| 0 | 9.15 | 8.96 | 9.04 | 9.05 ± 0.10 | - | y = 3.976-0.0067x | 44.92 |
| 3 | 8.98 | 9.05 | 8.88 | 8.97 ± 0.09 | 0.88 | | |
| 7 | 7.94 | 8.1 | 7.96 | 8.00 ± 0.09 | 11.60 | | |
| 15 | 7.18 | 6.97 | 7.08 | 7.08 ± 0.11 | 21.80 | | |
| 30 | 5.58 | 5.71 | 5.55 | 5.61 ± 0.09 | 37.97 | | |
| 60 | 4.32 | 4.48 | 4.26 | 4.35 ± 0.11 | 51.90 | | |
| 90 | 3.12 | 3.22 | 3.24 | 3.19 ± 0.06 | 64.71 | | |
| 120 | 1.54 | 1.62 | 1.52 | 1.56 ± 0.05 | 82.76 | | |
| 150 | 0.67 | 0.82 | 0.73 | 0.74 ± 0.08 | 91.82 | $R^2 = 0.97$ | |

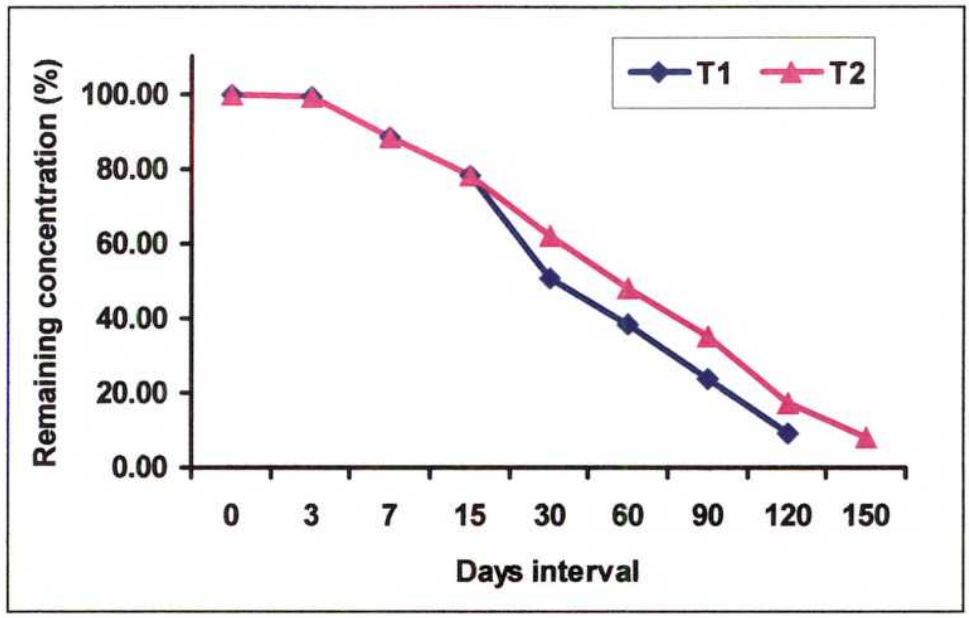



Figure 25: Percent remaining of flubendiamide in new alluvial soil

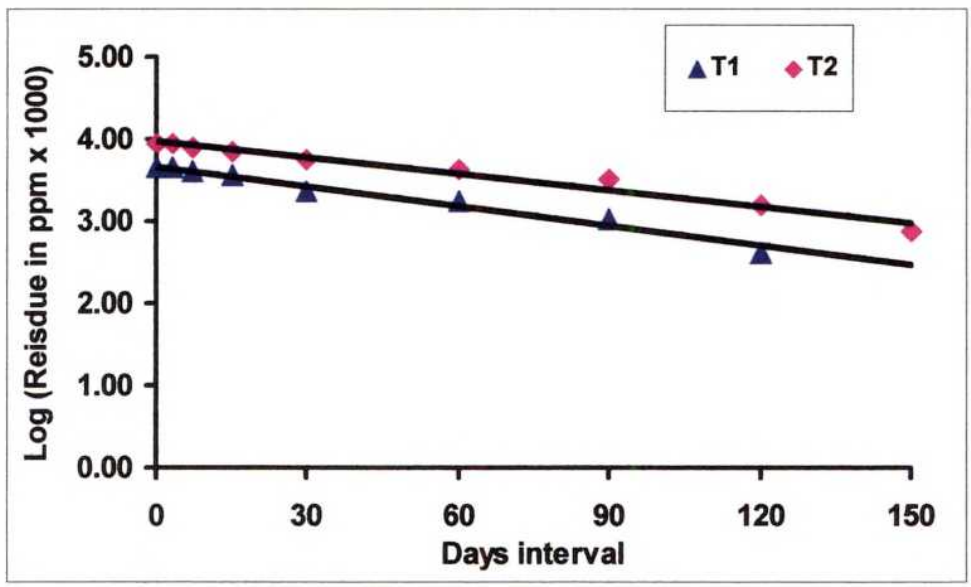



Figure 26: Linear plots of dissipation of flubendiamide in new alluvial soil

Interestingly, the des-iodo flubendiamide metabolite was also identified and quantified in mondouri soil. The analytical data are presented in table 46-47. The formation of des-iodo flubendiamide after 2 hours of application was found to be 0.17 and 0.29 ppm for $T_1$ and $T_2$ respectively. The residue increased to a level of 0.28-0.62 ppm at day seven and there after, a progressive declination of the metabolite occurred and 72.58 – 82.14 % dissipated out at 90 days irrespective of doses.

Table 46: Persistence and dissipation of des-iodo flubendiamide (5 ppm) in new alluvial soil

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | 0.16 | 0.17 | 0.18 | 0.17 ± 0.01 | - |
| 3 | 0.20 | 0.22 | 0.20 | 0.21 ± 0.01 | - |
| 7 | 0.32 | 0.28 | 0.24 | 0.28 ± 0.04 | - |
| 15 | 0.22 | 0.18 | 0.21 | 0.20 ± 0.02 | 27.38 |
| 30 | 0.12 | 0.14 | 0.13 | 0.13 ± 0.01 | 53.57 |
| 60 | 0.09 | 0.12 | 0.10 | 0.10 ± 0.02 | 63.10 |
| 90 | 0.05 | 0.06 | 0.04 | 0.05 ± 0.01 | 82.14 |
| 120 | BDL | BDL | BDL | BDL | 100.00 |
| 150 | BDL | BDL | BDL | BDL | 100.00 |

Table 47: Dissipation and persistence of des-iodo flubendiamide (10 ppm) in new alluvial soil

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | 0.25 | 0.34 | 0.27 | 0.29 ± 0.05 | - |
| 3 | 0.35 | 0.37 | 0.33 | 0.35 ± 0.02 | - |
| 7 | 0.60 | 0.65 | 0.61 | 0.62 ± 0.03 | - |
| 15 | 0.64 | 0.51 | 0.58 | 0.58 ± 0.07 | 6.99 |
| 30 | 0.38 | 0.39 | 0.34 | 0.37 ± 0.03 | 40.32 |
| 60 | 0.25 | 0.26 | 0.24 | 0.25 ± 0.01 | 59.68 |
| 90 | 0.19 | 0.18 | 0.14 | 0.17 ± 0.03 | 72.58 |
| 120 | BDL | BDL | BDL | BDL | 100.00 |
| 150 | BDL | BDL | BDL | BDL | 100.00 |

### 4.3.5 Persistence and dissipation behaviour of flubendiamide in red and lateritic (Jhargram) soil under laboratory condition

The results of dissipation of flubendiamide with time in red and lateritic soil are presented in table 48-49. No residue of flubendiamide could be detected in control samples during the study period. The results indicated that the initial deposits (after 2 hours of application) of flubendiamide in jhargram soil at 5 ppm ($T_1$) and 10 ppm ($T_2$) were found to be 4.38 and 8.90 ppm respectively. After 3 days, the residue declined to 4.28 and 8.47 ppm corresponds to $T_1$ and $T_2$, thus indicating a loss of only 2.36% and 4.79%. At day seven, the residue was found to be 3.99 ($T_1$) and 7.91 ($T_2$) ppm with a corresponding reduction of 8.98 and 11.09 % respectively. Moreover, 57% flubendiamide remained in the soil after 30 days in $T_1$ and ~ 47% remained on 60 days in case of $T_2$. Flubendiamide dissipated out 87.67% at 120 days in case of $T_1$ but 86.62% dissipation occurred in case of $T_2$ at 150 days (Figure 27). However, no residue could be quantified at 150 days below the level of quantification of 0.01 ppm in case of $T_1$.

The data on flubendiamide residues in soil at different days was subjected to statistical analysis to give the regression equation and residual half-life ($T_{1/2}$) based on mean residue values (Table 48-49). The half-life value calculated was found to be 41.23 days ($T_1$) and 51.9 days ($T_2$). The dissipation rate followed first order kinetics irrespective of any treatments (Figure 28).

Table 48: Persistence and dissipation of flubendiamide (5 ppm) in red and lateritic soil

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) | Regression equation | $T_{(1/2)}$ days |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | | |
| 0 | 4.39 | 4.42 | 4.32 | 4.38 ± 0.05 | - | y = 3.644-0.0073x | 41.23 |
| 3 | 4.25 | 4.3 | 4.28 | 4.28 ± 0.03 | 2.36 | | |
| 7 | 4.01 | 3.85 | 4.1 | 3.99 ± 0.13 | 8.98 | | |
| 15 | 3.62 | 3.45 | 3.58 | 3.55 ± 0.09 | 18.95 | | |
| 30 | 2.36 | 2.64 | 2.44 | 2.48± 0.14 | 43.38 | | |
| 60 | 1.52 | 1.38 | 1.36 | 1.42 ± 0.09 | 67.58 | | |
| 90 | 1.25 | 1.22 | 1.15 | 1.21 ± 0.05 | 72.45 | | |
| 120 | 0.53 | 0.52 | 0.56 | 0.54 ± 0.02 | 87.75 | | |
| 150 | BDL | BDL | BDL | BDL | 100.00 | $r^2 = 0.98$ | |

Table 49: Persistence and dissipation of flubendiamide (10 ppm) in red and lateritic soil

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) | Regression equation | $T_{(1/2)}$ days |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | | |
| 0 | 8.85 | 8.95 | 8.91 | 8.90 ± 0.05 | - | y = 3.940-0.0058x | 51.9 |
| 3 | 8.38 | 8.49 | 8.55 | 8.47 ± 0.09 | 4.79 | | |
| 7 | 8.01 | 7.85 | 7.88 | 7.91 ± 0.09 | 11.09 | | |
| 15 | 6.96 | 7.08 | 7.04 | 7.03 ± 0.06 | 21.05 | | |
| 30 | 5.84 | 5.62 | 5.75 | 5.74 ± 0.11 | 35.54 | | |
| 60 | 4.05 | 4.25 | 4.12 | 4.14 ± 0.10 | 53.48 | | |
| 90 | 2.45 | 2.34 | 2.41 | 2.40 ± 0.06 | 73.03 | | |
| 120 | 1.73 | 1.85 | 1.76 | 1.78 ± 0.06 | 80.00 | | |
| 150 | 1.2 | 1.15 | 1.22 | 1.19 ± 0.04 | 86.63 | $r^2 = 0.99$ | |

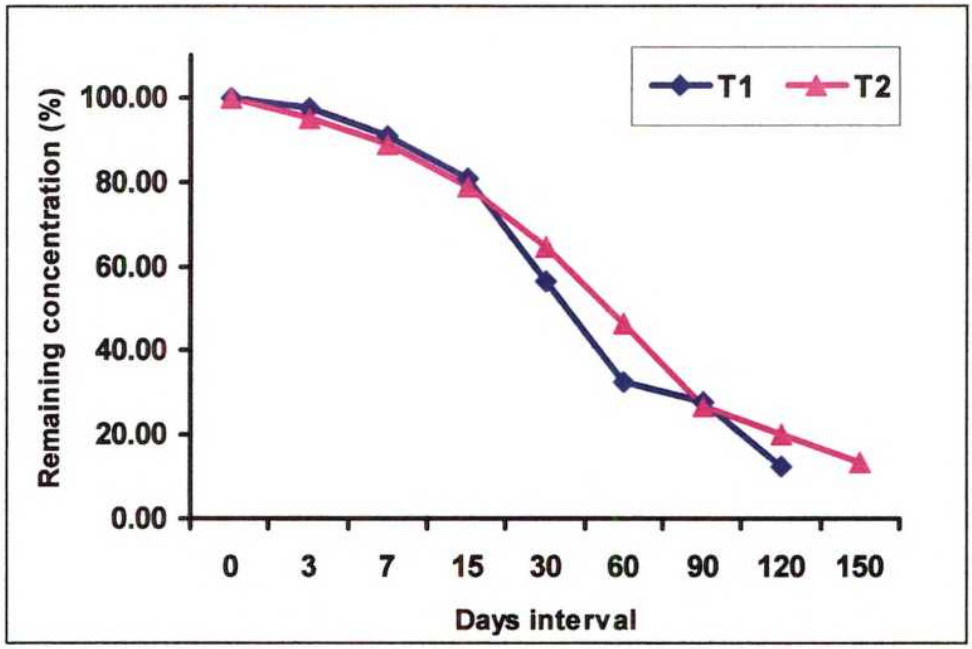



Figure 27: Percent remaining of flubendiamide in red and lateritic soil

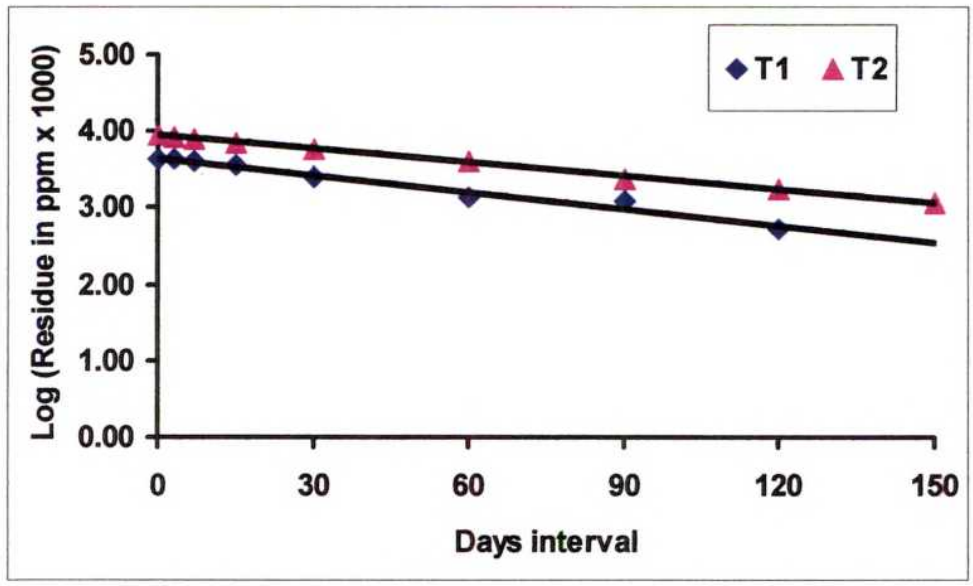



Figure 28: Linear plots of dissipation of flubendiamide in red and lateritic soil

Interestingly, the des-iodo flubendiamide metabolite was also identified and quantified in jhargram soil. The analytical data are presented in table 50-51. The formation of des-iodo metabolite after 2 hours of application was found to be 0.20 and 0.34 ppm for $T_1$ and $T_2$ respectively. The residue increased to a level of 0.40-0.84 ppm at day seven and thereafter, a progressive declination of the metabolite occurred and 80.83 – 82.14 % dissipated out at 90 days irrespective of treatments.

Table 50: Persistence and dissipation of des-iodo flubendiamide (5 ppm) in red and lateritic soil

| DAT | Residue in ppm | | | Mean residue | Dissipation |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | (ppm) ± S.D | (%) |
| 0 | 0.19 | 0.20 | 0.21 | 0.20 ± 0.02 | - |
| 3 | 0.23 | 0.19 | 0.22 | 0.21 ± 0.02 | - |
| 7 | 0.38 | 0.42 | 0.39 | 0.40 ± 0.02 | - |
| 15 | 0.21 | 0.18 | 0.20 | 0.20 ± 0.02 | 50.83 |
| 30 | 0.16 | 0.18 | 0.14 | 0.16 ± 0.01 | 60.00 |
| 60 | 0.09 | 0.12 | 0.10 | 0.10 ± 0.02 | 74.17 |
| 90 | 0.07 | 0.08 | 0.08 | 0.08 ± 0.01 | 80.83 |
| 120 | BDL | BDL | BDL | BDL | 100.00 |
| 150 | BDL | BDL | BDL | BDL | 100.00 |

Table 51: Persistence and dissipation of des-iodo flubendiamide (10 ppm) in red and lateritic soil

| DAT | Residue in ppm | | | Mean residue | Dissipation |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | (ppm) ± S.D | (%) |
| 0 | 0.38 | 0.28 | 0.35 | 0.34 ± 0.05 | - |
| 3 | 0.38 | 0.41 | 0.38 | 0.39 ± 0.02 | - |
| 7 | 0.78 | 0.84 | 0.89 | 0.84 ± 0.06 | - |
| 15 | 0.43 | 0.41 | 0.48 | 0.44 ± 0.04 | 47.62 |
| 30 | 0.27 | 0.31 | 0.30 | 0.29 ± 0.02 | 65.08 |
| 60 | 0.27 | 0.22 | 0.24 | 0.24 ± 0.03 | 71.03 |
| 90 | 0.12 | 0.17 | 0.16 | 0.15 ± 0.03 | 82.14 |
| 120 | BDL | BDL | BDL | BDL | 100.00 |
| 150 | BDL | BDL | BDL | BDL | 100.00 |

### 4.3.6 Persistence and dissipation behaviour of flubendiamide in coastal (Canning) soil under laboratory condition

The persistence and dissipation data of flubendiamide at different day's interval of two different doses in canning soil are presented in table 52-53. No residue of flubendiamide could be detected in control samples during the study period. The results indicated that the initial deposits (0 day) of flubendiamide in canning soil at 5 ppm ($T_1$) and 10 ppm ($T_2$) were found to be 4.51 and 8.97 ppm respectively. After 3 days, the residue declined to 4.41 and 8.53 ppm corresponds to $T_1$ and $T_2$, thus indicating a loss of only 2.22% and 4.87%. At day seven, the residue found to be 4.35 ($T_1$) and 8.11 ($T_2$) ppm with a corresponding reduction of 3.55 and 9.62 % respectively. The scrutiny of the data indicated that the residue remained in the soil up to fifteen days was higher in $T_1$ than $T_2$. At 30 days the percentage remained in the soil was same for $T_1$ and $T_2$ (Figure 29) and thereafter little bit faster dissipation occurred in $T_1$ than $T_2$. Flubendiamide dissipated out 81.89% at 120 days in case of $T_1$ but 85.06% dissipation occurred in case of $T_2$ at 150 days. However, no residue could be quantified at 150 days below the level of quantification of 0.01 ppm in case of $T_1$.

The data on flubendiamide residues in soil at different days was subjected to statistical analysis to give the regression equation and residual half-life ($T_{1/2}$) based on mean residue values (Table 52-53). The half-life value calculated was found to be 44.26 days ($T_1$) and 59.02 days ($T_2$). The dissipation rate followed first order kinetics irrespective of any treatments (Figure 30).

Table 52: Persistence and dissipation of flubendiamide (5 ppm) in coastal soil

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) | Regression equation | $T_{(1/2)}$ days |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | | |
| 0 | 4.54 | 4.51 | 4.48 | 4.51 ± 0.03 | - | y = 3.674-0.0068x | 44.26 |
| 3 | 4.37 | 4.42 | 4.44 | 4.41 ± 0.04 | 2.22 | | |
| 7 | 4.38 | 4.41 | 4.26 | 4.35 ± 0.08 | 3.55 | | |
| 15 | 3.85 | 3.95 | 4.01 | 3.94 ± 0.08 | 12.71 | | |
| 30 | 2.75 | 2.64 | 2.74 | 2.71 ± 0.06 | 39.91 | | |
| 60 | 1.85 | 1.96 | 2.01 | 1.94 ± 0.08 | 56.98 | | |
| 90 | 1.14 | 1.25 | 1.45 | 1.28 ± 0.16 | 71.62 | | |
| 120 | 0.62 | 0.64 | 0.69 | 0.65 ± 0.04 | 85.59 | | |
| 150 | BDL | BDL | BDL | BDL | 100.00 | $r^2 = 0.99$ | |

Table 53: Persistence and dissipation of flubendiamide (10 ppm) in coastal soil

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) | Regression equation | $T_{(1/2)}$ days |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | | |
| 0 | 8.85 | 8.95 | 9.1 | 8.97 ± 0.13 | - | y =3.9386-0.0051x | 59.02 |
| 3 | 8.58 | 8.57 | 8.45 | 8.53 ± 0.07 | 4.87 | | |
| 7 | 7.95 | 8.12 | 8.25 | 8.11 ± 0.15 | 9.62 | | |
| 15 | 6.68 | 6.75 | 6.84 | 6.76 ± 0.08 | 24.67 | | |
| 30 | 5.58 | 5.66 | 5.21 | 5.48 ± 0.24 | 38.87 | | |
| 60 | 4.76 | 4.48 | 4.62 | 4.62 ± 0.14 | 48.49 | | |
| 90 | 3.18 | 3.42 | 3.35 | 3.32 ± 0.12 | 63.02 | | |
| 120 | 2.3 | 2.26 | 2.41 | 2.32 ± 0.08 | 74.10 | | |
| 150 | 1.35 | 1.29 | 1.38 | 1.34 ± 0.05 | 85.06 | $r^2 = 0.98$ | |

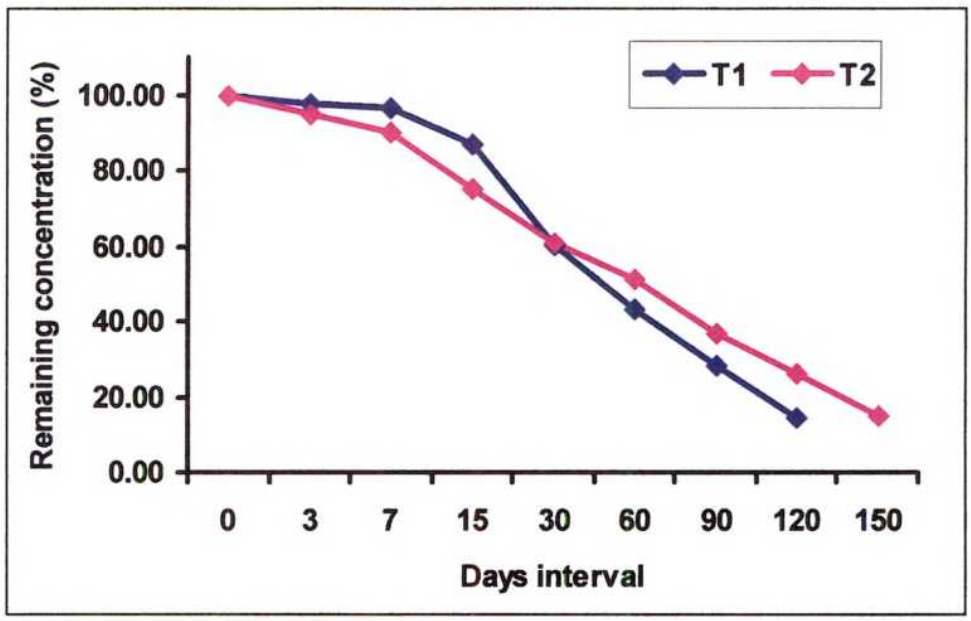



Figure 29: Percent remaining of flubendiamide in coastal soil

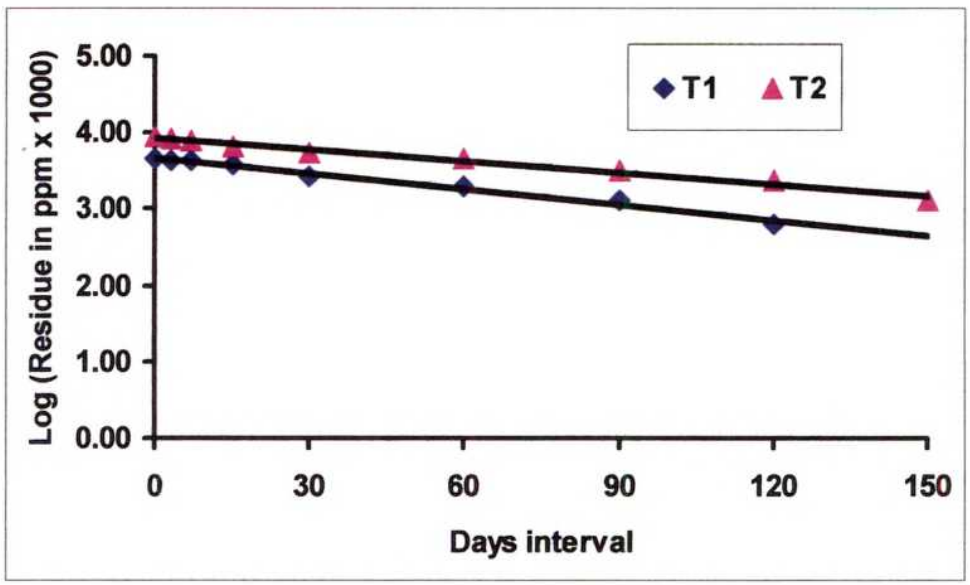



Figure 30: Linear plots of dissipation of flubendiamide in coastal soil

Interestingly, the des-iodo flubendiamide metabolite was also identified and quantified in canning soil. The analytical data are presented in table 54-55. The formation of flubendiamide metabolite after 2 hours of application was found to be 0.20 and 0.39 ppm for $T_1$ and $T_2$ respectively. The thorough scrutiny of the results revealed that the metabolite residue increased on day 7 to a concentration of 0.29 ppm and 0.56 ppm for $T_1$ and $T_2$ respectively. The metabolite residue decreased to a level of 0.17-0.36 ppm at day 30 and thereafter, a progressive declination of the metabolite occurred through out the study period and 72.41 – 78.57 % dissipated out at 90 days irrespective of treatments.

Table 54: Persistence and dissipation of des-iodo flubendiamide (5 ppm) in coastal soil

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | 0.22 | 0.18 | 0.21 | 0.20 ± 0.02 | - |
| 3 | 0.28 | 0.24 | 0.26 | 0.26 ± 0.02 | - |
| 7 | 0.32 | 0.28 | 0.27 | 0.29 ± 0.03 | - |
| 15 | 0.16 | 0.24 | 0.22 | 0.21 ± 0.04 | 27.59 |
| 30 | 0.15 | 0.19 | 0.18 | 0.17 ± 0.02 | 41.38 |
| 60 | 0.13 | 0.14 | 0.14 | 0.14 ± 0.01 | 51.72 |
| 90 | 0.08 | 0.10 | 0.0.7 | 0.08 ± 0.01 | 72.41 |
| 120 | BDL | BDL | BDL | BDL | 100.00 |
| 150 | BDL | BDL | BDL | BDL | 100.00 |

Table 55: Persistence and dissipation of des-iodo flubendiamide (10 ppm) in coastal soil

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | 0.36 | 0.42 | 0.4 | 0.39 ± 0.03 | - |
| 3 | 0.58 | 0.55 | 0.45 | 0.53 ± 0.07 | - |
| 7 | 0.58 | 0.52 | 0.57 | 0.56 ± 0.03 | - |
| 15 | 0.40 | 0.41 | 0.42 | 0.41 ± 0.01 | 26.79 |
| 30 | 0.36 | 0.34 | 0.38 | 0.36 ± 0.02 | 35.71 |
| 60 | 0.26 | 0.24 | 0.26 | 0.25 ± 0.01 | 55.36 |
| 90 | 0.15 | 0.12 | 0.10 | 0.12 ± 0.03 | 78.57 |
| 120 | BDL | BDL | BDL | BDL | 100.00 |
| 150 | BDL | BDL | BDL | BDL | 100.00 |

The overall comparison of persistence behaviour in three types of soil revealed that the half-life values ($t_{1/2}$) calculated from the regression equation for $T_1$ and $T_2$ in coastal soil, red and lateritic soil and new alluvial soil were found to be 44.26 – 59.02, 41.23 – 51.90 and 37.16 – 44.92 days respectively. The persistence of flubendiamide was in the order of coastal soil > red and lateritic soil > new alluvial soil. It appears that little differences in the dissipation kinetics of three soils were attributed to differences in soil pH, texture and the ability of indigenous microorganism to transform the insecticide.

The persistence data of all the three soils further revealed that flubendiamide dissipation rate was quite slow in coastal soil as compared to other two soils which might be due to high clay and higher organic matter content of the soil. Organic matter may bind flubendiamide or may form complex structure which prevents further microbial activity on the compound. Thus, the present study concludes that persistence of flubendiamide is more in coastal than neutral to acidic soil. Furthermore, low water solubility (Tsubata *et al.*, 2007) of flubendiamide gives an indication of low potential for mobility in the soil (Shane *et al.*, 2006). Hence probability of contamination of ground water by flubendiamide is minimal.

SECTION- D

## 4.4 Persistence and dissipation study of flubendiamide in water at different pH under laboratory condition

### 4.4.1 Analytical method for the estimation of flubendiamide and des-iodo flubendiamide residues

The analytical method adopted for the estimation of flubendiamide and des-iodo flubendiamide residue in water was by HPLC and the chromatogram of analytical standard mixture was already depicted in figure 8 of section A and the retention time (RT) was found to be 8.93 and 7.08 minutes respectively. The operating conditions for HPLC analysis were stated under 3.1.4.8 of Section A.

### 4.4.2 Results of recovery experiment of flubendiamide in water at different pH

The results of recovery experiment of flubendiamide in water at different pH are presented in table 56. From the table, it was observed that the recovery percentage of flubendiamide at the fortification level of 0.01 – 0.1 ppm varied in the range of 86% - 91.92% at pH – 4.0, 88.22% - 90.66% at pH – 7.0 and 89.16% - 95.66% at pH – 9.2 with corresponding RSD ranged from 0.99-2.02%, 0.92 – 2.80% and 1.26 – 1.49% respectively. Similarly, recovery results of des-iodo flubendiamide in water at different pH are presented in table 57. From the table, it was observed that the recovery percentage of flubendiamide at the fortification level of 0.01 – 0.1 ppm varied in the range of 90.08 - 94.90 % at pH – 4.0, 93.46- 94.31 % at pH – 7.0 and 93.62 - 97.25 % at pH – 9.2 with corresponding RSD ranged from 1.21-2.01%, 0.86 – 1.50% and 1.28 – 1.53% respectively. As the average recovery percentage was more than 89% for all three pH, hence the analytical method was quite satisfactory and adopted for the residue and dissipation study of flubendiamide in water at different pH under laboratory condition.

Table 56: Recovery of flubendiamide from water at different pH (4.0, 7.0 and 9.2)

| pH level | Fortification Level (ppm) | Recovery percentage | | | Mean ± S.D | RSD (%) |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | | |
| 4.0 | 0.01 | 87.50 | 86.45 | 84.10 | 86.02 ± 1.74 | 2.02 |
| | 0.05 | 89.85 | 90.50 | 88.75 | 89.70 ± 0.88 | 0.99 |
| | 0.10 | 93.02 | 92.51 | 90.25 | 91.92 ± 1.46 | 1.59 |
| 7.0 | 0.01 | 85.65 | 90.58 | 88.42 | 88.22 ± 2.47 | 2.80 |
| | 0.05 | 88.75 | 88.25 | 89.85 | 88.95 ± 0.82 | 0.92 |
| | 0.10 | 89.76 | 90.28 | 91.95 | 90.66 ± 1.14 | 1.26 |
| 9.2 | 0.01 | 89.56 | 87.68 | 90.24 | 89.16 ± 1.33 | 1.49 |
| | 0.05 | 92.36 | 90.78 | 93.25 | 92.13 ± 1.25 | 1.36 |
| | 0.10 | 94.27 | 96.25 | 96.45 | 95.66 ± 1.21 | 1.26 |

Table 57: Recovery of des-iodo flubendiamide from water at different pH (4.0, 7.0 & 9.2)

| pH level | Fortification Level (ppm) | Recovery percentage | | | Mean ± S.D | RSD (%) |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | | |
| 4.0 | 0.01 | 88.60 | 89.54 | 92.10 | 90.08 ± 1.81 | 2.01 |
| | 0.05 | 90.50 | 92.34 | 92.50 | 91.78 ± 1.11 | 1.21 |
| | 0.10 | 94.75 | 93.50 | 96.45 | 94.90 ± 1.48 | 1.56 |
| 7.0 | 0.01 | 92.15 | 94.50 | 94.65 | 93.77 ± 1.40 | 1.50 |
| | 0.05 | 93.25 | 92.78 | 94.35 | 93.46 ± 0.81 | 0.86 |
| | 0.10 | 93.45 | 93.75 | 95.74 | 94.31 ± 1.24 | 1.32 |
| 9.2 | 0.01 | 92.10 | 94.26 | 94.50 | 93.62 ± 1.32 | 1.41 |
| | 0.05 | 95.26 | 94.75 | 97.50 | 95.84 ± 1.46 | 1.53 |
| | 0.10 | 97.65 | 98.25 | 95.85 | 97.25 ± 1.25 | 1.28 |

### 4.4.3 Persistence and dissipation of flubendiamide in water at pH 4.0, 7.0 and 9.2 under laboratory condition

The laboratory experimental results of flubendiamide residues in water at different pH (4.0, 7.0 and 9.2) are presented in table 58-63. Flubendiamide was applied at two concentration level of 1.0 ppm ($T_1$) and 2.0 ppm ($T_2$) in each pH separately and the samples were collected at different day's interval. A vivid scrutiny of the results indicated that flubendiamide residue significantly decreased with time in all the pH selected for study. The untreated control samples for each pH were analyzed and no residue of flubendiamide could be detected.

In acidic medium (pH = 4.0), the initial deposit of flubendiamide was 0.88 ppm in $T_1$ and 1.84 ppm in $T_2$. It was observed that very slow dissipation occurred during the entire period of study (120 days). The dissipation percentage (Figure 31) at 3 days of application was 4.92% ($T_1$) and 2.54% ($T_2$). The residue gradually decreased with time and found that 25% dissipation occurred upto 30 days and little bit faster dissipation started thereafter. Around 50% residue remained at 90 days in $T_1$ and 120 days in $T_2$. The data revealed that the compound is hydrolytically stable in acidic medium.

Table 58: Persistence and dissipation of flubendiamide (1 ppm) in pH 4.0

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) | Regression equation | $T_{(1/2)}$ days |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | | |
| 0 | 0.92 | 0.88 | 0.85 | 0.88 ± 0.04 | - | y = 2.9052-0.0028x | 107.5 |
| 3 | 0.84 | 0.85 | 0.82 | 0.84 ± 0.02 | 4.92 | | |
| 7 | 0.75 | 0.73 | 0.71 | 0.73 ± 0.02 | 17.05 | | |
| 15 | 0.69 | 0.68 | 0.72 | 0.70 ± 0.02 | 20.83 | | |
| 30 | 0.64 | 0.66 | 0.67 | 0.66 ± 0.02 | 25.38 | | |
| 60 | 0.49 | 0.54 | 0.45 | 0.49 ± 0.05 | 43.94 | | |
| 90 | 0.42 | 0.47 | 0.44 | 0.44 ± 0.03 | 49.62 | | |
| 120 | 0.38 | 0.42 | 0.40 | 0.40 ± 0.02 | 54.55 | $r^2$ = 0.93 | |

Table 59: Persistence and dissipation of flubendiamide (2 ppm) at pH 4.

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) | Regression equation | $T_{(1/2)}$ days |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | | |
| 0 | 1.86 | 1.79 | 1.88 | 1.84 ± 0.05 | - | y = 3.2366-0.0027x | 111.5 |
| 3 | 1.79 | 1.81 | 1.78 | 1.79 ± 0.02 | 2.54 | | |
| 7 | 1.68 | 1.75 | 1.65 | 1.69 ± 0.05 | 7.97 | | |
| 15 | 1.45 | 1.62 | 1.53 | 1.53 ± 0.09 | 16.67 | | |
| 30 | 1.38 | 1.27 | 1.32 | 1.32 ± 0.06 | 28.08 | | |
| 60 | 1.12 | 1.08 | 1.02 | 1.07 ± 0.05 | 41.67 | | |
| 90 | 0.95 | 0.99 | 0.94 | 0.96 ± 0.03 | 47.83 | | |
| 120 | 0.95 | 0.91 | 0.89 | 0.92 ± 0.03 | 50.18 | $r^2 = 0.94$ | |

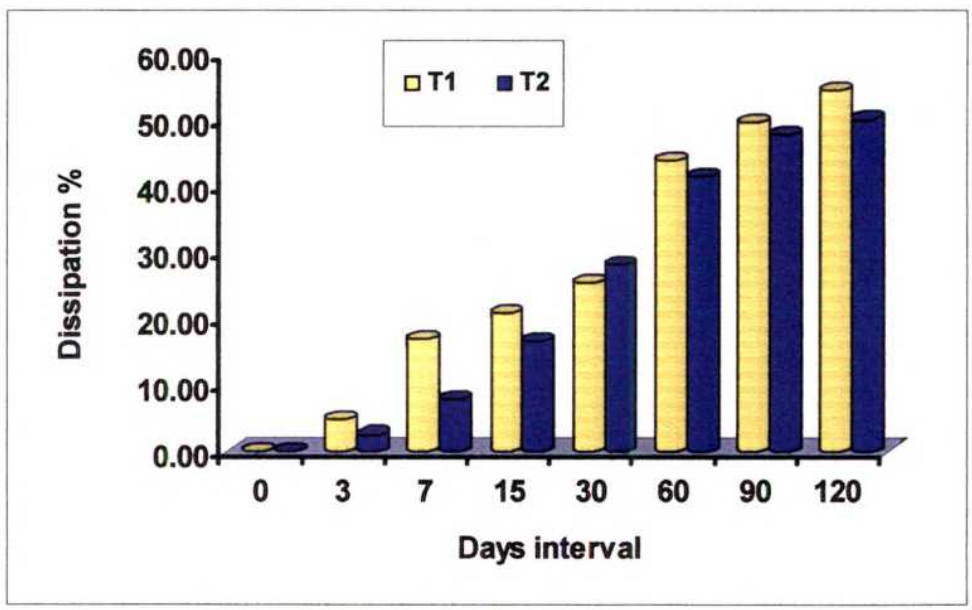



Figure 31: Dissipation percentage of flubendiamide (1 and 2 ppm) in pH 4.0

In neutral medium (pH = 7.0), the initial deposit of flubendiamide residues was found to be 0.93 ppm in $T_1$ and 1.85 ppm in $T_2$ which decreased gradually to 0.84 ppm and 1.71 ppm after 3 days of application thus representing a loss of 9.32% and 7.75% respectively. It was also observed that 50% residue dissipated out on 30 days in neutral condition (Figure 32). Finally, at 120 days the residue dissipated to about 63.24 – 68.82%

irrespective of $T_2$ and $T_1$. In contrast to acidic condition, faster dissipation occurred in neutral pH.

Table 60: Persistence and dissipation of flubendiamide (1 ppm) at pH 7.0

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) | Regression equation | $T_{(1/2)}$ days |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | | |
| 0 | 0.92 | 0.94 | 0.92 | 0.93 ± 0.01 | - | y = 3.1969-0.0036x | 81.36 |
| 3 | 0.83 | 0.84 | 0.86 | 0.84 ± 0.02 | 9.32 | | |
| 7 | 0.78 | 0.69 | 0.72 | 0.73 ± 0.05 | 21.51 | | |
| 15 | 0.61 | 0.58 | 0.54 | 0.58 ± 0.04 | 37.99 | | |
| 30 | 0.42 | 0.48 | 0.51 | 0.47 ± 0.05 | 49.46 | | |
| 60 | 0.41 | 0.43 | 0.45 | 0.43 ± 0.02 | 53.76 | | |
| 90 | 0.37 | 0.42 | 0.38 | 0.39 ± 0.03 | 58.06 | | |
| 120 | 0.32 | 0.28 | 0.27 | 0.29 ± 0.03 | 68.82 | $r^2 = 0.83$ | |

Table 61: Persistence and dissipation of flubendiamide (2 ppm) at pH 7.0

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) | Regression equation | $T_{(1/2)}$ days |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | | |
| 0 | 1.87 | 1.83 | 1.85 | 1.85 ± 0.02 | - | y = 3.1969-0.0036x | 83.60 |
| 3 | 1.73 | 1.71 | 1.68 | 1.71 ± 0.03 | 7.75 | | |
| 7 | 1.51 | 1.49 | 1.55 | 1.52 ± 0.03 | 18.02 | | |
| 15 | 1.48 | 1.48 | 1.41 | 1.46 ± 0.04 | 21.26 | | |
| 30 | 0.88 | 0.91 | 0.87 | 0.89 ± 0.02 | 52.07 | | |
| 60 | 0.82 | 0.79 | 0.84 | 0.82 ± 0.03 | 55.86 | | |
| 90 | 0.76 | 0.74 | 0.78 | 0.76 ± 0.02 | 58.92 | | |
| 120 | 0.68 | 0.69 | 0.67 | 0.68 ± 0.01 | 63.24 | $r^2 = 0.87$ | |

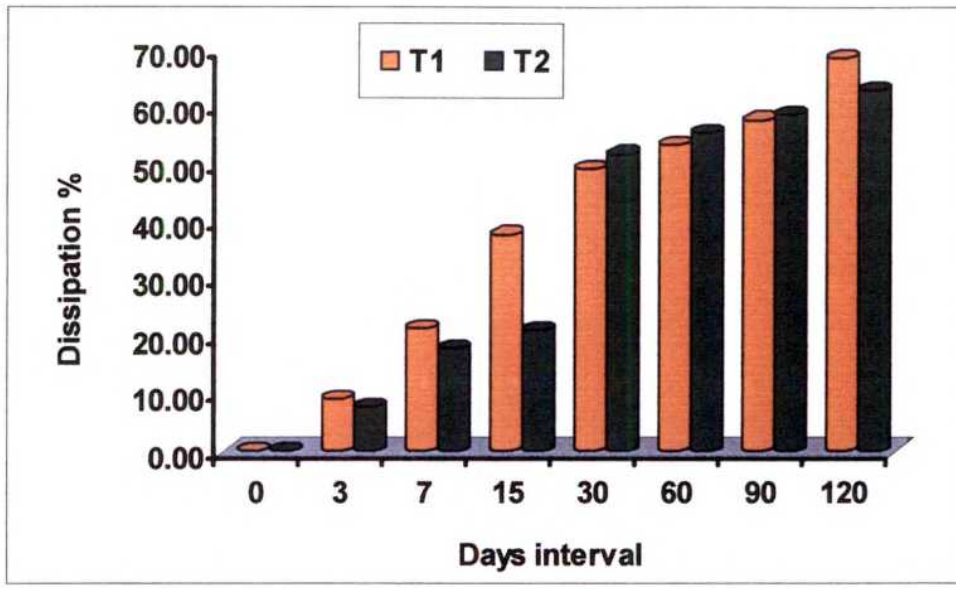



Figure 32: Dissipation percentage of flubendiamide (1 and 2 ppm) at pH 7.0

In alkaline medium (pH = 9.2) the initial concentration of flubendiamide was found to be 0.93 ppm in $T_1$ and 1.91 ppm in $T_2$. The amount of residues remained on 3, 7, 15, 30, 60, 90 and 120 days were 0.80, 0.71, 0.63, 0.58, 0.52, 0.47 and 0.40 ppm for treatment $T_1$ and 1.82, 1.53, 1.46, 1.24, 1.09, 1.03 and 0.92 ppm for treatment $T_2$. At 120 days the residue dissipated out 56.99% and 51.83% in $T_1$ and $T_2$ respectively (Figure 33). Thus, in alkaline medium the compound is also hydrolytically stable.

Table 62: Persistence and dissipation of flubendiamide (1 ppm) at pH 9.2

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) | Regression equation | $T_{(1/2)}$ days |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | | |
| 0 | 0.94 | 0.92 | 0.94 | 0.93 ± 0.01 | - | y = 2.8889-0.0026x | 115.78 |
| 3 | 0.81 | 0.78 | 0.82 | 0.80 ± 0.02 | 13.62 | | |
| 7 | 0.73 | 0.69 | 0.71 | 0.71 ± 0.02 | 23.66 | | |
| 15 | 0.65 | 0.63 | 0.62 | 0.63 ± 0.02 | 31.90 | | |
| 30 | 0.60 | 0.59 | 0.56 | 0.58 ± 0.02 | 37.28 | | |
| 60 | 0.52 | 0.49 | 0.54 | 0.52 ± 0.03 | 44.44 | | |
| 90 | 0.47 | 0.48 | 0.46 | 0.47 ± 0.01 | 49.46 | | |
| 120 | 0.38 | 0.41 | 0.41 | 0.40 ± 0.02 | 56.99 | $r^2 = 0.87$ | |

Table 63: Persistence and dissipation of flubendiamide (2 ppm) at pH 9.2

| DAT | Residue in ppm | | | Mean residue (ppm) ± S.D | Dissipation (%) | Regression equation | $T_{(1/2)}$ days |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | | |
| 0 | 1.91 | 1.89 | 1.93 | 1.91 ± 0.02 | - | y = 3.2233-0.0024x | 125.42 |
| 3 | 1.78 | 1.86 | 1.83 | 1.82 ± 0.04 | 4.54 | | |
| 7 | 1.54 | 1.52 | 1.53 | 1.53 ± 0.01 | 19.90 | | |
| 15 | 1.46 | 1.43 | 1.48 | 1.46 ± 0.03 | 23.73 | | |
| 30 | 1.27 | 1.24 | 1.21 | 1.24 ± 0.03 | 35.08 | | |
| 60 | 1.11 | 1.1 | 1.07 | 1.09 ± 0.02 | 42.76 | | |
| 90 | 1.04 | 0.96 | 1.08 | 1.03 ± 0.06 | 46.25 | | |
| 120 | 0.92 | 0.94 | 0.9 | 0.92 ± 0.02 | 51.83 | $r^2 = 0.87$ | |

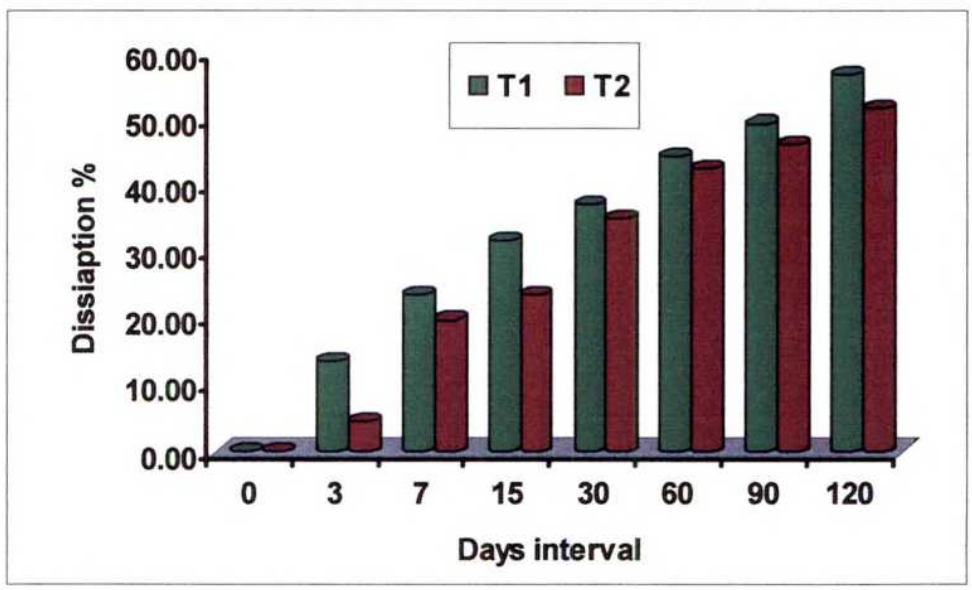



Figure 33: Dissipation percentage of flubendiamide (1 and 2 ppm) in pH 9.2

The dissipation of flubendiamide with time in water at all the pH under study followed the first order kinetics, since a straight line was obtained when log values of residues were plotted against time irrespective of any medium (Figure 34-36). The half-life ($T_{½}$) values calculated from the regression equation in acidic medium (pH = 4.0),

alkaline medium (pH = 9.2) and neutral medium (pH = 7.0) for $T_1$ treatment was found to be 107.5, 115.78 and 81.36 days whereas for $T_2$ it was 111.5, 125.42 and 83.6 days.

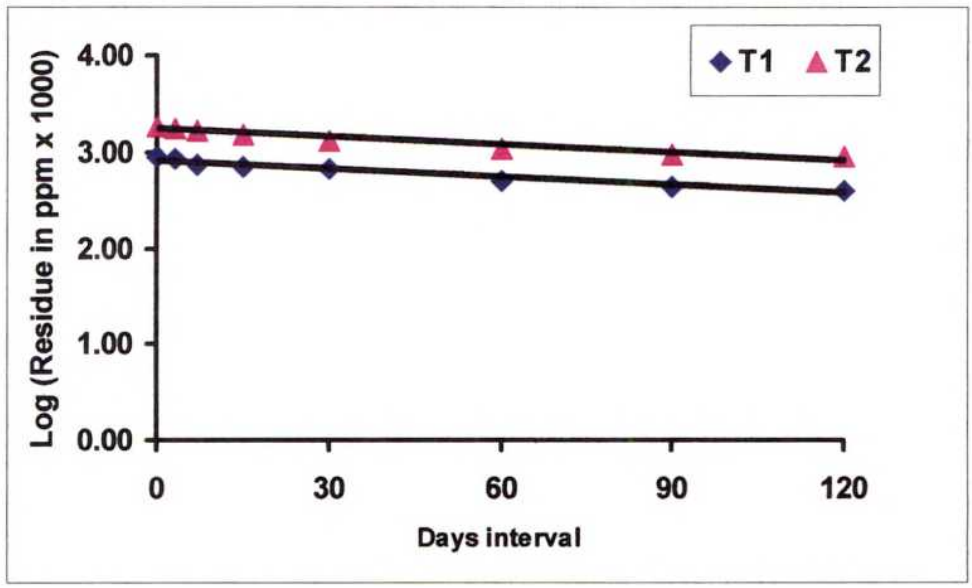



Figure 34: Linear plots of dissipation of flubendiamide in pH 4.0

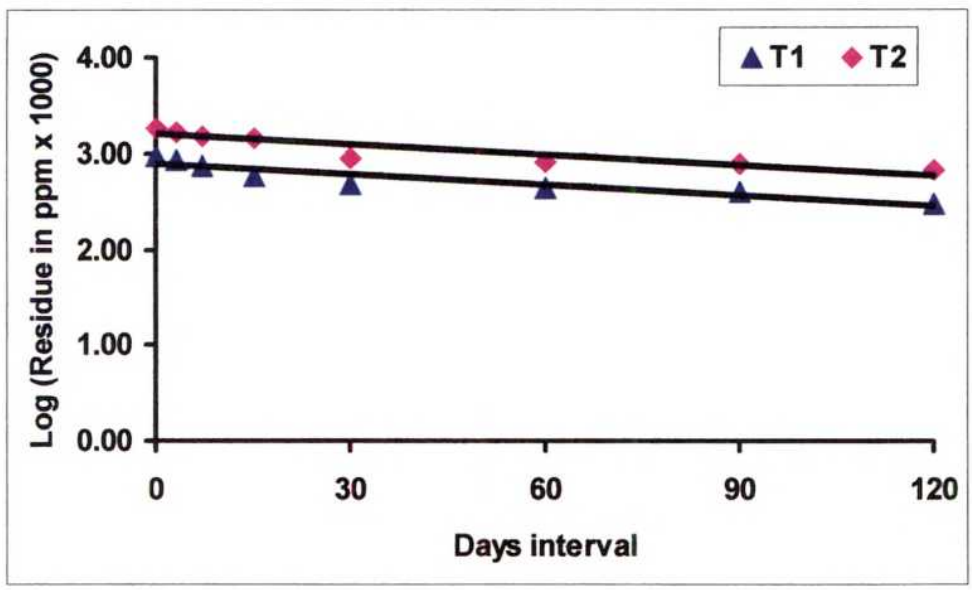



Figure 35: Linear plots of dissipation of flubendiamide in pH 7.0

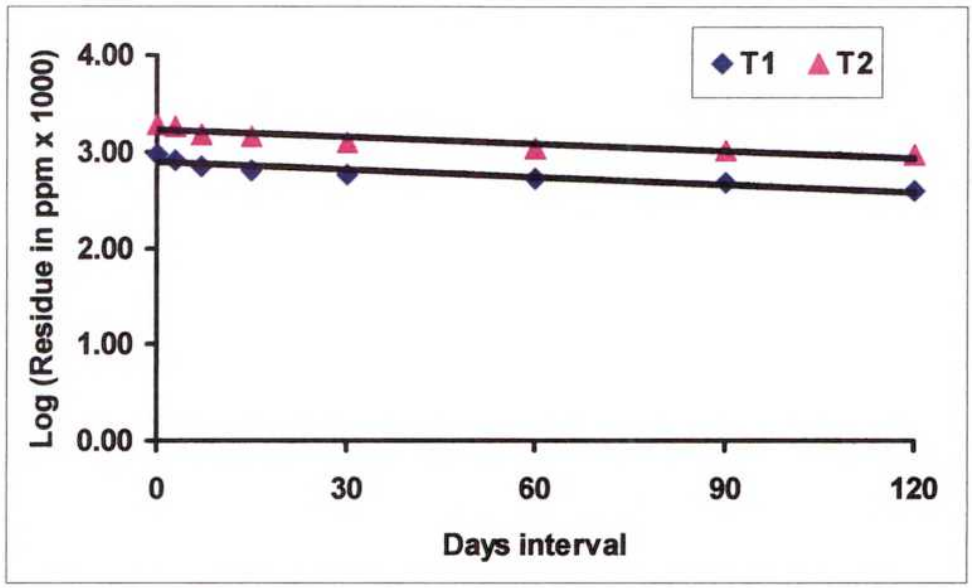



Figure 36: Linear plots of dissipation of flubendiamide in pH 9.2

The comparison of the percentage dissipation of flubendiamide with time was almost same at pH - 4.0 and 9.2, but little bit faster dissipation occurred in neutral medium (pH - 7.0). The persistence of flubendiamide was in the order of pH 9.2 > pH 4.0 > pH 7.0. Des-iodo flubendiamide was not detected in aqueous media. Thus, the present study indicated that the flubendiamide molecule is hydrolytically stable in acidic, neutral and alkaline medium which is in well agreement with that reported by Shane *et al.*, 2006.

# PART-II

*Photolytic degradation of flubendiamide in aqueous acetonitrile, methanol and isopropanol under UV and sunlight*

# Chapter 5

# Review of Literature

# Review of Literature

The review of literature pertaining to photochemical reactions of flubendiamide under sunlight and UV irradiation is discussed in the present chapter under the following categories.

5.1 Fundamental Principles of Photochemistry

5.2 Sources of irradiation

5.3 Choice of solvent

5.4 Photosensitizer

5.5 Analytical instruments used in photochemical studies

## 5.1 Fundamental Principles of Photochemistry

The two basic laws of photochemistry are the Grotthus – Draper Law and Stark – Einstein law. The Grotthus – Draper Law combined with the Beer-Lambert law, defines the absorption of light by the chemical while the Stark – Einstein law defines the efficiency with which the absorbed light transforms the chemicals into products. These laws are used to define the kinetics of photoreaction of a chemical.

The first law of photochemistry is The Grotthus – Draper Law which states that only radiations that are absorbed by the reacting system can be effective in producing chemical changes. Thus for photochemical transformation, it is necessary to know the amount of light absorb by the reacting chemical.

The second law of photochemistry is The Stark – Einstein law which states that each molecule directly taking part in a chemical reaction induced by light absorbs one quantum or photon of light. It applies to processes in which the absorption of a quantum of light leads to the disappearance of the molecule or the deactivation of the molecule to a non reactive state. (Source: Leifer, A. (1988). The Kinetics of environmental aquatic photochemistry: Theory and Practice, Published by ACS, Professional Refeence Book, p: 3-9.).

The photochemical reactions are selective because by using selective monochromatic light atoms or molecules are excited without exciting the surrounding medium. In photochemical reactions, the structures of the ground and excited states of

atom or molecules are generally different, thus exhibit significantly different chemical behavior. Excited state species generated by the absorption of light are high energy species e.g. triplet excited species, radicals, bi-radicals, etc. Photodegradation occurs by direct and indirect absorption of light. In direct photolysis, the substances absorbs UV-visible light energy and undergoes transformation whereas during indirect photolysis light energy is absorbed by other constituents of the media (water, soil). The excited species can then either transfer the energy to the substance or lead to the formation of reactive species, such as singlet oxygen or hydroxyl radical, which enter into a series of reactions.

The energies per Einstein varies widely with the wavelength (Table 64) and for the environmental photochemistry, the spectral range of interest is 290-800 nm, corresponding to an energy range of 98.6-35.8 kcal/mol. This energy is sufficient to break many chemical bonds. The lower wavelength limit for sunlight photoreaction has been set at 290 nm because ozone in the earth's atmosphere absorbs sunlight strongly at wavelength equal to or less than 290 nm. The upper wavelength limit set at 800 nm thermochemically because light at wavelength greater 800 nm (35.8 kcal/mol) does not possess sufficient energy to break the covalent bonds of ground state molecules. (Source: Leifer, A. (1988). The Kinetics of environmental aquatic photochemistry: Theory and Practice, Published by ACS, Professional Refeence Book, p: 3-9)

Table 64: Energies per Einstein at various wavelengths of light

| Wavelength (nm) | Spectral Range | Energy per Einstein ( kcal) |
|---|---|---|
| 200 | Ultraviolet | 143 |
| 250 | Ultraviolet | 114 |
| 300 | Ultraviolet | 95.3 |
| 300-350 | Ultraviolet | 95.3-81.7 |
| 400-450 | Visible (Violet) | 71.5-63.6 |
| 450-500 | Visible (Blue) | 63.6-57.2 |
| 500-575 | Visible (Green) | 57.2-49.7 |
| 575-590 | Visible (Yellow) | 79.7-48.5 |
| 590-650 | Visible (Orange) | 48.5-44.0 |
| 650-750 | Visible (Red) | 44.0-38.1 |
| 750-800 | Near infrared | 38.1-35.8 |

## 5.2 Source of irradiation

Photolysis is one of the major transformation processes affecting the fate of pesticides in the aquatic environment. In this context many light sources have been used by different researchers for carrying out model photolysis experiments i.e natural sunlight, sunset apparatus, mercury lamp, Xenon arc lamp, Halogen lamp, fluorescent lamp, etc. (Mallipudi *et al.*, 1991; Moza *et al.*, 1998; UV light (Hirahara *et al.*, 2001; Quivet *et al.*, 2004; Ikehata & El-Din, 2006), Visible light (Haggi *et al.*, 2002), X-ray beam (Trebse & Arcon, 2003; Rashed & Amin, 2007). It has been demonstrated that the use of different light sources under identical aqueous conditions can produce similar degradation products with the only differences in the kinetics of formation (Pal *et al.*, 1991). Although the use of a xenon arc lamp ($\lambda max > 290nm$) is useful since results obtained can be more easily compared to real sunlight condition (Mallipudi *et al.*, 1991) while the use of other light sources are recommended when the photodegradation of xenobiotics are slow, leading to faster degradation.

## 5.3 Choice of solvent

The choice of solvents consists of several considerations and among which environmental modeling is most important. In addition due to the low water solubility exhibited by many pesticides, use of photochemically inert organic solvents, such as acetonitrile, methanol and/or isopropanol is mandatory (Grunwell and Erickson,1973). To avoid the solubility problems many studies have been conducted even pure organic solvents such as Hexane (Keum *et al.*, 2002), methanol (Pal *et al.*, 1991; Bosca et al., 1998), oxygenated hexane or isopropanol (Schwack & Flosser-Muller, 1990).

Burkhard and Guth (1976) mentioned that the use of acetone as a photosensitiser on the rate of photodegradation of atrazine, atraton and ametryne in diluted aqueous solutions increased degradation 3- to 11-fold.

Schwack *et al.*, (1995) examined that the potential of the fungicide vinclozolin to undergo photochemical reactions on plant surfaces, model experiments ($\lambda > 280$ nm) were performed in various organic solvents simulating the plant cuticle environment. On irradiation in 2-propanol and n-propanol vinclozolin was completely degraded within 1 h, but the degradation was substantially lower in benzene, cyclohexane, cyclohexene, ethanol, methanol, and tert-butyl methyl ether (TBME). In 2-propanol, n-propanol, and cyclohexane, the main reaction was photoaddition of the solvent molecules to the vinclozolin vinyl group; the photoproducts in further reactions were dechlorinated. In the

presence of ethanol, photodehalogenation of the fungicide competed with photoaddition. On the other hand, in cyclohexene and benzene solutions, substitutions of the chlorines by solvent molecules were mainly observed. Photolysis in methanol or TBME yielded only dehalogenated photoproducts.

Bosca *et al.*, (1998) reported that photochemical and laser flash photolysis studies on dicloran have shown that this fungicide undergoes photo-reactions such as photoreduction of the nitro group and homolytic rupture of the C-$NH_2$ bond. Dramatic changes in the dicloran photoreactivity by the influence of the solvents have been observed. More efficient photodegradation of this fungicide was observed in diethyl ether and chloroform than in methanol or acetonitrile.

Kole *et al.*, (1999) reported that environmental photochemistry of pesticide is complicated as because these molecules may interact with many environmental components. Moreover, photolytic reactions are greatly influenced by meteorological conditions, especially sunshine hours and the results may vary with time of experiment. Therefore, most of the studies have been restricted to laboratory conditions or model experiments which may provide valuable information regarding the environmental behaviour of these molecules. Transformation of pesticide also occurs in plants but it is difficult to differentiate between photochemical and other transformation processes occurring in plants. Thus, studies in solution phase, however, may be undertaken in open or partially open glass wares placed under direct sunlight and or UV light. Aqueous solution of pesticides or solution in organic solvents (e.g. acetonitrile, methanol, isopropanaol, hexane etc) having low absorbance in UV region is generally used for such studies.

Sanz-Asensio *et al.*, (1999) carried out a comparative photodegradation kinetic study of ethiofencarb in aqueous and non-aqueous media (hexane and methanol). The degradation kinetics depends on the solvent polarity; the quickest pesticide transformation is in the aqueous medium and the slowest in hexane. The photoproducts are also dependent on the solvents. In the case of the aqueous solution, photocleavage of the carbon-sulphur bond gives 2-(methyl) phenyl-N-methylcarbamate as the main product, while methanol and hexane solutions show different photoproducts.

Somrani *et al.*, (2000) reported that photocatalytic degradation of Diuron in organic and semi-aqueous media is compared. The rates of disappearance fit Langmuir-Hinshelwood kinetics and the parameters k and K have been determined. A main

intermediate was detected in all cases and its structure has been determined. This product is obtained by oxidation of a methyl group of the urea moiety. Several compounds [trichloroaniline, dichloronitrobenzene, N-(3, 4-dichlorophenyl) formamide, dimethylurea] were detected by GC-MS analysis in both media while hydroxylated compounds were only observed in the semi-aqueous medium. Because the water solubility of Diuron at ambient temperature is very low (42 ppm) and our analytical technique was not suitable for detecting such weak concentrations, the photocatalyzed mineralization of Diuron was carried out in acetonitrile and in semi-aqueous 2.6 :1 (acetonitrile:water, by volume) media. The photocatalytic degradation of organic compounds in aqueous solutions has suggested the action of OH radicals. These radicals can be produced by the surface reaction of photoproduced holes with adsorbed water.

Kong and Lemley (2007) studied the effect of Triton X (TX) on the degradation kinetics and pathways of carbaryl undergoing anodic fenton treatment (AFT) to facilitate its practical application. The presence of Triton X-100 was found to slow down the carbaryl degradation rate. This result can be attributed to the consumption of hydroxyl radicals ($^{\bullet}OH$) by surfactants and the formation of a carbaryl...TX...$Fe^{3+}$ complex, resulting in the unavailability of carbaryl to $^{\bullet}OH$ attack. The modified AFT kinetic model shows an excellent fit to the carbaryl degradation profile ($R^2$>0.998), supporting the formation of a carbaryl...TX...$Fe^{3+}$ complex. The carbaryl degradation rate decreased as Triton X-100 concentration increased from 20 to 1000 mg $L^{-1}$. Both $^{\bullet}OH$ consumption by surfactants and complex formation are responsible for the degradation rate reduction below the critical micelle concentration (CMC), whereas the complex and micelle formation becomes a more dominant factor above the CMC. The effect of ethylene oxide (EO) numbers of a given nonionic surfactant mainly lies in the consumption of hydroxyl radicals, which increases with the length of the EO chain, but does not significantly affect the formation of the carbaryl...TX...$Fe^{3+}$ complex. Based on the GC–MS and LC–ESI–MS results, no evidence was found that the carbaryl degradation pathway was affected. Carbaryl was typically oxidized to 1-naphthol and 1,4-naphthoquinone similar to what is observed in the absence of surfactants. Triton X-100 was degraded via the breakdown of EO chains and $\omega$-oxidation of the terminal methyl group, which resulted in the production of a series of ethoxylate oligomers.

## 5.4 Photosensitizers

Tsao and Eto (1991) studied that photolysis of the flutolanil, in an aqueous solution and on solid surfaces (silica gel and glass) by irradiating with UV light under laboratory conditions. The photolysis of flutolanil, was accelerated more by carbonyl compounds such as benzophenone, acetophenone, flavone and xanthone than by dye photosensitizers like rose bengal, chlorophyllin and riboflavin.

Kole *et al.*, (1999) reported that sensitization process may be catalytic in nature when the chromophore in the sensitizer molecules regenerated by the process of energy transfer and cyclic redox reactions. Photosensitizers transfer the absorbed light energy to the acceptor molecule of pesticides.

Zaleska *et al.*, 1999; Zhao *et al.*, 2004; Chu & Wong, 2004; Ikehata & El-Din, 2006; Rashed & Amin, 2007) studied the role of some semiconductors like $TiO_2$, $H_2O_2$ and Fenton as photocatalysts in pesticide degradation for their efficiency in generating the singlet oxygen.

Substantial enhancement in the photodegradation of pesticides has been observed by soil organic matter like fulvic acid and humic acid (Zeng *et al.*, 2003; Sawinska *et al.*, 2004), dyes like rose Bengal, methylene blue (Kuo *et al.*, 2006), seconadary plant metabolites like riboflavin, pyridoxine, tyrosine etc.(Akhtar *et al.*, 1997).

Da Silva *et al.*, (2003) studied the photochemistry of triadimefon and triadimenol in aqueous solution and in methanol/water mixtures, in controlled and natural conditions. The photodegradation kinetics and product distribution are strongly dependent on the solvent and on the irradiation wavelength. The kinetics is faster in water than methanol. Direct photoreaction is an important dissipation pathway of triadimefon in natural water systems while triadimenol is stable in these conditions. 1, 2, 4-Triazole and 4-chlorophenol are two of the major photodegradation products. The formation of the 4-chlorophenoxyl radical was detected for both pesticides in methanol and methanol/water mixtures. In methanol/water mixtures the reaction of both pesticides also occurs with 4-chlorophenolate formation, which increases with the water content. The photochemical studies of pesticides and other pollutants should be made in conditions as similar as possible to those observed in environmental systems.

Singh *et al.*, (2003) investigated the photocatalysed degradation of bromacil herbicide in aqueous suspensions of titanium dioxide under a variety of conditions. The degradation was studied by monitoring the depletion in total organic carbon (TOC)

content as a function of irradiation time. The degradation kinetics of the model compound was studied under different conditions such as pH, catalyst concentration, substrate concentration, different types of $TiO_2$ and in the presence of electron acceptors such as hydrogen peroxide ($H_2O_2$) and potassium bromate ($KBrO_3$) besides molecular oxygen. The degradation rate was found to be strongly influenced by all the above factors. Higher degradation rate was observed with Degussa P25 as compared with other photocatalysts. The addition of bromate ion has been found to enhance the degradation rate markedly.

Daneshvar *et al.*, (2005) investigated the photodegradation efficiency of phosalone in water in the presence of $TiO_2$ particles under UV light illumination (1000 W). The influence of the basic photocatalytic parameters such as pH of the solution, amount of $TiO_2$, irradiation time, stirring rate, and distance from UV source was measured. The degradation rate of phosalone was not high when the photolysis was carried out in the absence of $TiO_2$ and it was negligible in the absence of UV light. The half-life ($DT_{50}$) of a 20 ppm aqueous solution of phosalone was 15 min in optimized conditions.

Malouki *et al.*, (2006) reported that ate of degradation of methabenzthiazuron (1 μM) was accelerated by $NO_3^-$ (0.1 mM) by a factor of 10. The irradiation of methabenzthiazuron (0.1 mM) in the presence of $NO_3^-$ (1 mM) or $NO_2^-$ (0.1 mM) yielded numerous intermediary photoproducts.

Quivet *et al.*, (2006) reported that photodegradation of imazapyr in aqueous solutions in the presence of $Na^+$, $Ca^{2+}$ or $Cu^{2+}$ and their concomitant anionic species ($Cl^-$, $NO_3^-$) plays major role in photo transformation processes of pesticides at various pesticide/metal ion molar ratios. The study showed the major role of metal ions in the degradation of imazapyr and its main photoproducts.

## 5.5 Analytical instruments used in photochemical studies

Analytical instruments like UV spectrophotometer, Infrared spectrophotometer, $^1$H-NMR, $^{13}$C-NMR and Mass Spectrometer are used for structural elucidation of any compound. The UV spectrophotometer gives an indication about the characteristic chromophoric group, Infrared spectrophotometer states about the nature of functional group, $^1$H-NMR determines the absorption position of protons in various structural environments, $^{13}$C-NMR indicates the number of carbons present in the molecule and Mass Spectrometer determines the molecular weight and the fragmentation pattern of the

molecule by the impact of electrons. With advancement of technology the structural confirmation of a molecule are carried out by the highly sophisticated instruments like GC-MS, LC-MS and Q-TOF micro mass spectrometer.

Lemiere (2001) mentioned the key strengths of Hybrid Quadrupole-TOF analyzer are its large mass-range, high degree of sensitivity, and essentially simultaneous detection of ions of all masses It is also fast and easy to use, and it has a simple experimental design (no tuning). The term Q-TOF is used to describe a type of hybrid mass spectrometer system, in which a quadrupole analyzer (Q) is used in conjunction with a time- of flight analyzer (TOF).By using two analyzer together (hybridized), distinct advantages accurate, which outweigh those of either analyzer individually. In the Q-TOF, the quadrupole is used in one of two modes to select the ions to be examined; the TOF analyzer measures the actual mass spectrum. The Q-TOF micro can provide information on elemental composition, structural characteristics and excellent specificity for identifying the complex matters. The ability of Q-TOF hybrid instruments to provide an accurate mass determination of the product ions in MS–MS for structure elucidation. This instrument provides an excellent sensitivity in MS/MS for product-ion scan and accurate mass for fragment identification.

Bobeldijk *et al.*, (2001) investigated surface water and identification of the non-target water pollutants with modern data acquisition possibilities of hybrid quadrupole-orthogonal acceleration time of flight mass spectrometers (Q-TOF).

Ferrer and Thurman (2003) reviewed on the importance of unequivocally detecting emerging contaminants, as well as establishing their presence in the environment by accurate mass spectrometric measurement techniques. The environmental issue of emerging contaminants is tied to the analysis of wastewater samples using the new analytical methods of the last decade, especially liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) and time-of-flight mass spectrometric (TOF/MS) techniques. Because of the high complexity of some environmental samples (i.e. wastewater and sludge samples), high-resolving power techniques are needed to provide additional structural information. Unique types of structural information for these compounds are derived from TOF/MS and quadrupole (Q)-TOF/MS instruments for the analysis of emerging contaminants in water and sediments. This review explains the unique features of these instruments and gives examples of their applications.

Ibanez *et al.*, (2004) reported that the high resolution and exact mass capabilities of hybrid quadrupole time-of-flight (QTOF) mass spectrometry can provide an ultimate confirmation in target analysis mode and aid in discovery and elucidation of unknown analytes. In this paper, the latter approach has been applied to study the transformation products of selected pesticides (terbuthylazine, simazine, terbutryn, terbumeton) in environmental waters. The high sensitivity in full-scan mode allowed elucidating minor metabolites even below 2% of the total peak area. Besides, the MS/MS capability of this tandem instrument was very useful for differentiation between isomeric transformation products. This work shows that hyphenated LC-QTOF is a powerful approach for the rapid screening and confirmation of unknown pesticide transformation products in environmental water.

Pico *et al.*, (2004) reviewed the different tandem MS instruments (triple and ion-trap quadrupoles, and hybrid quadrupole time-of-flight) including their advantages and drawbacks, typical detection limits, and ion signals at low concentrations. They emphasized on the instrumental set up, as well as LC and mass spectrometric experimental conditions, so as to increase the performance of the analytical system. The capacity of each instrument was to provide useful data for the identification of pesticides, and the possibility to obtain structural information for the identification of target and non-target compounds.

Aguera *et al.*, (2005) used GC/MS and LC/MS techniques for ascertaining chemical structures of a compound because of their sensitivity and selectivity. By this only tentative degradation pathways can be proposed. GC/MS has an important drawback for analyzing the very polar, less volatile compounds typically generated by these photo processes. In this case, LC/MS is a technique suitable for the analytes with a wide range of polarities, and has been shown to be a powerful tool for identification of the metabolites and unknown compounds in environmental samples.

Thurman *et al.*, (2005) studied the combination of liquid chromatography/ time-of-flight MS for accurate mass measurements to generate elemental compositions of ions and LC/ion trap multiple MS providing complementary structural information, which is useful for the elucidation of unknown organic compounds at trace levels in complex food extracts.

Hernandez *et al.*, (2005) discussed different strategies for developing sensitive multi-residue methods for pesticides in water, able to correctly quantify and confirm

compounds detected in samples. They discussed the use of different analyzers, triple quadrupole and hybrid quadrupole time-of-flight and compare their performances for screening purposes, and for the quantification and confirmation of positive samples.

Ibanez *et al.*, (2006) reported that Liquid chromatography (LC) coupled to hybrid quadrupole time-of-flight (QTOF) mass spectrometry (MS) is a useful analytical tool in the elucidation and confirmation of transformation products (TPs)/metabolites of pesticides with a wide range of polarity, in both environmental and biological samples. Firstly, the versatility of LC allows the determination of very distinct TPs/metabolites as chromatographic conditions can be easily changed and optimized depending on the analytical problem. Secondly, the mass accuracy provided by the TOF analyser allows the assignment of a highly probable empirical formula for each compound and the differentiation between nominal isobaric compounds. Finally, the possibility of performing MS/MS spectra with accurate mass measurements can be used for the final characterization of the TPs/metabolites detected and for the differentiation of isomeric compounds.

Lacorte and Fernandez (2006) reviewed the use of LC coupled to (quadrupole) time-of-flight mass spectrometry (LC-(Q) TOF-MS) to determine the presence of target and non-target pesticides in water and food. This technique gives accurate masses for both parent and fragment ions and enables the measurement of the elemental formula of a compound achieving compound identification. In addition, the combination of quadrupole-TOF permits tandem mass spectrometry, provides more structural information, and enhances selectivity.

Hernandez *et al.*, (2008) reported that many pesticide transformation products (TPs) can reach environmental waters as a consequence of their normally having a higher polarity than their parent pesticides. This makes the development of analytical methodology for reliable identification and subsequent quantification at the sub-microgram per liter levels necessary, as required under current legislation. They also reported that the photodegradation of several pesticides frequently detected in environmental waters from the Spanish Mediterranean region using the high-resolution and exact-mass capabilities of hybrid quadrupole time-of-flight mass spectrometry (QTOF MS) hyphenated to liquid chromatography (LC).

Chapter 6

# Material and Methods

# Material and Methods

The materials used and methods employed for the photo degradation of flubendiamide with three different solvent systems viz. acetonitrile: water, methanol: water and Isopropanol: water in order to investigate the transformation suffered by the compound under UV light ($\lambda max \leq 290$ nm) and Sunlight. A short account of the experimental design and methods employed for this study in different systems has been summarized in this chapter.

**6.1 Chemicals and solvents**

Analytical grade flubendiamide (96.7% purity) and formulation (20% WG) was supplied by M/s Rallis India Ltd, Bangalore. All the solvents viz. acetonitrile, methanol, isopropanol and water used under study were of HPLC grade.

**6.2 Preparation of flubendiamide solution for irradiation experiment**

Analytical grade flubendiamide (10mg) was dissolved in 1litre of solvent mixture of acetonitrile: water (1:1, v/v), methanol: water (1:1 v/v) and Isopropanol: water (1:1, v/v) separately to prepare 10 ppm concentration. Similarly flubendiamide (20% WG) formulation (50mg) was also dissolved in one litre of solvent mixture of acetonitrile: water (1:1, v/v), methanol: water (1:1 v/v) and Isopropanol: water (1:1, v/v) separately to prepare 10 ppm concentration.

**6.3 Irradiation under Sunlight**

The flubendiamide (analytical grade and formulation) each of 10 ppm concentration was prepared in a 1 litre Erlenmeyer flask (borosilicate glass) separately with acetonitrile: water (1:1, v/v), methanol: water (1:1 v/v) and isopropanol: water (1:1, v/v). The mouths of the flasks were tightly covered with rubber cork wrapped with aluminium foil to prevent any contamination. The flasks were then exposed to sunlight for kinetics study. Sunlight irradiated samples (5 mL) were withdrawn from each flasks separately at an interval of 0, 1, 3, 7, 15, 30, 40, 50, 60, 70, and 80 hours. Samples were then filtered through 0.2 μm Millipore discs and subjected to HPLC analysis in order to study the rate of degradation of flubendiamide. The control samples of flubendiamide and

formulation was kept in dark with all the three solvents and no reaction was observed in the control samples during the entire irradiation period.

### 6.4 Irradiation under UV light

Irradiation of 10 ppm analytical grade flubendiamide was carried out in a 1 L Pyrex-glass immersion photochemical reactor, with each of the selected solvent mixture [acetonitrile: water; methanol: water and isopropanol: water (1:1, v/v)] separately and cutting the radiation shorter than 290 nm. Similarly, 10 ppm flubendiamide formulation was also irradiated. A high-pressure mercury lamp (HPK 125W Philips), was placed in a water-jacketed Pyrex tube (diameter 3 cm), centred in the reactor. The reaction medium was stirred before illumination for at least half an hour in the dark in order to achieve the maximum adsorption of the flubendiamide onto solutions. During irradiation experiment, the solution was magnetically stirred continuously to keep it slurry. The progress of the reaction was followed by successive samplings (5 mL) at time intervals of 0, 1, 3, 5, 7, 10, 15, 30, 40 and 50 hours. Samples were then filtered through 0.2 μm Millipore discs and subjected to HPLC analysis in order to study the rate of degradation of flubendiamide. The control samples of flubendiamide and formulation was kept in dark with all the three solvents and no reaction was observed in the control samples during the entire irradiation period.

### 6.5 Analytical procedures for kinetic study

Reverse phase high pressure liquid chromatography (Agilent 1200 series) was used for analysis of the irradiated samples under UV light and sunlight. The operating parameters of HPLC are same as mentioned in table 7 and interpretation of residue data in section 3.2.1.4 of Chapter 3 in Part – I respectively.

### 6.6 Characterization of the flubendiamide photo products by Q-TOF micro mass spectrometer

There are various analytical methods for the identification of photo degraded products of which mass spectrometry is the most common. Very recently, quadrupole ion trap (QIT) and hybrid quadrupole/time-of-flight (TOF) instruments have joined the previous ones to increase the possibilities of identification. Now- a- days, Micromass ® Q-TOF micro™ Mass Spectrometer, one of the most modern tools is used for separation and identification of the compounds in mixture. The ability of Q-TOF hybrid instruments

is to provide an accurate mass determination of the product ions in MS–MS for structure elucidation. TOF analyzers are the simplest and fastest mass spectrometers. All of the sample ions are accelerated with the same amount of energy, and travel toward the detector over the same length of an evacuated flight tube (typically 1–2 m). Ions typically have a kinetic energy of 1,000–10,000 eV, and move through the analyzer in approximately 20 μs. Because the ions have the same energy but different m/z values, they will separate from each other and reach the detector at different times. The term Q-TOF is used to describe a type of hybrid mass spectrometer system, in which a quadrupole analyzer (Q) is used in conjunction with a time- of flight analyzer (TOF). By using two analyzer together (hybridized), distinct advantages accurate, which outweigh those of either analyzer individually. In the Q-TOF, the quadrupole is used in one of two modes to select the ions to be examined; the TOF analyzer measures the actual mass spectrum. The Q-TOF micro can provide information on elemental composition, structural characteristics and excellent specificity for identifying the complex matters. Hybrid quadrupole-TOF delivers simple exact mass measurement of precursor and product ions with maximum sensitivity to yield the highest confidence in structural elucidation and databank search results.

Considering the advantage of accurate mass determination of trace amount of unknown compounds from a mixture by Q-TOF, the present investigator carried out the characterization of the structures of the flubendiamide photoproducts from irradiated samples (sunlight and UV light) by using Micromass ® Q-TOF micro™ Mass Spectrometer with Electron Spray Ionization (positive mode), Water CaplC® pump and Auto sampler using Mass Lynx ™ 4.0 software.

Chapter 7

# Results and Dicussion

# Results and Discussion

The present chapter deals with the photolytic study of flubendiamide (analytical grade and formulation) under UV and sunlight in different aqueous organic solvent system. The results of the photo kinetic study and the characterization of different photoproducts form the subject matter of discussion.

## 7.1 Kinetic study of flubendiamide under sunlight

### 7.1.1 Analytical grade and flubendiamide formulation in aqueous acetonitrile

The results of the kinetic study of flubendiamide in acetonitrile: water (1:1, v/v) has been summarized in table 65.The analytical grade and formulations (10 ppm) were irradiated under sunlight for 80 hours. The scrutiny of the results indicated that in both the cases the rate of disappearance of flubendiamide was increased with increment of irradiation time. It was observed that after 15 hours, the dissipation percentage of analytical grade flubendiamide was found to be 18.61 % and that of formulation was found to be 22.40 % of their initial concentration (Figure 37). Around 50% of flubendiamide remained after 50 and 60 hours (Table 65) in analytical grade and formulation respectively. Dissipation of flubendiamide followed first order reaction kinetics (Figure 38) in both the cases as a straight line was obtained in each case when log values of the residue were plotted against different time intervals. The significant correlation co-efficient ($r^2$ = 0.95-0.98) indicated statistical conformity of the dissipation data to first order kinetics (Table 65). The calculated half life value from the regression equation was found to be 55.75 hours (analytical grade) and 79.22 hours (formulation).

Table 65: Rate of disappearance of flubendiamide (analytical and formulation) in acetonitrile: water (1:1) under sunlight

| Irradiation time (hr) | Analytical | | Formulation | |
|---|---|---|---|---|
| | Concentration (ppm) | Dissipation (%) | Concentration (ppm) | Dissipation (%) |
| 0 | 10.00 | - | 10.00 | - |
| 1 | 9.82 | 1.80 | 9.70 | 3.04 |
| 3 | 9.69 | 3.06 | 9.14 | 8.59 |
| 7 | 9.60 | 3.96 | 8.30 | 17.00 |
| 15 | 8.14 | 18.61 | 7.76 | 22.40 |
| 30 | 7.32 | 26.83 | 7.57 | 24.31 |
| 40 | 6.56 | 34.43 | 7.17 | 28.34 |
| 50 | 5.67 | 43.33 | 6.56 | 34.45 |
| 60 | 5.21 | 47.90 | 5.97 | 40.28 |
| 70 | 3.96 | 60.36 | 4.87 | 51.33 |
| 80 | 3.66 | 63.37 | 4.60 | 53.95 |
| Regression equation | y = 4.0088-0.0054x | | y = 3.9791-0.0038x | |
| $r^2$ | 0.98 | | 0.95 | |
| $DT_{50}$ (hr) | 55.75 | | 79.22 | |

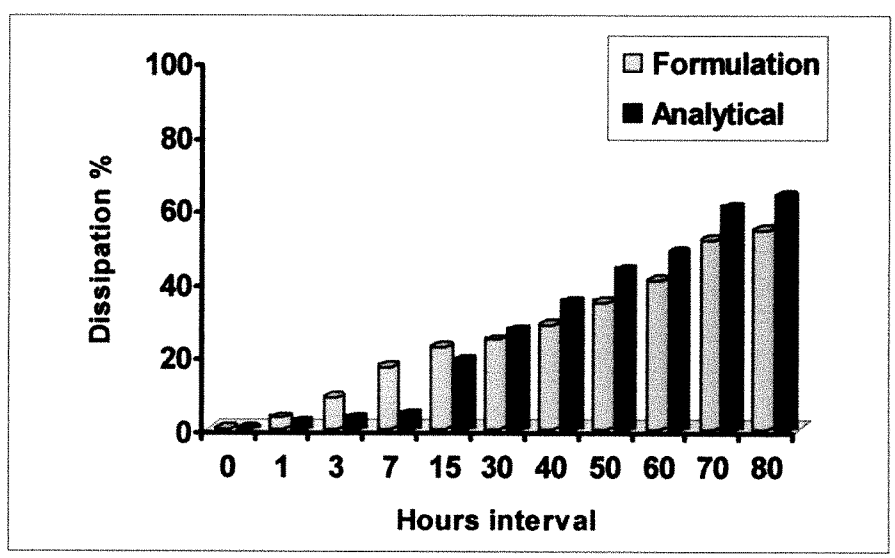



Figure 37: Dissipation percentage of flubendiamide analytical grade and formulation in aqueous acetonitrile under sunlight

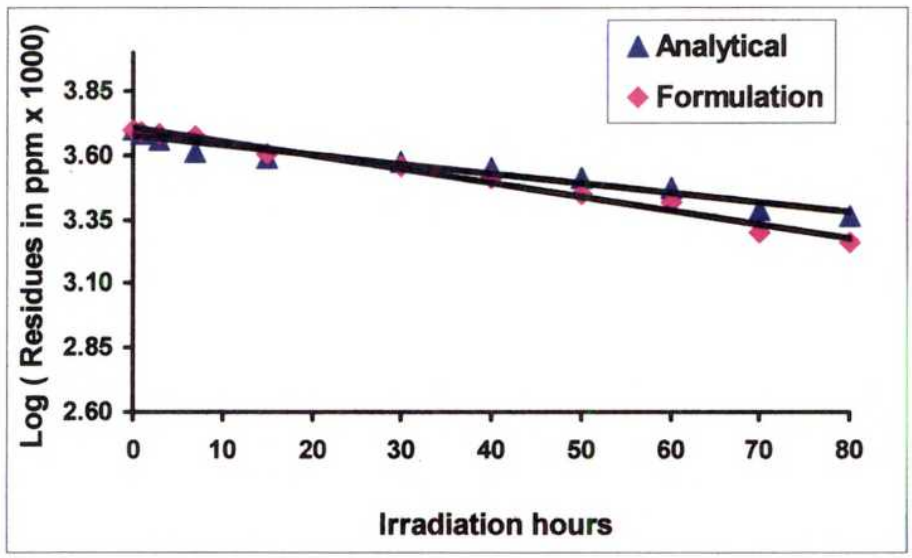



Figure 38: Linear plot of first order reaction kinetics of flubendiamide in aqueous acotonitrile under influence of sunlight

### 7.1.2 Analytical grade and flubendiamide formulation in aqueous methanol

The results of the kinetic study of flubendiamide in methanol: water (1:1, v/v) is summarized in table 66.The analytical grade and formulations (10 ppm) were irradiated under sunlight for 80 hours. The scrutiny of the results indicated that in both the cases the degradation rate of flubendiamide increased with irradiation time. It was observed that after 15 hours, the dissipation percentage of analytical grade flubendiamide was found to be 28.25 % and that of formulation was found to be 10.82% of their initial concentration (Figure 39). Dissipation of flubendiamide residue followed first order reaction kinetics (Figure 40) in both the cases as a straight line was obtained in each case when log values of the residue were plotted against different time intervals. The significant correlation co-efficient ($r^2$ = 0.96) indicated statistical conformity of the dissipation data to first order kinetics (Table 66). The calculated half life value from the regression equation was found to be 73.42 hours (analytical grade) and 158.43 hours (formulation).

Table 66: Rate of disappearance of flubendiamide (analytical and formulation) in methanol: water (1:1) under sunlight

| Irradiation time (hr) | Analytical | | Formulation | |
|---|---|---|---|---|
| | Concentration (ppm) | Dissipation (%) | Concentration (ppm) | Dissipation (%) |
| 0 | 10.00 | - | 10.00 | - |
| 1 | 9.20 | 7.98 | 9.69 | 3.07 |
| 3 | 8.50 | 15.02 | 9.39 | 6.08 |
| 7 | 7.54 | 24.64 | 9.39 | 6.12 |
| 15 | 7.18 | 28.25 | 8.92 | 10.82 |
| 30 | 6.66 | 33.32 | 8.93 | 10.72 |
| 40 | 6.20 | 37.97 | 8.36 | 16.36 |
| 50 | 5.66 | 43.43 | 8.09 | 19.14 |
| 60 | 5.20 | 47.99 | 7.56 | 24.39 |
| 70 | 4.62 | 53.79 | 6.93 | 30.69 |
| 80 | 4.08 | 59.16 | 6.73 | 32.74 |
| Regression equation | y = 3.9504-0.0041x | | y = 3.9912-0.0019x | |
| $r^2$ | 0.96 | | 0.96 | |
| $DT_{50}$ (hr) | 73.42 | | 158.43 | |

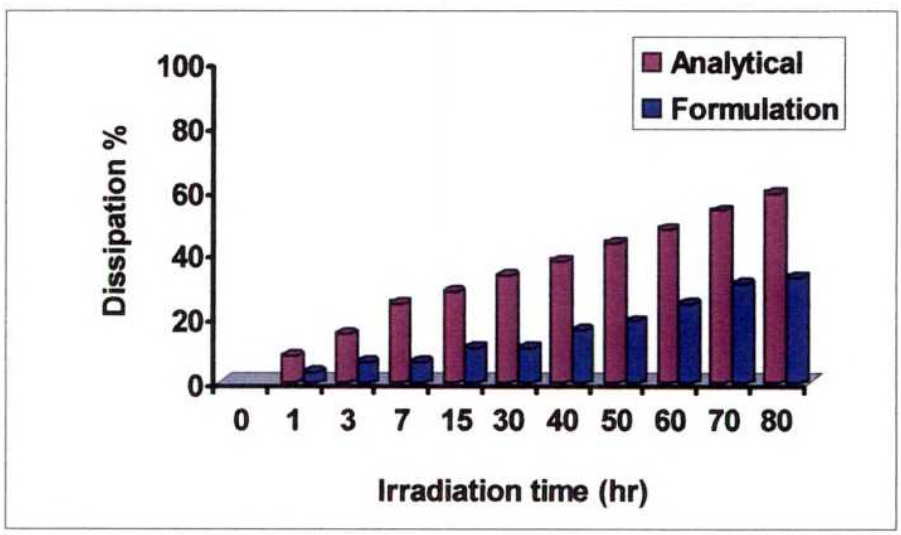



Figure 39: Dissipation percentage of flubendiamide analytical grade and formulation in aqueous methanol under sunlight

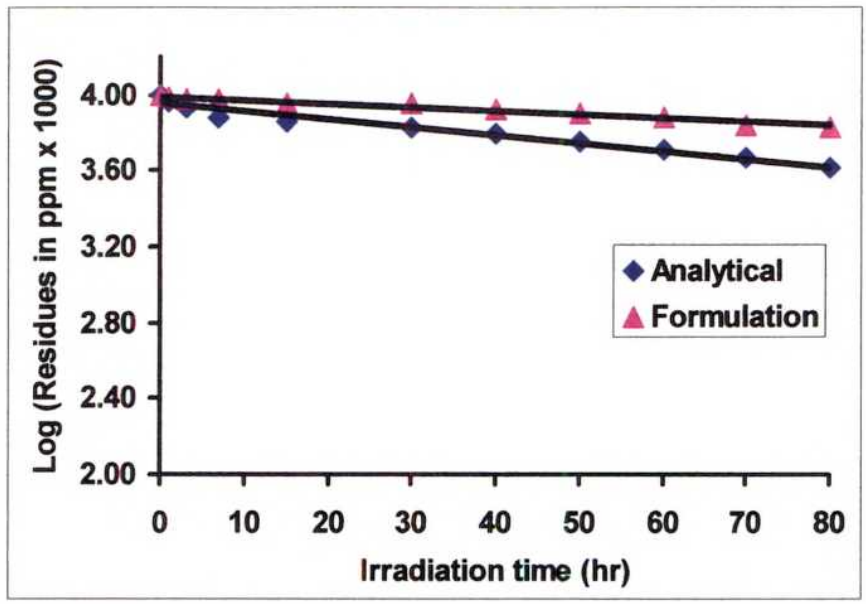



Figure 40: Linear plot of first order reaction kinetics of flubendiamide in aqueous methanol under influence of sunlight

### 7.1.3 Analytical grade and flubendiamide formulation in aqueous isopropanol

The results of the kinetic study of flubendiamide in isopropanol: water (1:1, v/v) has been summarized in table 67.The analytical grade and formulations (10 ppm) were irradiated under sunlight for 80 hours. The results indicated that in both the cases the rate of disappearance of flubendiamide increased with increment of time of irradiation. It appeared that the dissipation percentage of analytical grade flubendiamide after 15 hours was found to be 9.92 % and that of formulation was found to be 7.24% of the initial concentration (Figure 41). Flubendiamide remaining percentage was found to be 52-66% after 80 hours (Table 67) in analytical grade and formulation respectively. A first order reaction kinetics (Figure 42) of flubendiamide was obtained under the influence of sunlight in the presence of aqueous isopropanol. The significant correlation co-efficient ($r^2 = 0.95$-$0.96$) indicated statistical conformity of the dissipation data to first order kinetics (Table 65). The calculated half life value from the regression equation was found to be 94.01 hours (analytical grade) and 150.51 hours (formulation).

Table 67: Rate of disappearance of flubendiamide (analytical and formulation) in isopropanol: water (1:1) under sunlight

| Irradiation time (hr) | Analytical | | Formulation | |
|---|---|---|---|---|
| | Concentration (ppm) | Dissipation (%) | Concentration (ppm) | Dissipation (%) |
| 0 | 10.00 | - | 10.00 | - |
| 1 | 9.88 | 1.17 | 9.65 | 3.48 |
| 3 | 9.80 | 1.99 | 9.56 | 4.39 |
| 7 | 9.24 | 7.64 | 9.55 | 4.53 |
| 15 | 9.01 | 9.92 | 9.28 | 7.24 |
| 30 | 8.59 | 14.13 | 9.05 | 9.55 |
| 40 | 8.11 | 18.95 | 8.69 | 13.13 |
| 50 | 7.40 | 25.96 | 8.14 | 18.55 |
| 60 | 6.71 | 32.92 | 7.67 | 23.33 |
| 70 | 5.75 | 42.47 | 7.18 | 28.19 |
| 80 | 5.20 | 48.00 | 6.62 | 33.81 |
| Regression equation | y = 4.007-0.0032x | | y = 3.99.78-0.002x | |
| $r^2$ | 0.95 | | 0.96 | |
| $DT_{50}$ (hr) | 94.07 | | 150.51 | |

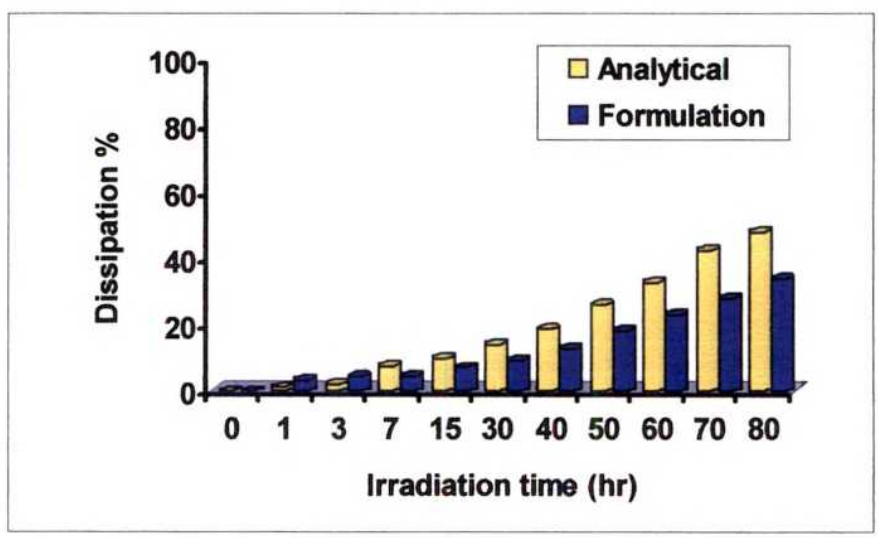



Figure 41: Dissipation percentage of flubendiamide analytical grade and formulation in aqueous isopropanol under sunlight

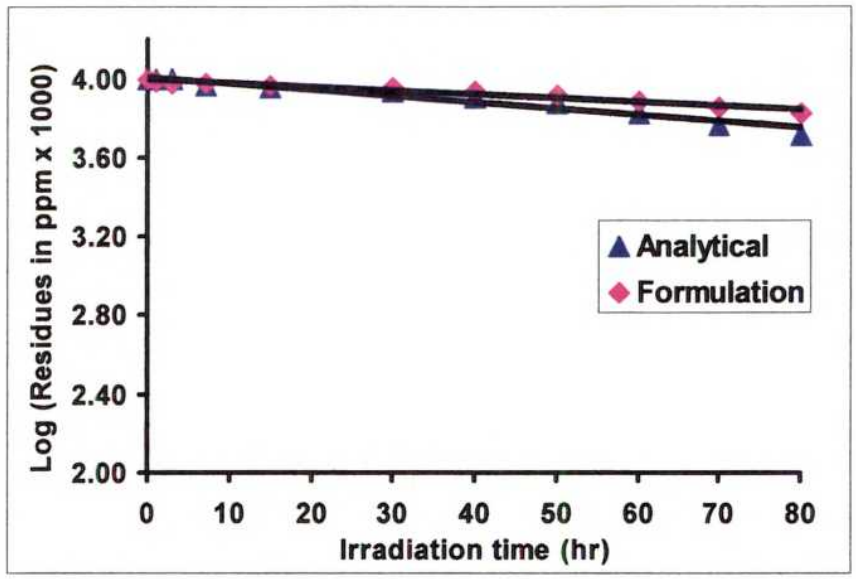



Figure 42: Linear plot of first order reaction kinetics of flubendiamide in aqueous isopropanol under influence of sunlight

The comparison of the photolytic half-life revealed that the flubendiamide degradation occurred faster in aqueous aceonitrile compared to methanolic and isopropanolic aqueous solution irrespective of analytical and formulation. Furthermore, upon comparison of the half life between analytical and formulation in three different solvent system it revealed that faster degradation occurred in the analytical grade than the formulation. The probable reason for slower rate of degradation of formulation may be attributed to the consumption of hydroxyl radicals (•OH) by surfactants and the formation of a substrate-surfactant complex, resulting in the unavailability of flubendiamide to •OH attack. The same type of phenomenon was observed with carbaryl as reported by Kong and Lemley (2007).

### 7.2 Kinetic study of flubendiamide under UV light

#### 7.2.1 Analytical grade and flubendiamide formulation in aqueous acetonitrile

The results of the kinetic study of flubendiamide in acetonitrile: water (1:1, v/v) has been summarized in table 68.The analytical grade and formulations (10 ppm) were irradiated under UV light for 30 hours. The results indicated that in both the cases the rate of disappearance of flubendiamide increased with irradiation time. It was observed that the initial concentration of flubendiamide (10 ppm) reached to 6.14 and 9.32 ppm in an hour which further decreased to 2.98 and 7.50 ppm in three hour corresponding to the loss of 70% and 25 % for analytical and formulation respectively. In case of

analytical grade, it was further observed that after three hours of irradiation, degradation rate was steady and 96.86 % dissipation (Figure 43) occurred at 15 hours. Disappearance of flubendiamide formulation was slower than analytical grade and it was found that 75 % dissipation took place at 15 hours. Analytical flubendiamide disappeared 100% and that of formulation was 93.35 % at 30 hour. Dissipation of flubendiamide residue followed first order reaction kinetics (Figure 44) in both the cases as a straight line was obtained in each case when log values of the residue were plotted against different time intervals. The significant correlation co-efficient ($r^2$=0.98--0.99) indicated statistical conformity of the dissipation data to first order kinetics (Table 68). The calculated half life value from the regression equation was found to be 3.17 hours (analytical grade) and 7.60 hours (formulation).

Table 68: Rate of disappearance of flubendiamide (analytical and formulation) in acetonitrile: water (1:1) under UV light

| Irradiation time (hr) | Analytical | | Formulation | |
|---|---|---|---|---|
| | Concentration (ppm) | Dissipation (%) | Concentration (ppm) | Dissipation (%) |
| 0 | 10.00 | - | 10.00 | - |
| 1 | 6.14 | 38.55 | 9.32 | 6.80 |
| 3 | 2.98 | 70.16 | 7.50 | 24.97 |
| 5 | 2.87 | 71.34 | 6.69 | 33.12 |
| 7 | 1.85 | 81.50 | 5.63 | 43.70 |
| 10 | 0.83 | 91.66 | 4.43 | 55.73 |
| 15 | 0.31 | 96.86 | 2.42 | 75.75 |
| 30 | 0.00 | 100.00 | 0.67 | 93.35 |
| Regression equation | y = 3.9005-0.0951x | | y = 4.0119-0.0396x | |
| $r^2$ | 0.98 | | 0.99 | |
| $DT_{50}$ (hr) | 3.17 | | 7.60 | |

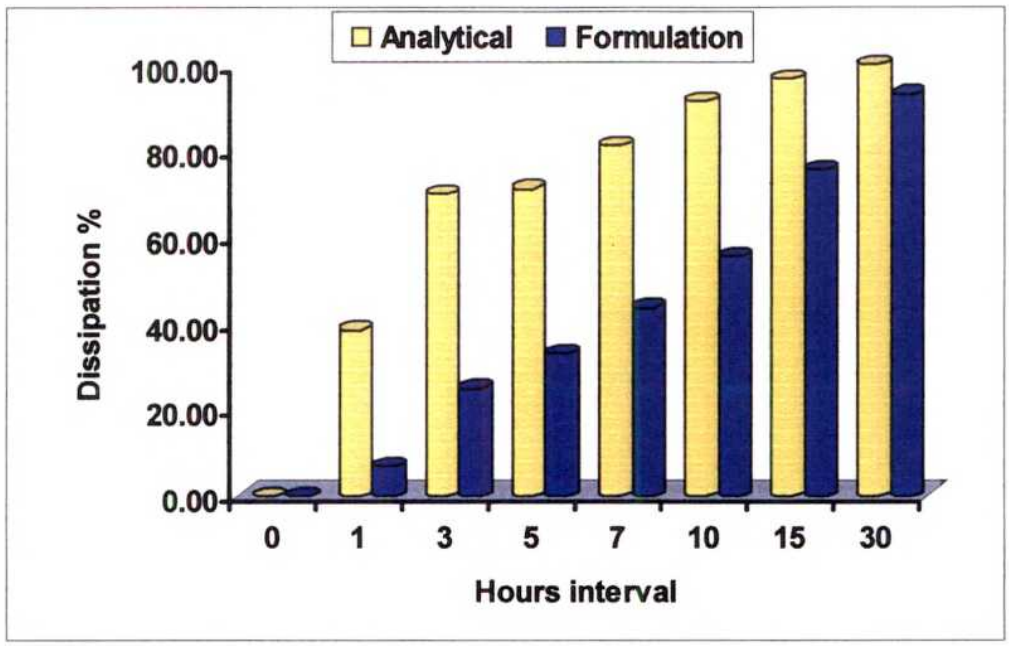



Figure 43: Dissipation percentage of flubendiamide analytical grade and formulation in aqueous acetonitrile under UV light

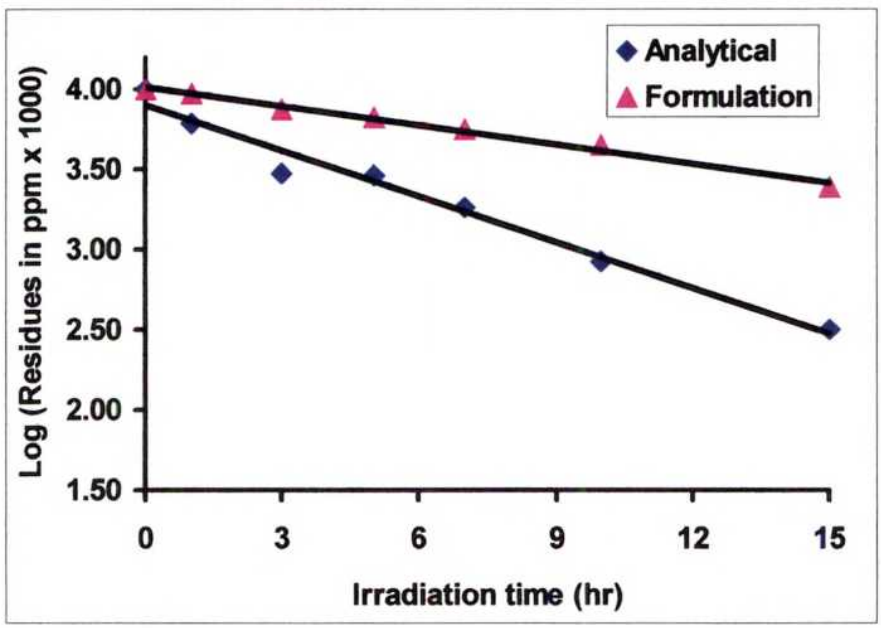



Figure 44: Linear plot of first order reaction kinetics of flubendiamide in aqueous acotonitrile under influence of UV light

### 7.2.2 Analytical grade and flubendiamide formulation in aqueous methanol

The results of the kinetic study of flubendiamide in methanol: water (1:1, v/v) has been summarized in table 69. The analytical grade and formulations (10ppm) were irradiated under UV light for 60 hours. The results indicated that in both the cases the

rate of disappearance of flubendiamide increased with exposure of irradiation time. It was observed that the initial concentration (100%) of flubendiamide (analytical and formulation) remained 53.20% and 79.95 % respectively after 7 hours of exposure. The flubendiamide dissipated to 96.25% (analytical) and 86.35% (formulation) after 60 hours (Figure 45). Dissipation of flubendiamide residue followed first order reaction kinetics (Figure 46) in both the cases as a straight line was obtained in each case when log values of the residue were plotted against different time intervals. The significant correlation co-efficient ($r^2$=0.97--0.99) indicated statistical conformity of the dissipation data to first order kinetics (Table 69). The calculated half life value from the regression equation was found to be 12.04 hours (analytical grade) and 21.81 hours (formulation).

Table 69: Rate of disappearance of flubendiamide (analytical and formulation) in methanol: water (1:1) under UV light

| Irradiation time (hr) | Analytical | | Formulation | |
|---|---|---|---|---|
| | Concentration (ppm) | Dissipation (%) | Concentration (ppm) | Dissipation (%) |
| 0 | 10.00 | - | 10.00 | - |
| 1 | 9.04 | 9.58 | 9.32 | 6.78 |
| 3 | 7.62 | 23.77 | 8.63 | 13.68 |
| 7 | 5.32 | 46.80 | 7.60 | 24.05 |
| 15 | 2.80 | 71.97 | 5.70 | 43.02 |
| 30 | 1.06 | 89.41 | 3.54 | 64.56 |
| 45 | 0.51 | 94.89 | 2.56 | 74.41 |
| 60 | 0.37 | 96.25 | 1.36 | 86.35 |
| Regression equation | y = 3.9187-0.025x | | y = 3.9816-0.0138x | |
| $r^2$ | 0.97 | | 0.99 | |
| $DT_{50}$ (hr) | 12.04 | | 21.81 | |

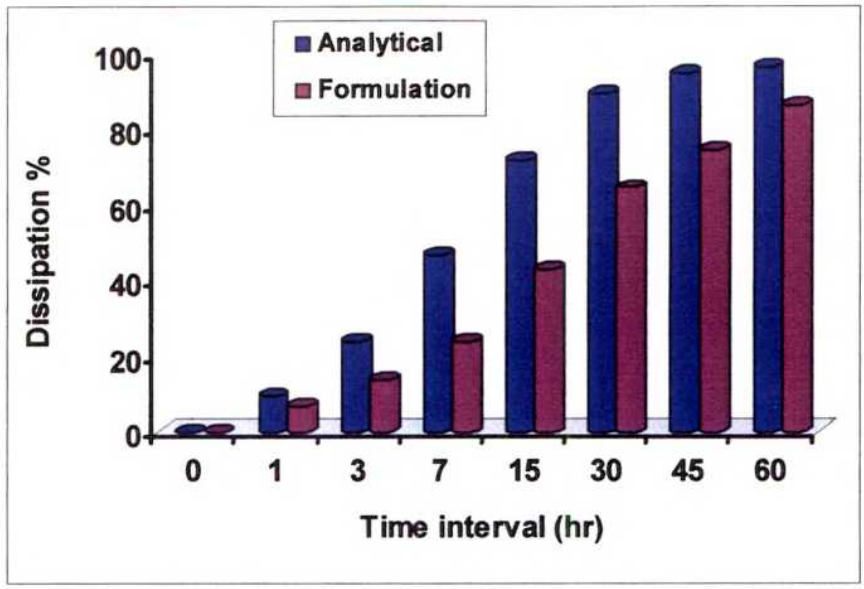



Figure 45: Dissipation percentage of flubendiamide analytical grade and formulation in aqueous methanol under UV light

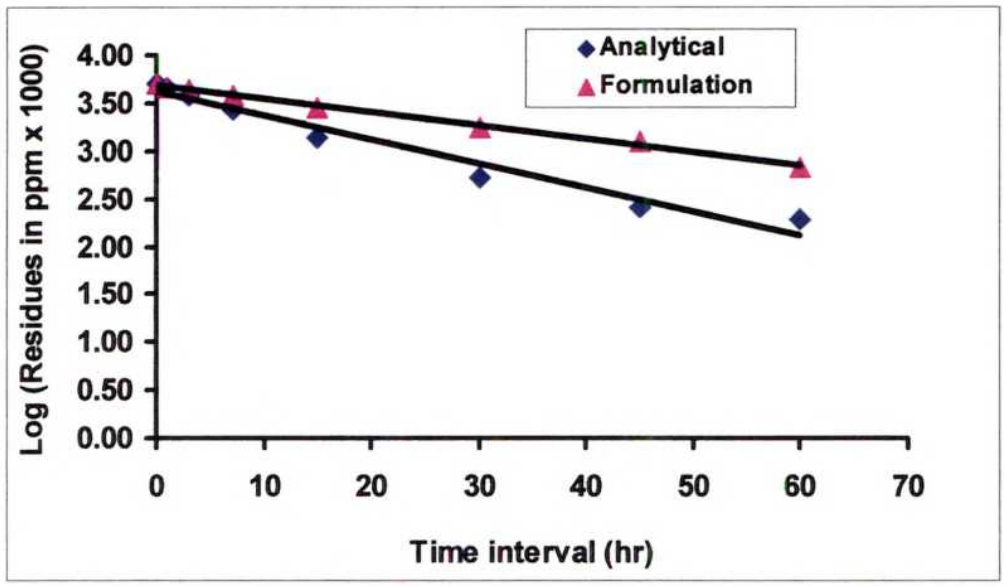



Figure 46: Linear plot of first order reaction kinetics of flubendiamide in aqueous methanol under influence of sunlight

### 7.2.3 Analytical grade and flubendiamide formulation in aqueous isopropanol

The results of the kinetic study of flubendiamide in isopropanol: water (1:1, v/v) is summarized in table 70. The analytical grade and formulations (10 ppm) were irradiated under UV light for 80 hours. The results indicated that in both the cases the rate of disappearance of flubendiamide increased with exposure of irradiation time. It

was observed that the initial concentration (100%) of flubendiamide (analytical and formulation) remained 74.22% and 84.19 % respectively after 7 hours of exposure. The flubendiamide dissipated to 72.01% (analytical) and 53.01% (formulation) after 60 hours (Figure 47). Dissipation of flubendiamide residue followed first order reaction kinetics (Figure 48) in both the cases as a straight line was obtained in each case when log values of the residue were plotted against different time intervals. The significant correlation co-efficient ($r^2$=0.98-0.99) indicated statistical conformity of the dissipation data to first order kinetics (Table 70). The calculated half life value from the regression equation was found to be 36.26 hours (analytical grade) and 55.74 hours (formulation).

Table 70: Rate of disappearance of flubendiamide (analytical and formulation) in isopropanol: water (1:1) under UV light

| Irradiation time (hr) | Analytical | | Formulation | |
|---|---|---|---|---|
| | Concentration (ppm) | Dissipation (%) | Concentration (ppm) | Dissipation (%) |
| 0 | 10.00 | - | 10.00 | - |
| 1 | 9.14 | 8.64 | 9.70 | 3.00 |
| 3 | 8.51 | 14.94 | 9.04 | 9.60 |
| 7 | 7.42 | 25.78 | 8.42 | 15.81 |
| 15 | 6.48 | 35.20 | 7.64 | 23.61 |
| 30 | 5.04 | 49.64 | 6.68 | 33.20 |
| 40 | 3.68 | 63.18 | 5.92 | 40.80 |
| 50 | 3.14 | 68.64 | 5.22 | 47.81 |
| 60 | 2.80 | 72.01 | 4.70 | 53.01 |
| 70 | 2.58 | 74.17 | 4.02 | 59.80 |
| 80 | 2.08 | 79.19 | 3.50 | 65.02 |
| Regression equation | y = 3.9504-0.0083x | | y = 3.981-0.0054x | |
| $r^2$ | 0.98 | | 0.99 | |
| $DT_{50}$ (hr) | 36.26 | | 55.74 | |

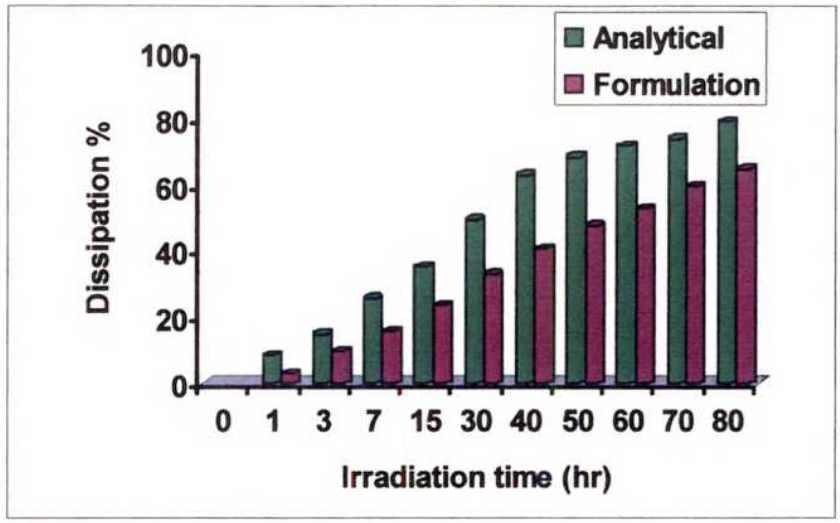



Figure 47: Dissipation percentage of flubendiamide analytical grade and formulation in aqueous isopropanol under UV light

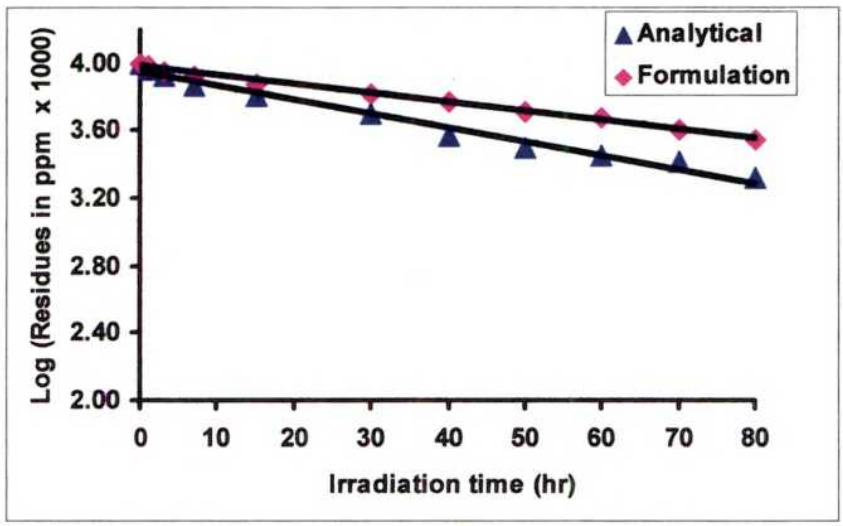



Figure 48: Linear plot of first order reaction kinetics of flubendiamide in aqueous isopropanol under influence of UV light

The comparison of photolytic half life of flubendiamide under the influence of UV light in three different aqueous organic solvent system revealed that degradation rate was faster in the order of acetonitrile: water > methanol: water > isopropanol: water for the analytical and formulation respectively. It was further observed that disappearance of analytical was faster than formulation in all the three solvent systems.

The comparison of irradiation type, it was found that faster degradation occurred under influence of UV light irrespective of flubendiamide analytical and formulation as

well as solvent system when compared to sunlight. The faster degradation in UV light might be accounted by the fact that flubendiamide $\lambda_{max}$ is 210 nm and the energy (95.3 kcal / mol) at $\lambda \leq 300$ nm is sufficient to break many chemical bonds. Also, the distance between the flubendiamide solutions and the UV light source was shorter as compared to sunlight.

## 7.3 HPLC monitoring of photo transformed products

### 7.3.1 Monitoring of sunlight and UV irradiated samples of flubendiamide in aqueous acetonitrile

The sunlight and UV irradiated samples of flubendiamide (analytical and formulation) in aqueous acetonitrile, methanol and isopropanol at different time intervals were monitored by HPLC using UV detector at wavelength of 210 nm. The HPLC chromatogram at different hours of sunlight irradiated samples in acetonitrile: water (1:1) revealed that various photo transformed products were formed with the continuous degradation of flubendiamide whether it was analytical or formulation. For both the cases, it was observed that other than flubendiamide (9.70 minute), one additional peak was found at the retention time (RT) of 7.701 minutes which resembles to des-iodo flubendiamide metabolite as compared with the authentic ones. This peak height was increased with increment of time exposure. Very interestingly, one more peak was found at 15 hours having RT of 8.331 minutes However, the additional peak heights were also increased with time and one more peak was found at 50 hours having the RT of 3.053 minutes. At 80 hours four more additional peaks were found having the retention time of 2.585, 3.861, 4.816 and 5.66 minutes (Figure 49). Thus, samples irradiated under sunlight give rise to seven (7) photo peaks in addition to flubendiamide.

The HPLC chromatogram at different hours of UV irradiated samples in acetonitrile: water (1:1) revealed that various photo transformed products were formed with the continuous degradation of flubendiamide whether it was analytical or formulation. It was observed that within 30 hours 100% flubendiamide disappeared. The peak at retention time (RT) 7.706 minutes was found at 1 hour and thereafter at five hours one more peak was found with RT of 4.029 minutes. Another peak with RT 4.839 minutes was observed at 15 hours. Thus, altogether three photo products were formed under UV light (Figure 50). However, out of seven peaks observed in sunlight three peaks matched under UV light condition.

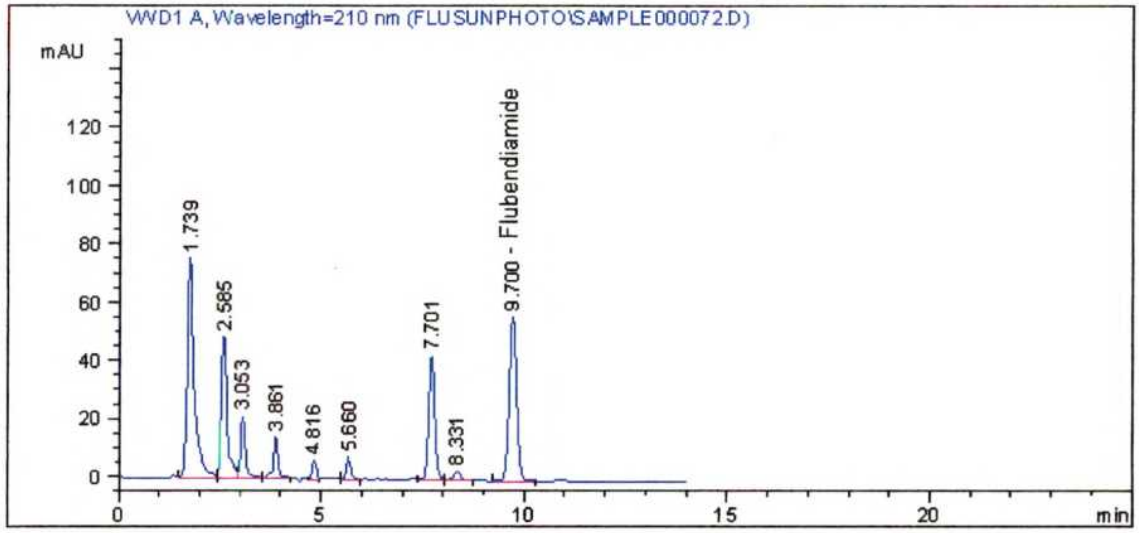



Figure 49: HPLC chromatogram of sunlight irradiated analytical flubendiamide at 80 hours in acetonitrile: water (1:1)

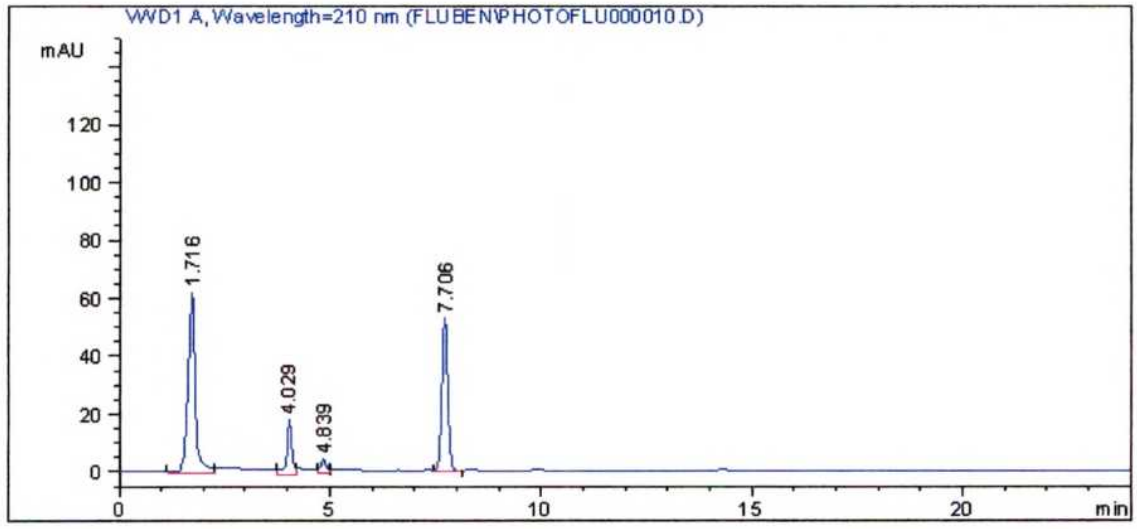



Figure 50: HPLC chromatogram of UV irradiated analytical flubendiamide at 30 hours in acetonitrile: water (1:1)

### 7.3.2 Monitoring of sunlight and UV irradiated samples of flubendiamide in aqueous methanol

The monitoring of sunlight and UV irradiated flubendiamide (analytical and formulation) samples in aqueous methanol at different time intervals was carried out by HPLC. The sunlight irradiated samples revealed that flubendiamide disappearance was very slow and one peak was observed at RT 7.625 minutes (Figure 51). This compound at RT 7.625 minutes started to form from 1 hour and gradually the peak height was increased upto 80 hours.

In the HPLC chromatogram at different hours of UV irradiated samples; one additional peak was found with RT 5.945 minutes in addition to the peaks found in sunlight. In altogether two photo products at 60 hours were formed under UV light and the parent compound degraded 96.25% at 60 hours (Figure 52). It was further observed that the peak height at RT-7.714 minutes was more than what found in sunlight. This peak was identified as des-iodo flubendiamide by comparing with the external standard. Schwack *et al.*, (1995) also reported that photolysis in methanol yielded dehalogenated photoproducts.

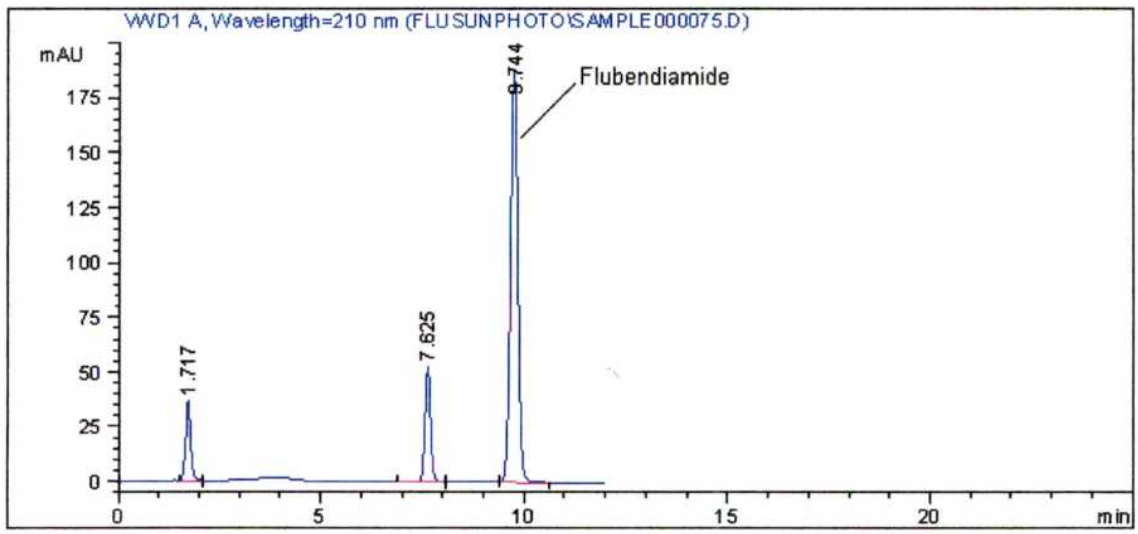



Figure 51: HPLC chromatogram of sunlight irradiated analytical flubendiamide at 80 hours in methanol: water (1:1)

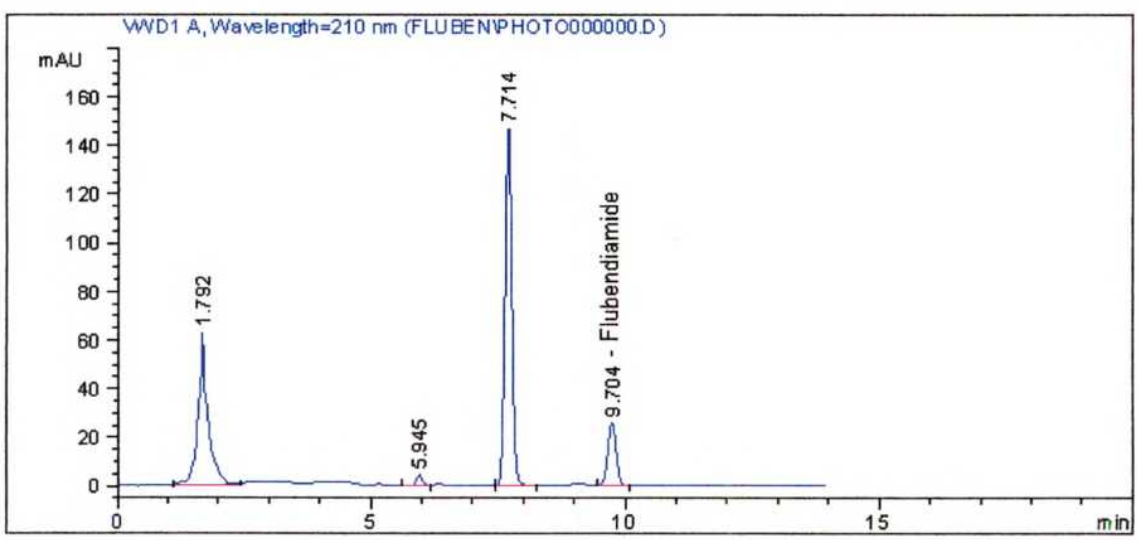



Figure 52: HPLC chromatogram of UV irradiated analytical flubendiamide at 60 hours in methanol: water (1:1)

### 7.3.3 Monitoring of sunlight and UV irradiated samples of flubendiamide in aqueous isopropanol

The monitoring of sunlight irradiated flubendiamide (analytical and formulation) samples was carried out by HPLC in aqueous isopropanol at different time intervals. At 80 hours the irradiated sample chromatogram showed the presence of photo transformed peak at RT 7.757 and 8.309 minutes (Figure 52). The peak at RT 7.757 and 9.698 minutes corresponds to des-iodo flubendiamide and flubendiamide as had been compared with the external standard. In UV irradiated samples; only one peak was found with RT 7.694 minutes in addition to flubendiamide peak (Figure 53).

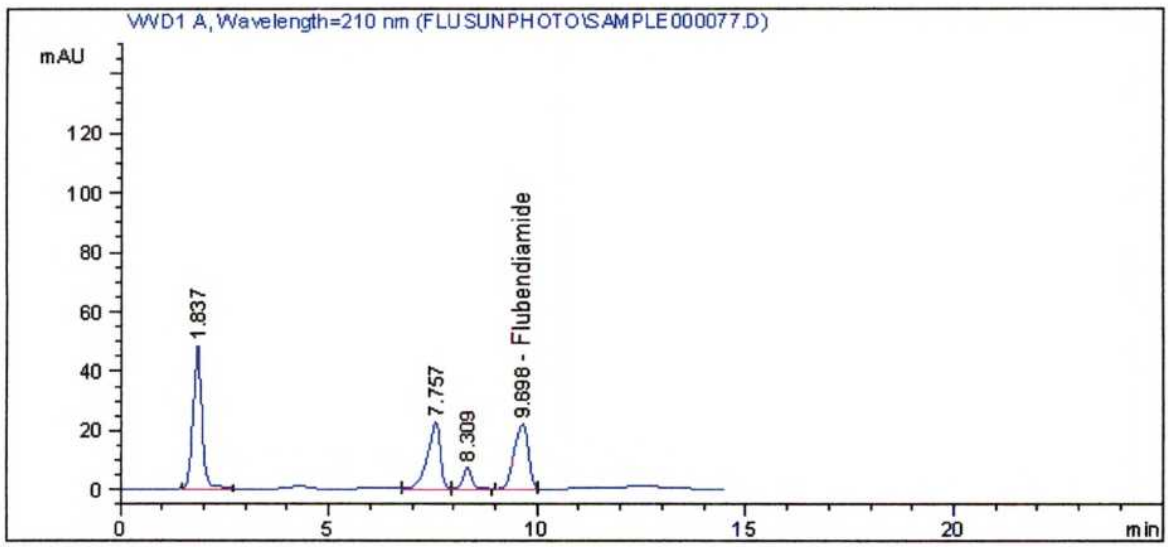



Figure 53: HPLC chromatogram of sunlight irradiated analytical flubendiamide at 80 hours in isopropanol: water (1:1)

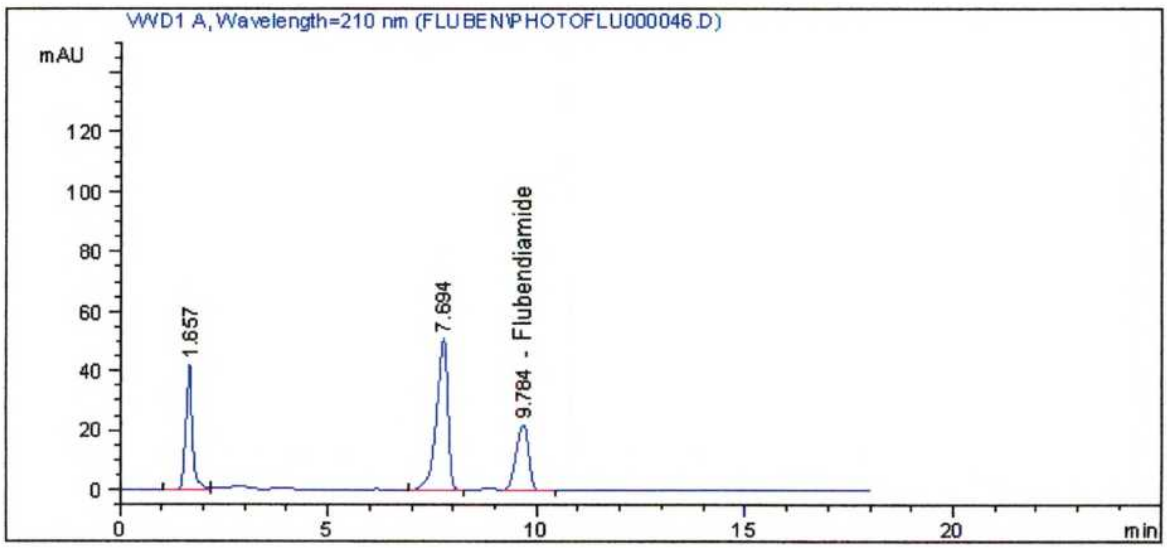



Figure 54: HPLC chromatogram of UV irradiated analytical flubendiamide at 80 hours in isopropanol: water (1:1)

The overall photokinetic studies under UV and sunlight showed that flubendiamide (analytical and formulation) undergoes faster photodegradation in aqueous acetonitrile followed by aqueous methanol and aqueous isopropanol. Moreover, the calculated photolytic half – life values revealed that the rate of degradation of flubendiamide occurred faster under UV light as compared to sunlight. Furthermore, from the monitoring results it was observed that seven peaks at different retention time (Table 71) found in sunlight irradiated aqueous acetonitrile solution excluding flubendiamide peak whereas in other solvent system (aqueous methanol / isopropanol) showed less number of peaks with same RT matched with aqueous acetonitrile either in sunlight or UV. This variation in the distribution of photo products might possibly be due to variation in wavelength and time of irradiation. Therefore, different products formed from flubendiamide and present as mixture in sunlight irradiated aqueous acetonitrile solution was chosen for characterization by Q-TOF micro mass spectrometer, considering the separation of each product by m/z value, the unique feature of the instrument.

Table 71: HPLC monitoring results of photo transformed products of flubendiamide

| Irradiation source | Peak (Retention time) in minutes | | |
|---|---|---|---|
| | Acetonitrile: water (1:1) | Methanol: water (1:1) | Isopropanol: water (1:1) |
| Sunlight | 2.585 | - | - |
| | 3.053 | - | - |
| | 3.861 | - | - |
| | 4.816 | - | - |
| | 5.680 | - | - |
| | 7.701 | 7.625 | 7.757 |
| | 8.331 | - | 8.309 |
| | (flubendiamide) 9.70 | 9.744 | 9.698 |
| UV | 4.029 | - | - |
| | 4.839 | - | - |
| | - | 5.945 | - |
| | 7.706 | 7.714 | 7.694 |
| | - | 9.704 | 9.784 |

## 7.4 Characterization of flubendiamide photo products present in the aqueous acetonitrile solution by Q-TOF micro mass spectrometer

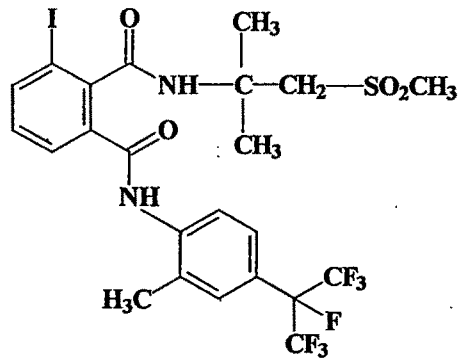
Under the operating conditions of the Q-TOF micro mass spectrometer the molecular ion peaks or fragment ion peaks appeared at (M-1) / (M+1) / (M+19) / (M+23) due to the presence of H, K and Na ion respectively in the system. In the present study most of the peaks for the molecular ion ($M^+$) appeared as (M+1) or (M+23).

The sunlight irradiated aqueous acetonitrile solution was subjected to analysis by Q-TOF micro mass spectrometer. The total scan of the mixture leads to several molecular ion peak ($M^+$) either in the form of (M+1) or (M+23) as shown in table 72.

Table 72: Molecular ion peaks of the photometabolites of flubendiamide irradiated under sunlight

| S.no | m/z value | Relative intensity (%) | m/z-1 | m/z -23 |
|---|---|---|---|---|
| 1. | 705.25 | 20 | 704.25 | 682.25 |
| 2. | 595.26 | 5 | 594.26 | 572.26 |
| 3. | 579.26 | 50 | 578.26 | 556.26 |
| 4. | 413.38 | 20 | 412.38 | 390.38 |
| 5. | 365.28 | 8 | 364.28 | 342.28 |
| 6. | 315.25 | 9 | 314.25 | 292.25 |
| 7. | 301.24 | 100 | 300.24 | 278.24 |
| 8. | 282.19 | 12 | 281.19 | 259.19 |
| 9. | 245.16 | 9 | 244.16 | 222.06 |
| 10. | 196.18 | 14 | 195.18 | 173.18 |
| 11. | 152.12 | 5 | 151.12 | 129.12 |
| 12. | 149.08 | 15 | 148.08 | 126.08 |

The m/z - 23 values (682.25) corresponds to the molecular ion peak ($M^+$) of the parent compound flubendiamide (I). All the peaks thus obtained were further subjected to MS-MS for characterization of the photo products except (I). However, characterization was possible only for m/z -23 values of **572.26, 556.26, 390.38** and m/z-1 value of **281.19**. These four photo metabolites were designated as $F_1$, $F_2$, $F_3$ and $F_4$ respectively.




Flubendiamide **(I)**

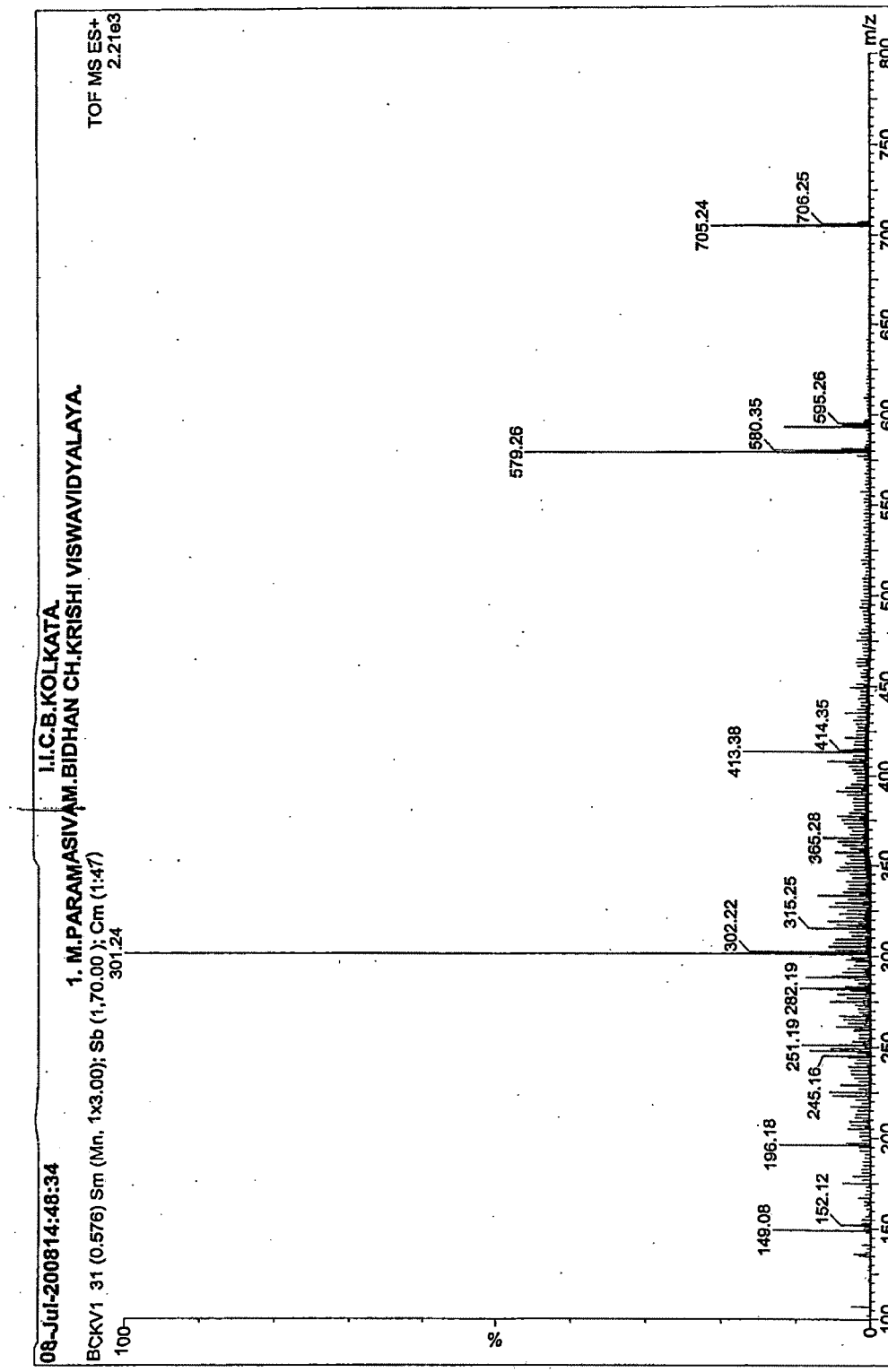
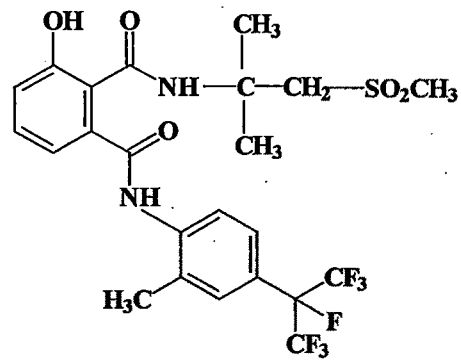



Figure 55: TOF MS ES+ chromatogram of sunlight irradiated aqueous acetonitrile solution

### 7.4.1 Characterization of the photo product with m/z value of 572.26 ($F_1$)

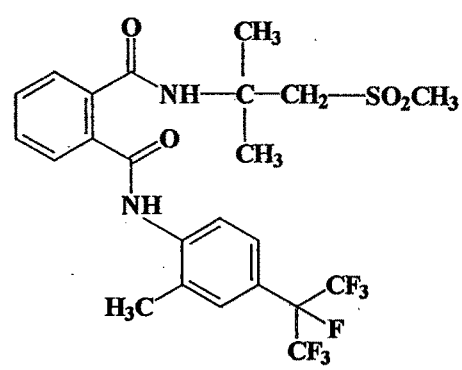
The MS-MS spectrum of the photo metabolite $F_1$ showed the molecular ion peak at m/z value of 572.26 ($M^+$) with some diagonistic peaks at m/z – 1 value of 556, 281. The molecular ion peak may lose an OH· to form a cation (m/z = 556) which may further fragment to form a positively charged substituted phthalimide species (m/z = 281) and a neutral molecule flubendiamide aniline (mol. wt 275). Based on mass fragmentation the structure of $F_1$ appeared to be **3-hydroxy flubendiamide**. The MS-MS spectra and mass fragmentation of $F_1$ are shown in figure 56 and 57 respectively.




3-hydroxy flubendiamide ($F_1$)

### 7.4.2 Characterization of the photo product with m/z value of 556.26 ($F_2$)

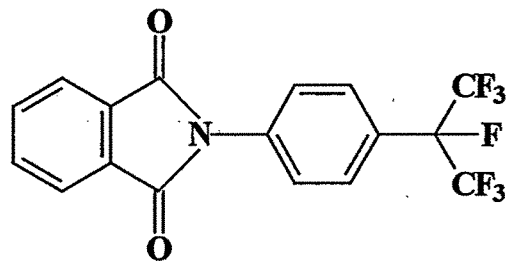
The radical ion peak of $F_2$ corresponding to the m/z – 23 values of 556.26 when subjected to MS-MS produced characteristic peaks at m/z 281, 147 and 134. $F_2$ undergoes rearrangement along with the elimination of the aniline moiety to produce a substituted phthalimide species with m/z 281 (100%) which further undergoes cleavage to produce a peak at m/z = 147 corresponding to $M^+$ of phthalimide and at m/z = 134 corresponding to the sulfone moiety. The probable structure of $F_2$ could be assigned as **des-iodo flubendiamide**. The MS-MS spectra and mass fragmentation of $F_2$ are shown in figure 58 and 59 respectively.




Des-iodo flubendiamide ($F_2$)

### 7.4.3 Characterization of the photo product with m/z value of 390.38 ($F_3$)

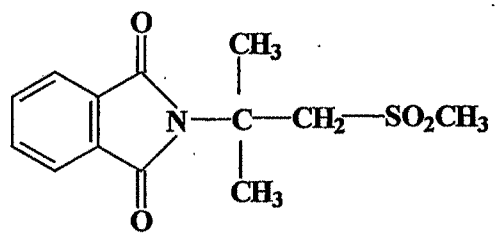
The MS-MS spectrum of the photo metabolite **$F_3$** showed $M^+$ at m/z = 390 is suspected to be a phthalimide derivative. The cation formed from it loses a carbon monoxide molecule to form a cation with m/z = 362 which finally loses a fluorine atom to give a peak at m/z = 343. The probable structure of $F_3$ could be assigned as **N-[4'-{1,2,2,2-tetrafluoro-(1-trifluoromehtyl) ethyl}-phenyl]-phthalimide.** The MS-MS spectra and mass fragmentation of $F_3$ are shown in figure 60 and 61 respectively.




N-[4'-{1,2,2,2-tetrafluoro-(1-trifluoromehtyl) ethyl}-phenyl]-phthalimide (**$F_3$**)

### 7.4.4 Characterization of the photo product with m/z value of 281.19 ($F_4$)

The MS-MS spectrum of the photo metabolite **$F_4$** showed the molecular ion peak at m/z – 1 value of 281. The peak at m/z = 281 may be due to another phthalimide derivative. The radical ion of which loses the phthalimide part to form a cation with m/z = 135 or it may otherwise fragment to give a cationic species having m/z value of 147. The probable structure of $F_4$ could be assigned as **N'(2-mesyl-1,1-dimethylethyl) phthalimide.** The MS-MS spectra and mass fragmentation of $F_4$ are shown in figure 62 and 63 respectively.




N'(2-mesyl-1,1-dimethylethyl)phthalimide (**$F_4$**)

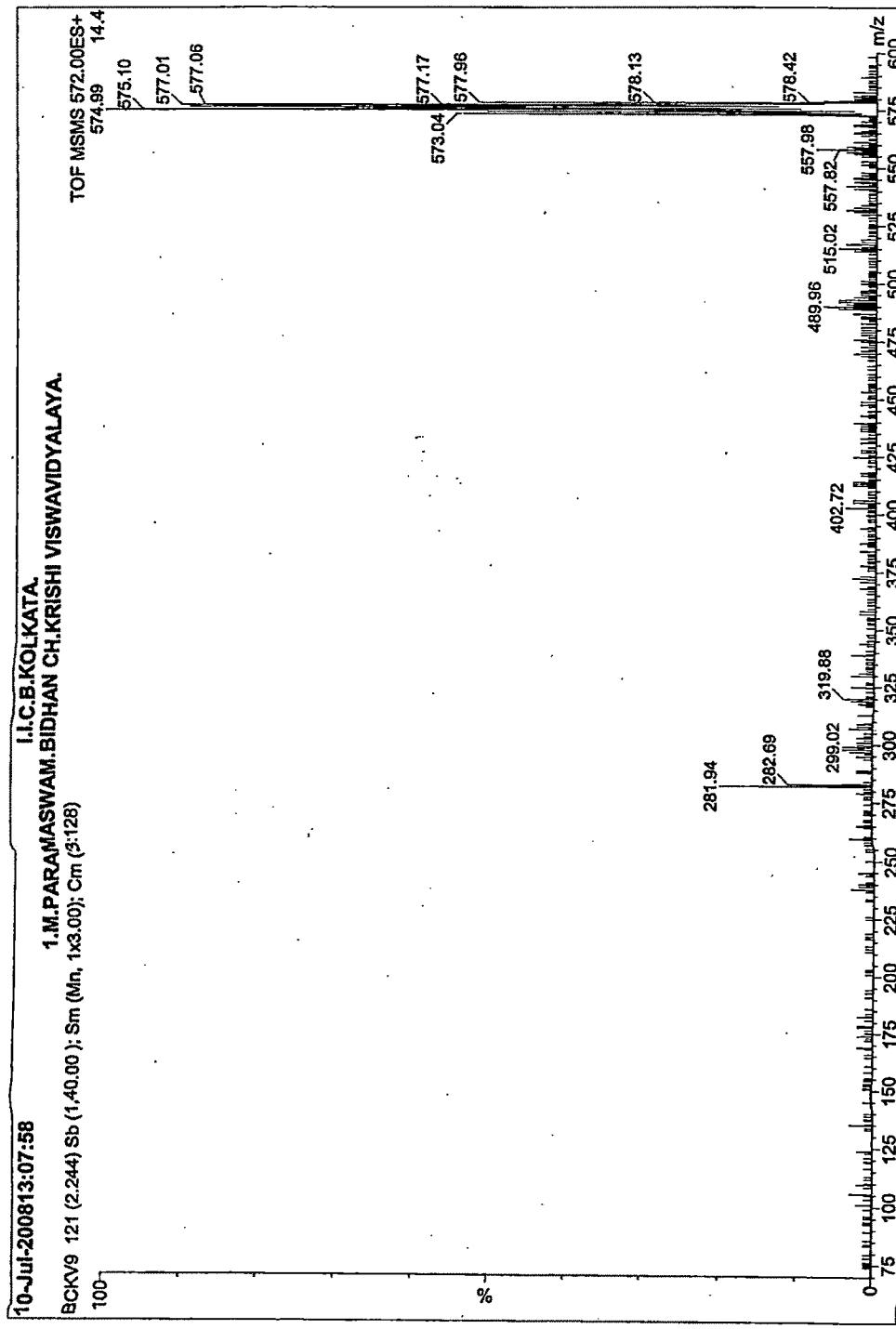



Figure 56: Mass spectra of $F_1$

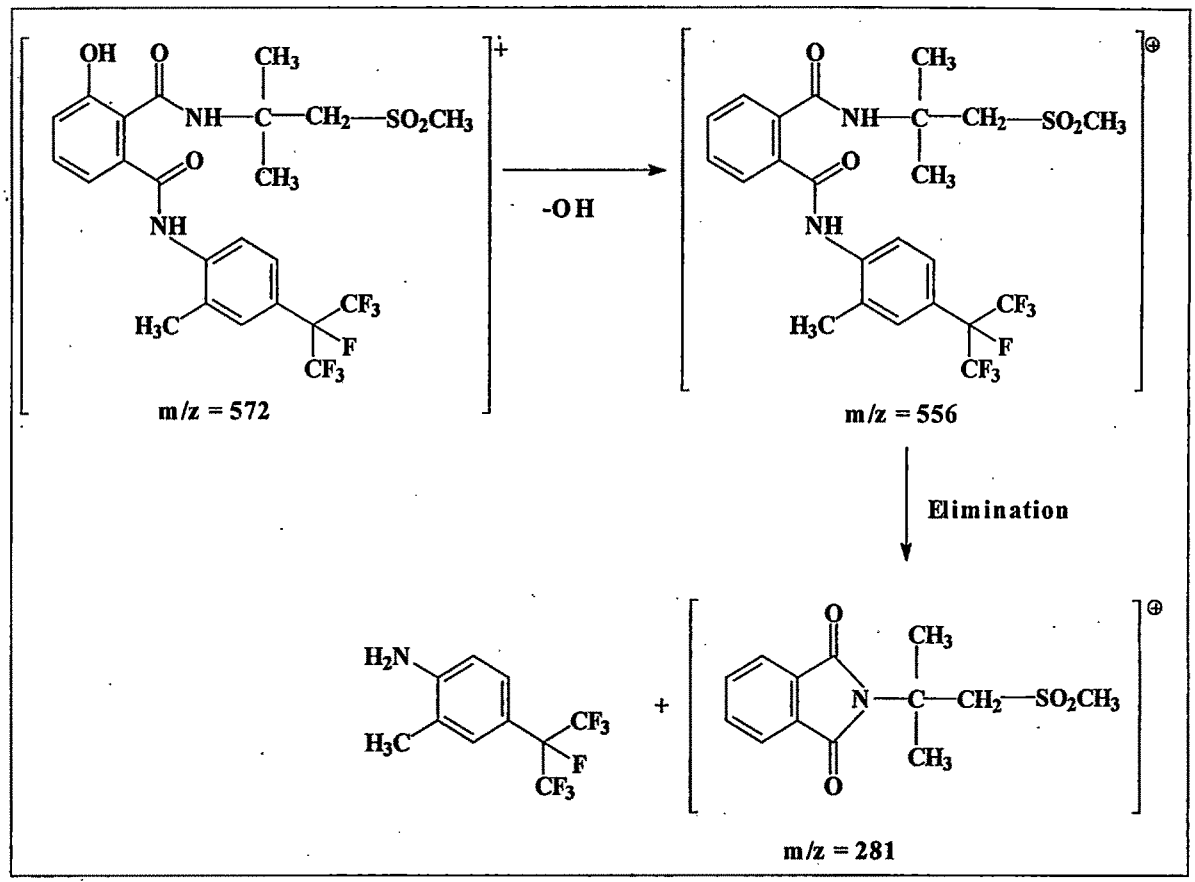



Figure 57: Mass fragmentation of $F_1$

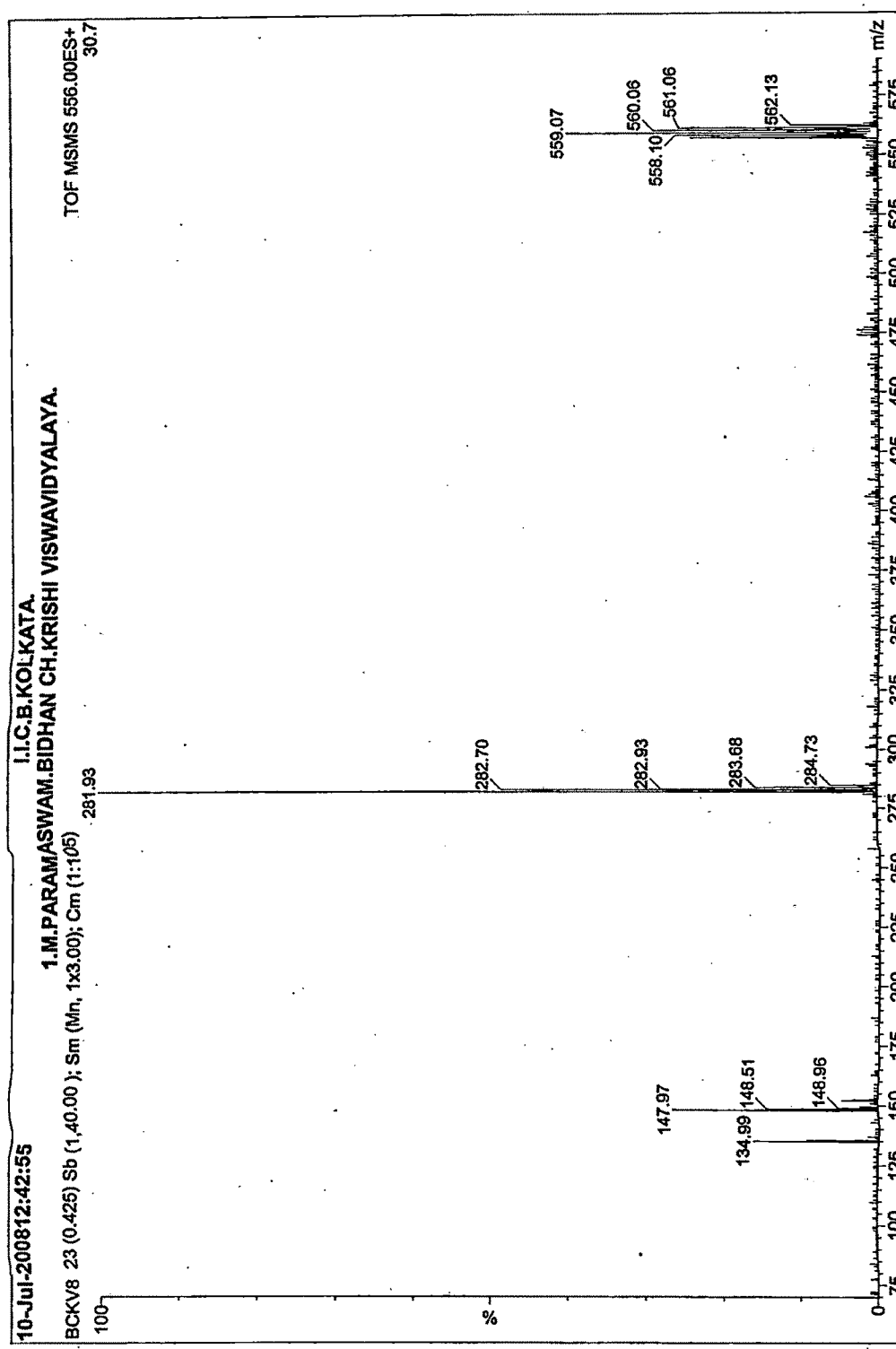



Figure 58: Mass spectra of $F_2$

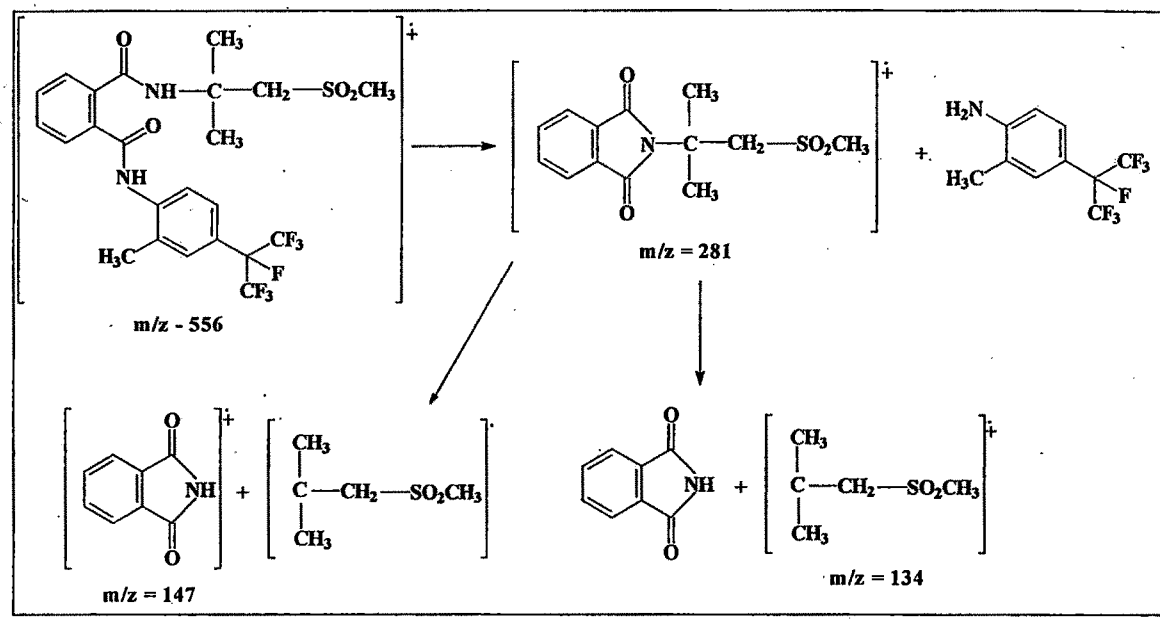



Figure 59: Mass fragmentation of $F_2$

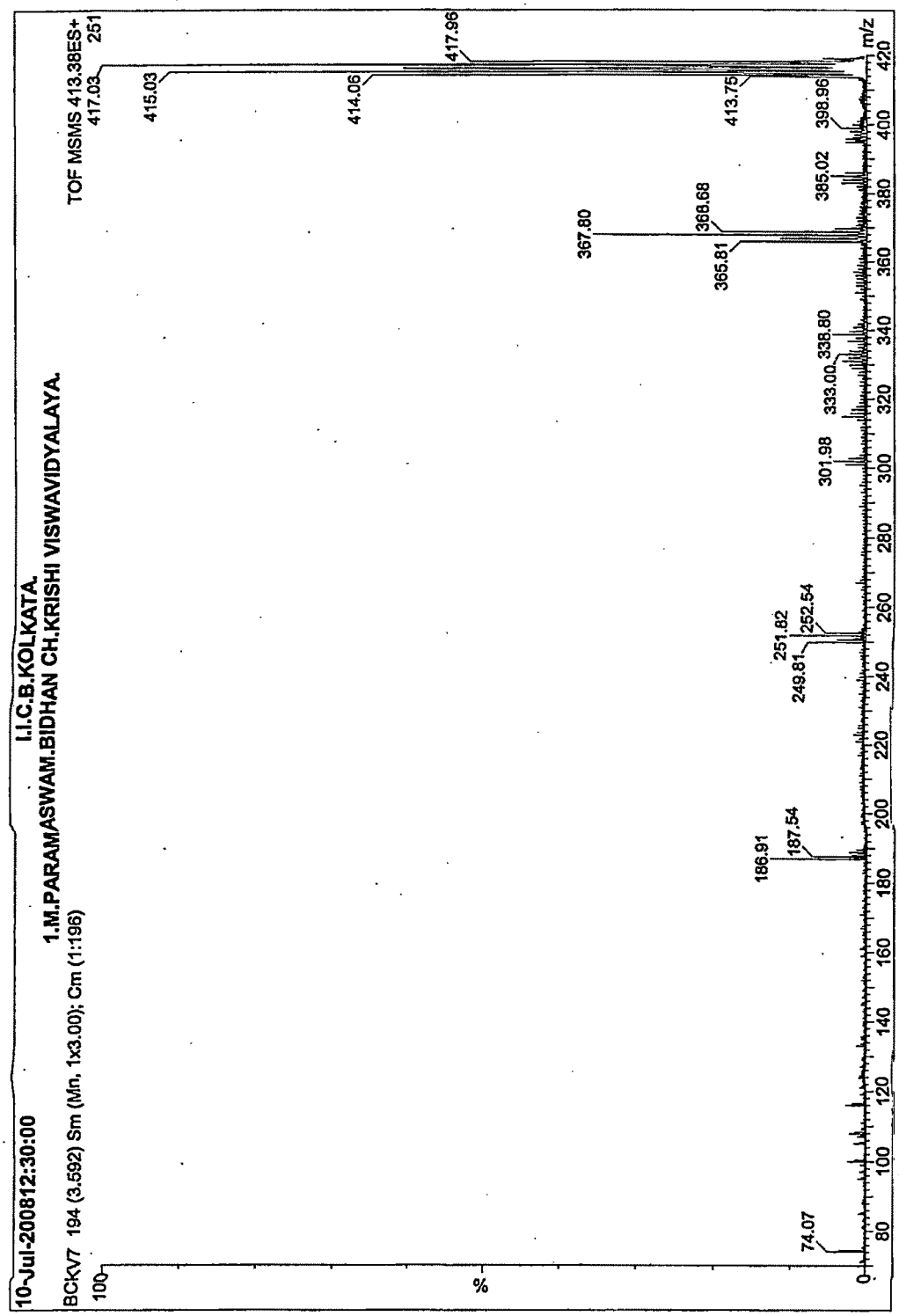



Figure 60: Mass spectra of $F_3$

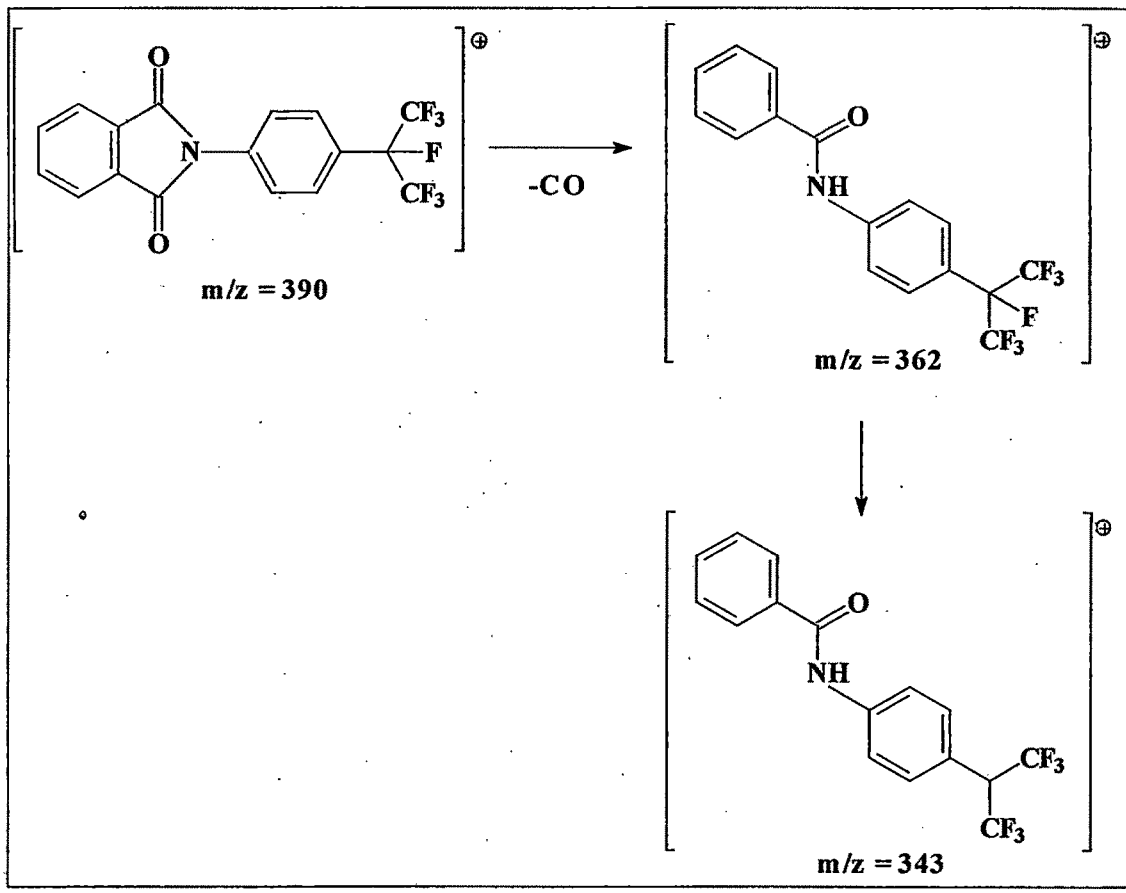



Figure 61: Mass fragmentation of $F_3$

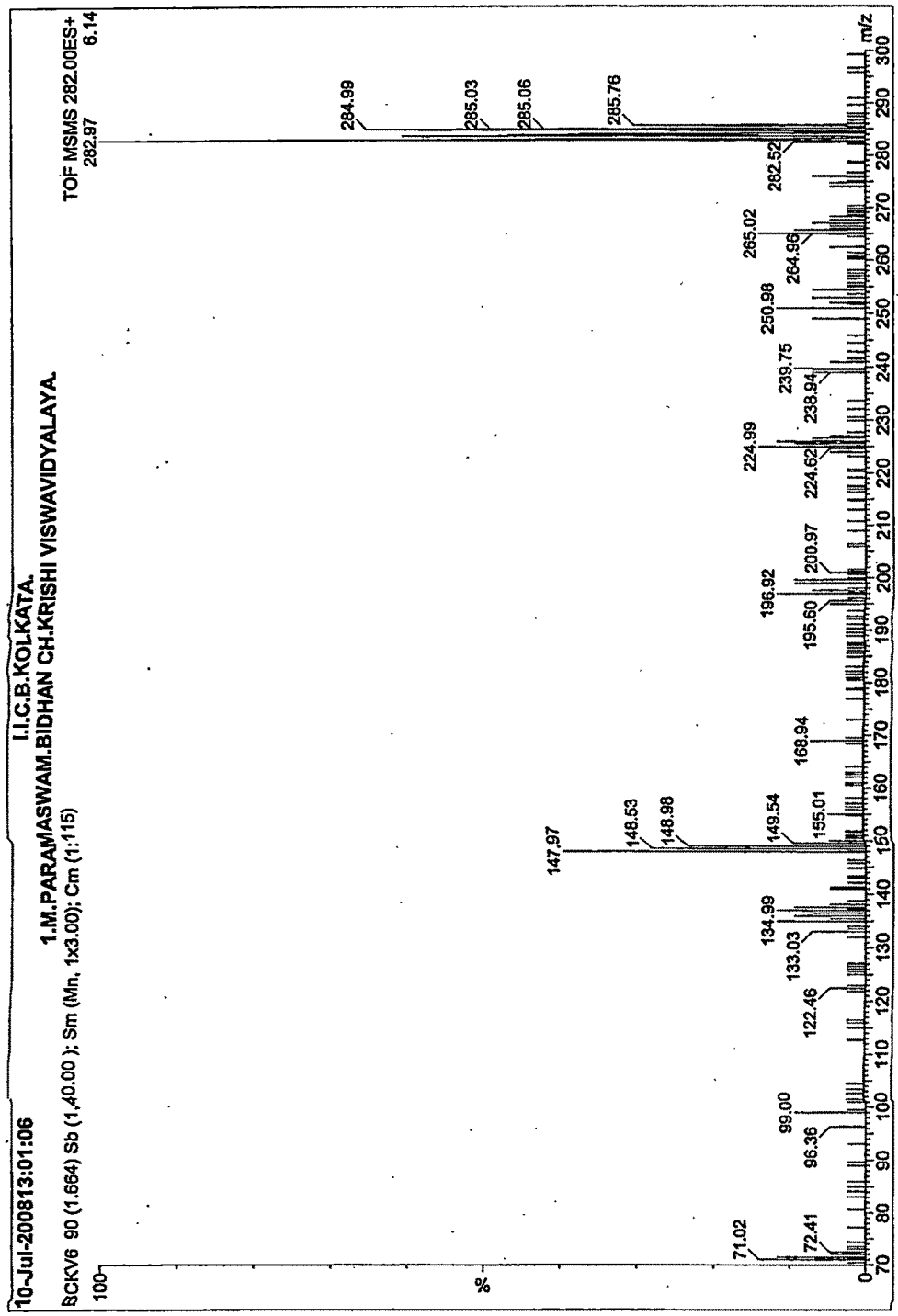



Figure 62: Mass spectra of $F_4$

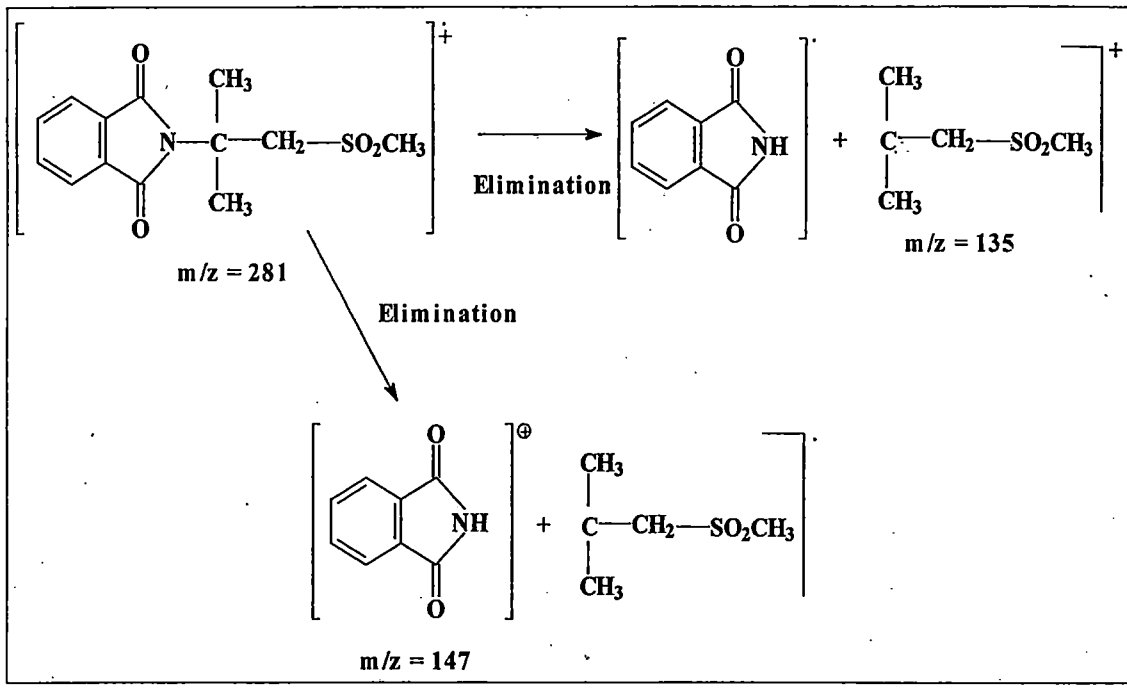



Figure 63: Mass fragmentation of $F_4$

The plausible pathway for the formation of four photo products ($F_1$, $F_2$, $F_3$ and $F_4$) from flubendiamide is shown in figure 64. The photo product $F_1$ was formed by des-iodination followed by hydroxylation at the phthalic acid ring. Dehalogenation of the parent compound led to the formation of photo product $F_2$ and the other photo product $F_4$ was possibly formed from either $F_1$ or $F_2$ or both. The photo product $F_3$ may possibly be formed from flubendiamide via the formation of intermediary flubendiamide –iodo phthalimide (m/z = 531), flubendiamide phthalimide (m/z = 405) and removal of a methyl group from m/z 405. The photo products $F_1$, $F_2$ and $F_4$ were also formed in cabbage, tomato and apple from flubendiamide as reported by Justus *et al.*, (2007). Interestingly, during my research work on the persistence study of flubendiamide in cabbage, tomato and soil, the compound $F_2$ (des-iodo flubendiamide) was formed and quantified. Pesticides suffer photo degradation by hydroxyl radicals that have been shown to be present in sunlight – irradiated natural waters. Accordingly, the reactivity of flubendiamide toward hydroxyl radicals and nature of the products formed would be the same to what may be expected in natural water. Thus, it seems that a potential exists for the application of photolysis in removal of traces of flubendiamide. The present study

indicates that the photo transformation pathway involves the degradation processes *viz.* dehalogenation, hydrolysis and elimination. The photoproducts $F_1$, $F_2$, $F_3$ and $F_4$ have been reported for the first time.

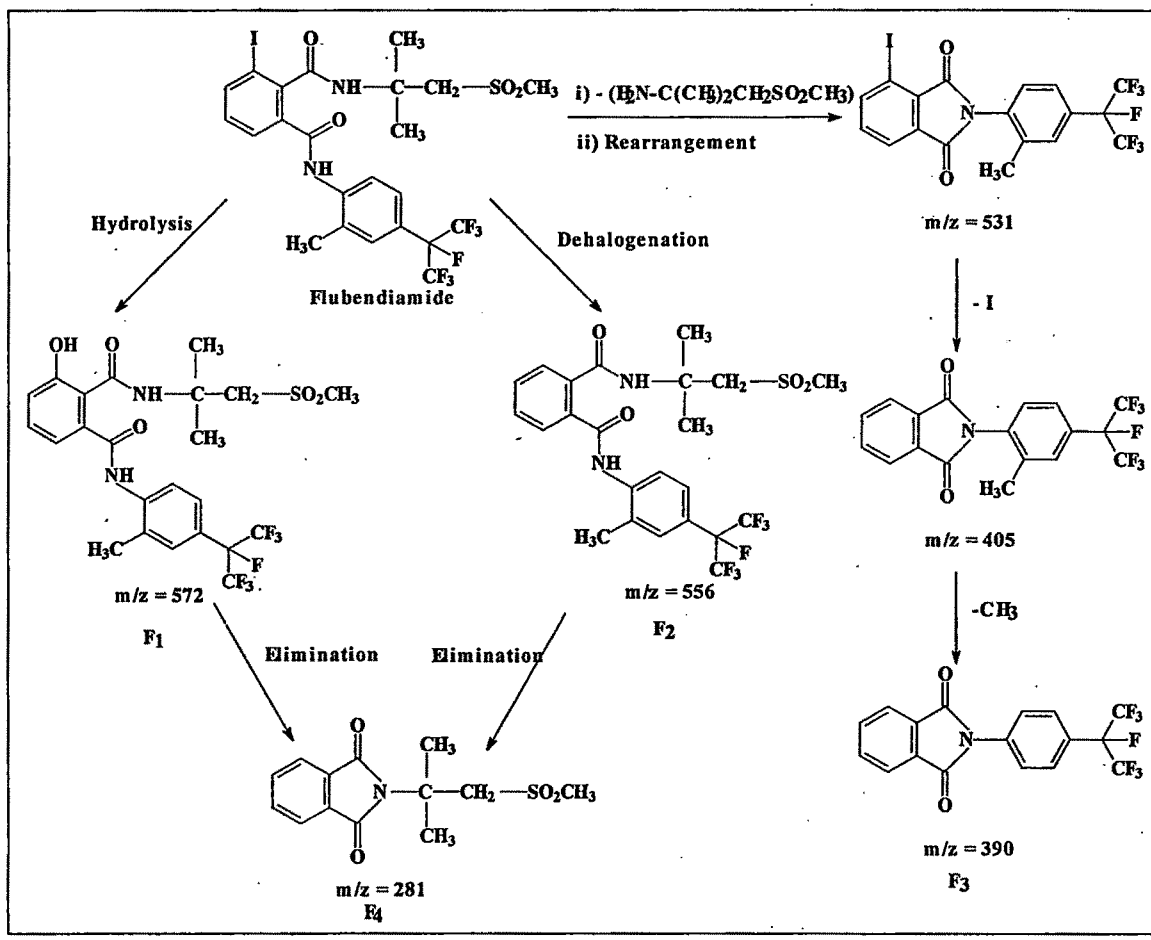



Figure 64: Plausible pathway for the formation of the photo products $F_1$, $F_2$, $F_3$ and $F_4$ from flubendiamide

Chapter 8

# Summary and Conclusion

# Summary and Conclusion

The research work embodied in the thesis entitled **"Studies on the Fate and Behaviour of Flubendiamide in Tropical Environment"** have been carried out at AINP on Pesticide Residue Laboratory; BCKV, consisting of two parts:

**PART-I**: Fate and behaviour of flubendiamide in crops, soil and water

**PART-II**: Photolytic degradation of flubendiamide in aqueous acetonitrile, methanol and isopropanol under UV and sunlight.

Brief resume of Part -I:

The novel insecticide flubendiamide was standardized in HPLC along with its metabolite des-iodo flubendiamide. The analytical method for residue analysis in cabbage head, tomato and pigeon pea (grain, shell and straw) was developed by using new emerging technique known as Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS). This method involves initial single phase extraction of 10 g samples with 10 mL acetonitrile, followed by liquid-liquid partitioning formed by addition of 4 g anhydrous $MgSO_4$ plus 1 g NaCl. Removal of residual water and cleanup are performed simultaneously by using a rapid procedure called dispersive SPE in which $MgSO_4$ and primary secondary amine (PSA) were used. The amount of PSA was standardized and it was found that PSA amount varied with the substrate. 10 mg was sufficient for excellent clean up in tomato fruit and pigeon pea grain, 25 mg for cabbage head and pigeon pea shell, and 50 mg for pigeon pea straw. The residue method was validated by conducting recovery experiments with different substrate under study. By using this technique, the limit of quantification (0.01 mg/kg) in all substrates was achieved. The individual recovery rates for determination of flubendiamide and its metabolite des-iodo flubendiamide in plant matrices ranged from 87.26 - 97.30% and 88.45-98.25% respectively with RSD typically less than 5%. Thus, the method complies with all guideline requirements for determination flubendiamide and its metabolite residue in different plant matrices.

The flubendiamide (20% WG) was sprayed on cabbage at the head formation stage @ 12.5 (recommended dose, $T_1$) and 25 g a.i $ha^{-1}$ (double the recommended dose, $T_2$). The cabbage head samples were collected at 0, 1, 3, 5, 7, 10 and 15 days after second

spray and processed samples are subjected to HPLC for estimation of flubendiamide and des-iodo flubendiamide residues. The total residue data of flubendiamide along with des-iodo flubendiamide in cabbage head revealed that the initial deposit of total residue was found to be 0.16 ppm and 0.31 ppm in case of $T_1$ and $T_2$ respectively which further dissipated to 12.5% ($T_1$) and 9.68% ($T_2$) on day 1 and steady reduction of residue occurred with increment of time. The residue became non detectable below 0.01 ppm on day 10 at the recommended dose and on day 15 at double the recommended dose. First order reaction kinetics was observed and the half life values in cabbage head were found to be 3.4-3.6 days irrespective of dose. Further scrutiny of the residue data revealed that in $T_1$ the flubendiamide dissipated to 50% on day 3 of which 77.5% was accounted for by the formation of des-iodo flubendiamide metabolite and similarly in $T_2$ it was 22.6% on day 1 of which 70.0% metabolite was formed from this dissipated amount. Thus, des-iodo flubendiamide is the major metabolite formed via the process of deiodination in cabbage head. Based on LOQ value of 0.01 ppm, the suggested safe waiting period found to be 14.54 and 16.79 days at $T_1$ and $T_2$ respectively. **Thus, from the residue point of view, consumption of cabbage head is safe after 14 days of application of flubendiamide 20% WG @ 12.5 g a.i ha$^{-1}$.**

In case of tomato, flubendiamide 20% WG was sprayed twice at an interval of 15 days @ 50 ($T_1$) and 100 ($T_2$) g a.i ha$^{-1}$ (recommended and double the recommended dose) and tomato fruit samples were collected after the second spray at an interval of 0, 1, 3, 5, 7 and 10 days. The tomato fruit sample was subjected to residue analysis as followed in cabbage head sample. The results indicated that initial deposit of flubendiamide was found to be 0.27 ($T_1$) and 0.46 ($T_2$) ppm respectively with corresponding reduction of 43-44 % irrespective of treatment on first day. Further reduction took place more than 80% on fifth day. Residues of flubendiamide dissipated below detectable level of 0.01 ppm on 7$^{th}$ day at both the doses. The calculated half life ranged from 1.64-1.98 days irrespective of the doses. Furthermore, from the total reportable residue data, it was revealed that flubendiamide dissipated to 44.4 % on day 1 of which 81.66 % was accounted for by the formation of des-iodo flubendiamide metabolite ($T_1$) and in $T_2$, dissipation was 43.5 % on day 1 of which 92.5 % metabolite was formed from this dissipated amount. Thus, des-iodo flubendiamide is the major metabolite formed via the process of deiodination in tomato fruits. Based on LOQ (0.01 mg/kg), the suggested safe waiting period calculated was found to be 7.7 and 10.6 days irrespective of $T_1$ and $T_2$. **Based on the residue data,**

**it was concluded that after application of flubendiamide 20% WG at recommended dose (50 g a.i. / ha) during fruiting stage, the tomato fruit may be plucked at 7 days for safe consumption.**

Harvest time residue of flubendiamide and its metabolite des-iodo flubendiamide were analyzed in pigeon pea grain, shell and straw @ 50 and 100 g a.i $ha^{-1}$. No flubendiamide and des-iodo flubendiamide residue could be detected at harvest (42 days). **Thus, flubendiamide 20% WG can suitably fit in plant protection practices.**

Harvest soil samples (cabbage, tomato and pigeon pea) collected after last spray of flubendiamide did not show the presence of either flubendiamide or des-iodo metabolite at detection limit of 0.01 ppm. It indicates that minimum chances of flubendiamide residues will occur in the succeeding crop.

Laboratory dissipation study of flubendiamide at a concentration of 5 ($T_1$) and 10 ($T_2$) ppm was conducted in three types of soil (New alluvial, Red & lateritic and Coastal) for a period of 150 days. The half-life values ($T_{1/2}$) of all the soil irrespective of doses were calculated and found to be 44.26 – 59.02 (coastal soil), 41.23 – 51.90 (red and lateritic soil) and 37.16 – 44.92 (new alluvial soil) days. First order reaction kinetics followed in all the three soils. The persistence of flubendiamide was in the order of coastal soil > red and lateritic soil > new alluvial soil. Insignificant differences in the dissipation kinetics of three soils were attributed to differences in soil pH, texture and the ability of indigenous microorganism to transform the insecticide.

The persistence data of all the three soils further revealed that flubendiamide dissipation rate was quite slow in coastal soil as compared to other two soils which might be due to high clay and higher organic matter content of the soil. Organic matter may bind flubendiamide or may form complex structure which prevents further microbial activity on the compound. Des-iodo flubendiamide also quantified with various amount in three soil types and it persisted upto 90 days. Thus, the present study concludes that persistence of flubendiamide is more in coastal than neutral to acidic soil and coupled with its low water solubility gives an indication of minimal contamination of ground water.

A study on the persistence and dissipation behaviour of flubendiamide in water with three different pH (4.0, 7.0 and 9.2) was carried out in the laboratory for a period of 120 days at a concentration level of 1 ($T_1$) and 2 ($T_2$) ppm. The initial deposit of flubendiamide was found to be 0.88, 0.93 and 0.93 ppm ($T_1$) and 1.84, 1.85 and 1.91 ppm

($T_2$) irrespective of pH 4.0, 7.0 and 9.2. The range of dissipation percentage of flubendiamide in all the pH at 120 days irrespective of $T_1$ and $T_2$ was found to be 50.18 – 68.82%. Flubendiamide dissipation in water at all the pH under study followed the first order reaction kinetics. The half-life ($T_{½}$) value at pH - 4.0, 9.2 and 7.0 for $T_1$ was found to be 107.5, 115.78 and 81.36 days whereas for $T_2$ it was 111.5, 125.42 and 83.6 days. The persistence of flubendiamide was in the order of pH 9.2 > pH 4.0 > pH 7.0. **Thus, the present study indicated that flubendiamide molecule is hydrolytically stable in acidic, neutral and alkaline medium.**

Brief resume of Part - II:

The photolytic degradation of flubendiamide analytical and its formulation (10 ppm) was carried out in aqueous acetonitrile, methanol and isopropanol under UV and sunlight. The photolytic half life value of analytical grade flubendiamide under sunlight was found to be 55.75, 73.42 and 94.01 hours and that of formulation was 79.22, 158.43 and 150.51 hours while in UV light, for analytical grade it was 3.17, 12.04 and 36.26 hours and that of formulation was 7.60, 21.81 and 55.74 hours in aqueous acetonitrile, methanol and isopropanol respectively. The overall photokinetic studies under UV and sunlight showed that flubendiamide (analytical and formulation) undergoes faster photodegradation in aqueous acetonitrile followed by aqueous methanol and aqueous isopropanol. Furthermore, faster degradation occurred in the analytical grade than the formulation. The probable reason for slower rate of degradation of formulation may be attributed to the consumption of hydroxyl radicals ($^{\bullet}OH$) by surfactants and the formation of a substrate-surfactant complex, resulting in the unavailability of flubendiamide to $^{\bullet}OH$ attack. Flubendiamide degradation rate was faster under UV light as compared to sunlight. Moreover, from the monitoring results of HPLC, it was observed that seven peaks at different retention time was found in sunlight irradiated aqueous acetonitrile solution whereas in other solvent system (aqueous methanol / isopropanol) showed less number of peaks with same RT matched with aqueous acetonitrile either in sunlight or UV. This variation in the distribution of photo products might possibly be due to variation in wavelength and time of irradiation. The sunlight irradiated aqueous acetonitrile solution was subjected to analysis by Q-TOF micro mass spectrometer and four photo products ($F_1$, $F_2$, $F_3$ and $F_4$) were characterized.

Based on MS-MS mass fragmentation pattern, the probable structure of photo metabolite $\mathbf{F_1}$ appeared to be 3-hydroxy flubendiamide, $\mathbf{F_2}$ as des-iodo flubendiamide, $\mathbf{F_3}$ as N-[4'-{1,2,2,2-tetrafluoro-(1-trifluoromehtyl) ethyl}-phenyl]-phthalimide and $\mathbf{F_4}$ as N'(2-mesyl-1,1-dimethylethyl)phthalimide.

The plausible pathways for the formation of four photo products ($F_1$, $F_2$, $F_3$ and $F_4$) from flubendiamide were also ascertained and the main photo transformation pathway involves viz. dehalogenation, hydrolysis and elimination. The photoproducts $F_1$, $F_2$, $F_3$ and $F_4$ have been reported for the first time.

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*abstract seen