“TO STUDY THE DEGRADATION AND DOWNWARD MOVEMENT OF OXYFLUORFEN IN SANDY, SANDY LOAM AND CLAYEY SOILS”

A
THESIS
SUBMITTED TO THE
ANAND AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE

OF
Master of Science
(AGRICULTURE)

IN
SOIL SCIENCE AND AGRICULTURAL CHEMISTRY

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2014
(Regd. No. 04-1575-2011)
DEDICATED TO
MY
BELOVED
PARENTS
AND
RESPECTED GUIDE
Abstract...
ABSTRACT

Oxyfluorfen is formulated for agricultural use as an emulsifiable liquid concentrate and as a granular product, although it is most frequently used in a liquid formulation for food crops and as a granular formulation for ornamental nursery crops for the weed control. Oxyfluorfen is used for the control of annual grasses and broad-leaved weeds in tropical and sub-tropical crops, by pre- or post-emergence application in maize, rice, gram, groundnut, soybean, cotton and onion crops. It is selective contact herbicide and absorbed more readily by the foliage than by the roots, with very little translocation. Its application as liquid or dry formulations on the crop or soil may contaminate soil or leave residues on crop produce.

Oxyfluorfen is moderately persistent in most soil environments, with a representative field half-life of about 30 to 40 days. Oxyfluorfen
Abstract

is not subjected to microbial degradation or hydrolysis. The main mechanism of degradation in soils may be photodegradation and evaporation/co-distillation in moist soils. In laboratory studies, its soil half-life was 6 months, indicating very low rates of microbial degradation.

Looking to the above facts, a laboratory study entitled "To study the degradation and downward movement of oxyfluorfen in sandy, sandy loam and clayey soils" was conducted at Pesticide Residue Laboratory, All India Network Project on Pesticide Residues, Centre for Organic Farming, ICAR Unit-9, Anand Agricultural University, Anand, Gujarat (India) with the following objectives:

1. To validate the pesticide residue method of oxyfluorfen for sandy, sandy loam and clayey soils.
2. To study the depth-wise distribution of oxyfluorfen in soil column.
3. To study the degradation of oxyfluorfen in sandy, sandy loam and clayey soils under laboratory conditions.
4. To study the level of oxyfluorfen in column leachates.

In order to study the persistence and degradation of oxyfluorfen from three different types of soils viz., sandy, sandy loam and clayey soil, sandy soil was collected from Agronomy Farm of Sardar Krushinagar Dantiwada Agricultural University, Dantiwada. sandy loam soil was collected from Bidi Tobacco Research Station Farm of Anand Agricultural University, Anand and clayey soil from Organic
Abstract

Farm of Navsari Agricultural University, Navsari. Prior to study, these soils were subjected to physico-chemical analysis with known history of pesticide applications. For depthwise distribution of oxyfluorfen study, a 36 cm long PVC column with an internal diameter (i.d.) of 6 cm was used by marking into sections each of 6 cm in length. The bottom ring was tied up with muslin cloth with thread to avoid contamination of leachate with soil particles. The column was filled with soil from bottom to 30 cm length with tapering. Before applying the oxyfluorfen, soil column was wetted to their apparent water holding capacity by applying 100 mL aqueous solution of 0.01 M CaCl₂ to the top of column at an interval of 24 h. After this initial equilibration 30 mg/column of oxyfluorfen was applied to the top of column. Polythene sheet was tied up on each column in order to prevent volatization losses. Initially, columns were irrigated with 100 mL of aqueous solution of 0.01 M CaCl₂ up to 10 days and at later stage 50 mL of above mentioned solution was applied to maintain the field capacity until the termination of experiment. Leachates were collected and analyzed. The experiment was conducted with three repetitions along with a control column. Prior to analysis of oxyfluorfen from soil, the method was validated in the laboratory. The leachates were collected on 0, 1, 3, 5, 7, 10, 15, 30, 45 and 60 days after application were filtered, extracted and analyzed by GLC for
residues of oxyfluorfen. The entire experiment was conducted at room temperature.

After end of experiment, each column was cut into 5 segments each of 6 cm and herbicide residues in soil from different depths i.e. 0-6 cm (D₁), 6-12 cm (D₂), 12-18 cm (D₃), 18-24 cm (D₄), 24-30 cm (D₅) was analyzed. The soil inside the columns was dried separately under shade and 10 g of soil was used for quantitative analysis of oxyfluorfen residues by GLC.

The oxyfluorfen residues in leachates persisted up to 60 days except 0 day in sandy soil. In sandy soil the residues showed increasing trend up to 7th day that is 13.759 ng g⁻¹ and decreased thereafter till the 60th day. In case of sandy loam and clayey soils the residues were below determination limit until the termination of experiment.

The downward movement of oxyfluorfen in the column under laboratory conditions revealed that after 60 days of application, the residues were mainly confined to the top three layers i.e. 0-6 cm, 6-12 cm and 12-18 cm in sandy soil, while in case of the sandy loam and clayey soil the residue were below determination limit even to the top layer of column. This confirms the low risk of sub-soil contamination in sandy loam and clayey soils compared to the sandy soil.
The persistence of oxyfluorfen in different soils followed first order degradation kinetics and dissipated in one phase.

The residues of oxyfluorfen recorded in sandy soil on 0 day was 0.408 µg g\(^{-1}\) with half-life (DT\(_{50}\)) of 43.00 days.

The residues of oxyfluorfen recorded in sandy loam soil on 0 day was 0.496 µg g\(^{-1}\) with half-life (DT\(_{50}\)) of 60.20 days.

The residues of oxyfluorfen recorded in clayey soil on 0 day was 0.498 µg g\(^{-1}\) with half-life (DT\(_{50}\)) of 75.25 days.

The order of persistence of oxyfluorfen in soil denoted by half-life i.e. DT\(_{50}\) observed in the experiment were as follows: Clayey soil (75.25 days) > Sandy loam soil (60.20 days) > Sandy soil (43.00 days).
This is to certify that the thesis entitled "To study the degradation and downward movement of oxyfluorfen in sandy, sandy loam and clayey soils" submitted by Kumbhar Mukesh Bhikhabhai, Reg. No. 04-1575-2011 in partial fulfillment of the requirements for the award of the degree of Master of Science (Agriculture) in Soil Science and Agricultural Chemistry of the Anand Agricultural University is a record of bona fide research work carried out by him under my personal guidance and supervision. The thesis has not previously formed the basis for award of any degree, diploma or other similar title.

Place: Anand
Date: 06/01/2014

(M. F. RAJ)
Guide
DECLARATION

This is to declare that the whole of the research work reported here in the thesis entitled "TO STUDY THE DEGRADATION AND DOWNWARD MOVEMENT OF OXYFLUORFEN IN SANDY, SANDY LOAM AND CLAYEY SOILS" for the partial fulfillment of the requirement for the award of the degree of MASTER OF SCIENCE in SOIL SCIENCE AND AGRICULTURAL CHEMISTRY by the undersigned is the result of investigation done by him under the direct guidance and supervision of Dr. M. F. Raj, Assistant Residue Analyst, AINP on Pesticide Residues, ICAR Unit-9, Anand Agricultural University, Anand and no part of the research work has been submitted for any other degree so far.

Place : Anand
Date : 06/01/2014

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ACKNOWLEDGEMENT

Acknowledgement is written at last, placed at first and read the least, but still it is the only opportunity to thank one and all who are conscientious in completion of this mite of nine to science.

I express deep sagacity of veneration to Dr. M. F. Raj, Assistant Residue Analyst, AINRP on Pesticide Residues, ICAR, Unit-9, Anand Agricultural University, Anand and chairman of my advisory committee for his encouragement and unquantifiable help and his rays of advice, inspiring subtle guidance and scrupulous perusal of manuscript of the thesis.

I register my sincere thanks to my minor guide Dr. B. D. Patel, Associate Research Scientist, DWSR Anand Centre, B. A. College of Agriculture, Anand Agricultural University, Anand other committee members Dr. P. G. Shah, Residue Analyst, AINRP on Pesticide Residues, ICAR, Unit-9, Anand Agricultural University, Anand and Dr. A. D. Kalola, Associate Professor, Department of Agri. Statistics, B. A. College of Agriculture, Anand Agricultural University, Anand, for their valuable comments and helpful suggestions during the course of this investigation.

I am greatly thankful to Dr. K. B. Kathiria, Director of Research & Dean, PG Studies and Dr. K. P. Patel Principal, B.A. College of Agriculture, Anand Agricultural University, Anand for providing necessary facilities throughout the period of studies.

I am equally grateful to Dr. V. R. Bhatt, Professor and Head, Department of Soil Science and Agricultural Chemistry, B. A. College of Agriculture, Anand Agricultural University, Anand for providing facilities for analysis of soil samples of my research work.

My affectionately acknowledge the help, co-operation and encouragement rendered to the members of my department. viz., Dr. Susheel Singh, Milanbhai Joshi, Dilipbhai J. Patel, Miteshbhai R. Patel, Kiranbhai M. Vaghela, Riteshbhai R. Panchal, Hiteshbhai A. Joshi, Badal V. Patel, Ms. Hetalben N. Gor, Ms. Hemlattaben K. Patel and Ms. Pritiben P. Solanki who gave support indirectly during my study.

I also wish to thank supporting staff viz., Vasantbhai R. Chavda, Sandeepbhai S. Solanki, Rameshbhai G. Gohel and Harshad A. Chavda of AINRP on Pesticide Residues, ICAR, Unit-9, Anand Agricultural University, Anand during laboratory work.
I express my sincere thanks to my friends Gajendra Kumar Rana, Vikas G. Damor, Kalpesh P. Bhuriya, Hiten N. Maheriya, Kaushik G. Khandayata, Amit S. Ninama, Rahul R. Kharadi, Himanshu H. Gadavi, Pinakin J. Prajapati, Suresh G. Prajapati and Bhavesh K. Patel for their help and suggestions directly or indirectly during the course of investigation.

Finally, my felicitousness overwhelms to express my deepest sense of reverence and indebtedness to my most-beloved parents, father Shri Bhikhabhhai, mother Smt. Ramilaben, my younger brother Mayank, my elder sister Kaminaben and Darshanaben whose blessings, love and moral support influence me immensely in realizing my goal.

Finally, I frankly admit that it is not possible to remember all the faces that stood behind the façade at this juncture and omission of any names does not mean lack of gratitudeness.

Now as I carry this thesis in my hand, I carry with me memories that will enrich my nostalgia. I have taken literary license but any factorial errors are my responsibility alone.

Place: Anand
Date: 06/10/2014

(Mukesh B. Kumbhar)
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<td>%</td>
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<td>@</td>
<td>At the rate of</td>
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</tr>
<tr>
<td>μg</td>
<td>Microgram</td>
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<tr>
<td>μV</td>
<td>Micro Volt</td>
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<tr>
<td>°C</td>
<td>Degree Celsius</td>
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<tr>
<td>a.i.</td>
<td>Active ingredient</td>
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<tr>
<td>AAU</td>
<td>Anand Agricultural University</td>
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<td>AICRP</td>
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<td>Anon.</td>
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<tr>
<td>BD</td>
<td>Bulk density</td>
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<td>BDL</td>
<td>Below Determination Limit</td>
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<tr>
<td>ca</td>
<td>Approximately</td>
<td></td>
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<tr>
<td>cm</td>
<td>Centimetre</td>
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<tr>
<td>D</td>
<td>Depth</td>
<td></td>
</tr>
<tr>
<td>DAA</td>
<td>Days after application</td>
<td></td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloro methane / Methylene chloride</td>
<td></td>
</tr>
<tr>
<td>dS m⁻¹</td>
<td>Desi Siemens per meter</td>
<td></td>
</tr>
<tr>
<td>DT₅₀</td>
<td>Dissipation Half-Life</td>
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<tr>
<td>EC</td>
<td>Emulsifiable Concentrate / Electrical Conductivity</td>
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<tr>
<td>et al.</td>
<td>et allii; and co-workers</td>
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<td>ctc.</td>
<td>Et cetera; and rest, so on</td>
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<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
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<td>Fig.</td>
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<tr>
<td>G</td>
<td>Gram</td>
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<tr>
<td>g/cc</td>
<td>Gram per cubic centimetre</td>
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</tr>
<tr>
<td>GLC</td>
<td>Gas Liquid Chromatograph</td>
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<tr>
<td>h</td>
<td>Hour</td>
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<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatograph</td>
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<tr>
<td>i.d.</td>
<td>Internal diameter</td>
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<tr>
<td>ICAR</td>
<td>India Council of Agricultural Research</td>
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<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
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<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemist</td>
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</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
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<tr>
<td>L</td>
<td>Liter</td>
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</tr>
<tr>
<td>LC50</td>
<td>The median lethal concentration of substance required to kill 50% of given test population</td>
<td></td>
</tr>
<tr>
<td>LD50</td>
<td>The median lethal dose of substance required to kill 50% of given test population</td>
<td></td>
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<tr>
<td>LOD</td>
<td>Limit of Determination</td>
<td></td>
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<td>LOQ</td>
<td>Limit of Quantitation</td>
<td></td>
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<tr>
<td>mL</td>
<td>Milliliter</td>
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<tr>
<td>MWHC</td>
<td>Maximum Water Holding Capacity</td>
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</tr>
<tr>
<td>N</td>
<td>Normal</td>
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</tr>
<tr>
<td>nA</td>
<td>Neno Ampere</td>
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</tr>
<tr>
<td>ND</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>OC</td>
<td>Organic carbon</td>
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</tr>
<tr>
<td>pH</td>
<td>Potential of hydrogen ion</td>
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</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
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<tr>
<td>PSA</td>
<td>Primary Secondary Amine</td>
<td></td>
</tr>
<tr>
<td>QuEChERS</td>
<td>Quick Easy Cheap Effective Rugged and Safe</td>
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</tr>
<tr>
<td>RH</td>
<td>Relative humidity</td>
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<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
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<tr>
<td>RSD</td>
<td>Relative Standard deviation</td>
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<tr>
<td>RSD\text{WR}</td>
<td>Relative Standard Deviation Within-Laboratory Reproducibility</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
<td></td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
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<td>viz.,</td>
<td>Namely</td>
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<td>vs.</td>
<td>Versus</td>
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Introduction
Herbicides are chemicals which are used to kill unwanted plants or 'weeds'. Many of these act by interfering with the natural growth of plants considered as 'weeds' by imitating plant hormones. There are different modes of action by which herbicides kill a plant – excessive oxidation, chemicals that act on the cell membrane, that mimic plant growth regulators like auxin, inhibition of DNA synthesis and synthesis of some amino acids etc. Herbicides are broadly classified under pesticides and like other pesticides can be either contact or systemic poisons. While some of them have broad spectrum action, others work on specific plants (monocots etc.). However, most end up impacting environmental resources and living organisms other than the intended ones.

The highest food grain losses are due to weeds, being 28 %. The losses due to insect and diseases account for 23 and 25 %, respectively (Anonymous, 2011a). In India the usage of insecticides, herbicides and fungicides was 64, 20 and 16 %, respectively during 2006-07. During the same period, the world consumption scenario was 24, 48 and 22 %, respectively for insecticides, herbicides, and fungicides (Varshney, 2009).

The indiscriminate and excessive use of pesticides leads to the problems of their residues in agricultural commodities and
environmental samples, pest resurgence and development of insect resistance to pesticides.

In countries like USA (where only around 1% of the population is engaged in farming), herbicides constitute about 70% of all pesticides used in farming; worldwide, herbicides constitute 48.7% of the world pesticides market, followed by insecticides (24.3%), fungicides (23.6%) and others (3.5%). In India, insecticides continue to be the largest used pesticides in agriculture, with 20% of the pesticides being herbicides/weedicides. It indicates that the use of herbicides is rapidly on the rise (Anonymous, 2011b).

Currently, herbicides are the primary methods for managing weeds in industrialized countries and are gaining popularity in developing countries. More than 250 chemicals are listed as herbicides by the Weed Science Society of America (Monaco et al., 1994) out of which only 44 are registered for sale in India. Although, herbicide consumption in India has risen manifold from a meager 15 tonnes in 1970 to about 11,000 tonnes in 2001-02, they are used only in approx. 20 million hectares amounting to about 10% of the present total cropped area. In contrast, in a developed country like U.S., the herbicide treated acreage exceeds 85% of the cropped area, with an average consumption of 2.24 kg herbicide/ha.

Although herbicides have benefited the agricultural community by substantially increasing crop yields, their use is not without
potential problems. Some of the unintended negative impacts of herbicide use are persistence in soil, pollution of ground-water, toxic residues in food (contamination), feed and fodder, adverse effect on non-target organisms and development of resistance in weeds.

Herbicides are applied to soil either as pre- or post-emergence treatments to manage weeds. The length of time and herbicide remain active in soil is called “soil persistence”. Herbicide persistence depends on soil physical, chemical, biological properties and the environment. High temperatures and soil moisture enhance soil microbial activity thereby hastening herbicide decomposition.

The basic role of ground-water as a primary and irreplaceable resource of good-quality water for human consumption raises public concern regarding its contamination by using herbicides. However, such a concern in developed countries is justified, where the herbicides accounting for almost 60 % of the pesticides used, are reported to be typical contaminants of groundwater.

Oxyfluorfen, a diphenyl ether herbicide is used for the control of annual broadleaf and grassy weeds in tropical and subtropical crops, by pre- or post-emergence application in maize, rice, gram, groundnut, soybean, cotton and onion crops. It is selective contact herbicide and absorbed more readily by the foliage than by the roots, with very little translocation.
It targets a specific enzyme, protoporphyrinogen oxidase, in the chlorophyll biosynthetic pathway. Inhibiting protoporphyrinogen oxidase in plants leads to an accumulation of phototoxic chlorophyll precursors which, in the presence of light, produce activated oxygen species which rapidly disrupt cell membrane integrity. It must contact plant foliage to cause effects. Plants that are actively growing are most susceptible to oxyfluorfen. By forming a chemical barrier on the soil surface, oxyfluorfen affects plants at emergence. This barrier is formed with adequate spray coverage or irrigation following granule application (to partially dissolve granules and promote dispersion of oxyfluorfen over the soil surface).

In India, oxyfluorfen is used in dry seeded rice @ 150-200 g a.i. ha⁻¹ at 0-6 days after sowing for the control of annual grasses and broad leaf weeds. It is also used in greengram, blackgram, pigeonpea, chickpea, peas @ 100-125 g a.i. ha⁻¹ at 0-3 days after sowing for the control of wide spectrum of weeds. In groundnut and sunflower the herbicide is used @ 250 g a.i. ha⁻¹ at 0-3 days after sowing for the control of wide spectrum of weeds. In onion and garlic it is used @ 250 g a.i. ha⁻¹ at pre- or early post-transplanting (Anonymous, 2011c).

Oxyfluorfen is moderately persistent in most soil environments, with a representative field half-life of about 30 to 40 days. Oxyfluorfen is not subject to microbial degradation or hydrolysis. The main
mechanism of degradation in soils may be photodegradation and evaporation/co-distillation in moist soils. In laboratory studies, its soil half-life was 6 months, indicating very low rates of microbial degradation. Soil binding is highest in soils with high organic matter and clay content. Once oxyfluorfen is adsorbed to soil particles, it is not readily removed. It is practically insoluble in water, and therefore is unlikely to be appreciably mobile in most instances, unless the sorptive capacity of the soil is exceeded. Oxyfluorfen did not leach below 10.2 cm in any soil except sand (Anonymous, 2011c). In water, oxyfluorfen is rapidly decomposed by light. Because oxyfluorfen is nearly insoluble in water and has a tendency to adsorb to soil, it will be sorbed to suspended particles or sediments. There is very little movement of oxyfluorfen within treated plants. It is not readily metabolized by plants, but since it is not readily taken up by roots, residues in plants are generally very low. Residues of oxyfluorfen accumulated in carrots and oats grown on previously treated fields, but not in cotton or lettuce. The determination of selected pesticides in groundwater was studied by Hernandez et al. (1993).

Looking to the above facts, a study entitled "To study the degradation and downward movement of oxyfluorfen in sandy, sandy loam and clayey soils" was conducted at Pesticide Residue Laboratory, AINP on Pesticide Residues, Anand Agricultural
University, Anand to study the degradation and downward movement of oxyfluorfen under laboratory conditions with the following objectives:

1. To validate the pesticide residue method of oxyfluorfen from sandy, sandy loam and clayey soils.
2. To study the depth-wise distribution of oxyfluorfen in soil columns.
3. To study the degradation of oxyfluorfen in sandy, sandy loam and clayey soils under laboratory conditions.
4. To study the level of oxyfluorfen in column leachates.
Review of Literature
II. REVIEW OF LITERATURE

In agriculture, the safe and effective use of agrochemicals, especially pesticides has successfully been carried out to solve various problems. These includes: pre- and post-harvest losses due to insects, weeds, fungi and effective control of vectors of human and animal disease.

Consumer concerns on food safety and society awareness of chemical contaminants in the environment have increased in the past few years. As a consequence, more restrictions in the use of chemical products have been imposed at national and international levels. Though use of pesticides has helped India and other countries in achieving self-sufficiency in food production, their indiscriminate use has considerably polluted the environment. It is now well established that the benefits of the pesticide conferred on human kind are great. It has been estimated that even after the effective use of pesticides, these losses would be astronomical in the absence of persistent chemical use. Nevertheless, chemical crop protection is profit-induced poisoning of the environment.

Attempts have been made to collect literature on various aspects of present investigation pertaining to herbicide contamination in the environment. The available literature related to present studies have been reviewed and presented hereunder in different headings.
2.1 Leaching of oxyfluorfen in soil

Soil column leaching study was conducted by Valliappan et al. (1990) using Poly Vinyl Chloride (PVC) tubes 30 cm long x 10 cm diameter. The columns were filled with sandy loam soil and 1.5 kg ha\(^{-1}\) benthicarb, 1.25 kg ha\(^{-1}\) butachlor, 0.625 kg ha\(^{-1}\) oxadiazon, 0.01 kg ha\(^{-1}\) pyrazosulfuron-ethyl and oxyfluorfen @ 0.1 kg ha\(^{-1}\) were applied to the opened ends. Oxyfluorfen was shown to be the least mobile, butachlor and benthicarb to be moderately mobile and oxyfluorfen and pyrazosulfuron-ethyl to have leached furthest.

Hoogeweg and Hornsby (1997) observed that leaching potential of the herbicides increased in the order norflurazon=oryzalin=oxyfluorfen<diuron<bromacil acid<simazine.

Ying and Williams (2000) studied the leaching behavior of norflurazon, oxadiazon, oxyfluorfen and trifluralin in South Australian soils on packed soil columns. On the packed soil columns, norflurazon was the most leachable herbicide. More of the herbicides were detected in the leachates from the sandy soils than from the clayey soils. Organic matter is generally low in these soils.

A field study was performed to evaluate the accuracy of six pesticide screening leaching indexes for herbicide movement by Kogan et al. (2007). Adsorption, dissipation and soil movement were studied in a vineyard in a sandy loam soil during 2005 season. Simazine, diuron, pendimethalin, oxyfluorfen and flumioxazin were applied to
bare soil at rates commonly used. They observed that simazine reached 120 cm, diuron 90 cm, flumioxazin 30 cm soil depth respectively. Pendimethalin and oxyfluorfen were retained up to 5 cm.

Leaching of sulfentrazone, isoxaflutole and oxyfluorfen in three soils one with a sandy loam texture and two clayey ones with low and high organic matter content was studied by Melo et al. (2010). Doses of 0.50 and 0.75 kg ha⁻¹ sulfentrazone, 0.113 and 0.169 kg ha⁻¹ isoxaflutole and 0.72 and 1.08 kg ha⁻¹ oxyfluorfen were applied on the surface of 30 cm PVC columns filled with three soils. Herbicide leaching was detected by bioassay method using sorghum (Sorghum bicolor) plants. Oxyfluorfen remained adsorbed in surface layers of soil only. When compared the types of soil, the highest herbicide activity and movement occurred in sandy loam soil. Oxyfluorfen showed low leaching potential.

Leaching potential of herbicides used in cotton crop under soil column conditions was studied by Inoue et al. (2010) to evaluate the influence of different irrigation depth on the leaching potential of four herbicides in sandy and clayey soils. A bioassay technique in soil column was conducted in which water depth of 0, 20, 40, 60, 80 and 100 mm were simulated after application of alachlor, oxyfluorfen, prometryne and S-metolachlor. Regardless of the irrigation depth applied in sandy soil, oxyfluorfen did not exceed the depth of 5-10 cm. In columns filled with clay soil, oxyfluorfen did not move beneath the
surface layer, even under the highest water depths. A more intense downward movement of the herbicide molecules was found in sandy soil than in clay soil.

Andrade et al. (2011) carried out an experiment to estimate the leaching potential of herbicides used in the intensive agricultural areas of Alto Paranaiba region (Brazil). They observed that alachlor, diuron, glyphosate, linuron, trifluralin, fluazifop-p-buthyl, paraquat, lactofen and oxyfluorfen were less mobile in soil.

2.2 Persistence of oxyfluorfen in soil

DeFrank et al. (1990) conducted an experiment with seven pre-emergence herbicides and identified oxyfluorfen as a promising herbicide for commercial taro (Colocasia esculenta) production in Hawaii. Oxyfluorfen was applied twice at 0.38, 0.56 and 1.11 kg ha\(^{-1}\) to taro crop grown under wetland flooded and upland conditions. They observed true levels in flood waters immediately after treatment dissipated to undefectable level within 24 h.

XiaoHua et al. (1994) studied the degradation dynamics of oxyfluorfen in rice and its ecosystem. The herbicide was sprayed at 150-240 g a.i. ha\(^{-1}\), 7 days after treatments of the crop. The result showed that oxyfluorfen residues were not detected in soil at harvest.

Movement and persistence of imazaquin, oxyfluorfen, flurochloridone and terbacil in a sandy loam soil under field
conditions for 2 years was studied by Milanova and Grigorov (1996). Oxyfluorfen @ 1.2 kg a.i. ha\(^{-1}\) showed least mobility in the soil profile. It was retained in the 0-5 cm layer throughout the experimental period in both the years.

Dissipation of oxyfluorfen and oxadiazon followed first order reaction kinetics with half-life of 8.8 and 12 days, respectively. (Das et al., 2003).

The dissipation and mobility of the herbicide oxyfluorfen in subtropical soil of Taiwan were investigated in the laboratory with six tea garden soils by JuiHung et al. (2003). The results indicated that the half-life of oxyfluorfen ranged from 72 to 160 days.

Dumas et al. (2008) studied the dissipation rate of prometryn, oxyfluorfen and imazethapyr in the Mollisol and the Vertisol soils from the south semi-arid coastal region of Puerto Rico. Herbicide rates were 2.24, 0.28 and 0.070 kg a.i. ha\(^{-1}\) for prometryn, oxyfluorfen and imazethapyr, respectively. Soil samples at 15 cm depth were collected at 0, 28, 56, 112 and 168 days after application. Field half-lives between 2 and 43, 31 and 88, and 19 and 46 days were found for prometryn, oxyfluorfen and imazethapyr, respectively.

A four-year field study was conducted to study the dissipation and soil movement of pendimethalin and oxyfluorfen in a sandy loam soil by Alister et al. (2009). Pendimethalin and oxyfluorfen were applied every year in August @ 1.33 and 0.75 kg ha\(^{-1}\), respectively.
They observed that more than 74% of the herbicide applied was detected at the top 2.5 cm layer for both herbicides and none was detected at 10 cm or deeper.

Sondhia (2010) at National Research Centre for Weed Science, Jabalpur (MP) studied the persistence and bioaccumulation of oxyfluorfen residues in onion. Oxyfluorfen was applied @ 250 and 500 g a.i. ha\(^{-1}\). Soil samples collected at harvest showed 0.003 μg g\(^{-1}\) of oxyfluorfen residues at both the doses.

Sharma et al. (2011) studied the persistence of oxyfluorfen in soil from garlic field experiment. They observed that oxyfluorfen did not leave residues in soil beyond harvest of the crop when applied @ 125, 250 and 500 g ha\(^{-1}\) on the garlic crop.

### 2.3 Depthwise distribution of oxyfluorfen in three different soils

Under field conditions, oxyfluorfen was applied @ 720 g a.i. ha\(^{-1}\) to soil and soil samples were collected from 0-5, 5-10 and 10-15 cm depth and water samples were collected at weekly interval after application. The results showed that herbicide residues in soil varied with sampling depth and initial concentration indicated that there was little or no loss during application. No residues of oxyfluorfen were found in the drainage water (Frank et al., 1991).
Materials
And
Methods
III. MATERIALS AND METHODS

This chapter deals with the particulars of materials used during the course of exploration and methods adopted in conducting the present investigation entitled “To study the degradation and downward movement of oxyfluorfen in sandy, sandy loam and clayey soils”.

3.1 Chemical

3.1.1 Oxyfluorfen

**Compound name:** Oxyfluorfen

**Chemical name:** 2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl) benzene

**Trade and other names:** Goal, Goal Tender, Galigan, Oxiflo

**Chemical family:** Diphenyl ether herbicide

**Case number:** 2490

**CAS registry number:** 42874-03-3

**OPP chemical code:** 111601

**Empirical formula:** C15H11ClF3NO4

**Molecular weight:** 361.72 g mole⁻¹
Materials and methods

Structural formula:

\[
\begin{align*}
\text{Vapour pressure:} & \quad 2 \times 10^{-6} \text{ power mm Hg at } 25 \, ^\circ\text{C.} \\
\text{Density:} & \quad 1.06 \, \text{g mL}^{-1} \\
\text{Melting point:} & \quad 83-84 \, ^\circ\text{C} \\
\text{Boiling point:} & \quad > 240 \, ^\circ\text{C} \\
\text{Solubility:} & \quad \text{Water: } 0.1 \, \text{mg L}^{-1} \\
& \quad \text{Acetone: } 725 \, \text{g kg}^{-1} \\
& \quad \text{Chloroform: } 500-550 \, \text{g kg}^{-1} \\
& \quad \text{Cyclohexanone: } 615 \, \text{g kg}^{-1} \\
\text{Solubility in other solvents:} & \quad \text{Soluble in most organic solvents} \\
\text{Organic-carbon sorption constant (mL g}^{-1}): & \quad \text{Average is } 100,000 \, \text{mL g}^{-1} \text{ (estimated)} \\
\text{Basic manufacturer:} & \quad \text{Dow Agro Sciences} \\
\text{Partition coefficient:} & \quad 4.4683
\end{align*}
\]
Adsorption coefficient: 100,000 (estimated)

RfD: 0.003 mg kg\(^{-1}\) day\(^{-1}\)

Mode of action: Oxyfluorfen targets a specific enzyme, protoporphyrinogen oxidase, in the chlorophyll biosynthetic pathway. Inhibiting protoporphyrinogen oxidase in plants leads to an accumulation of phototoxic chlorophyll precursors which, in the presence of light, produce activated oxygen species which rapidly disrupt cell membrane integrity. Oxyfluorfen must contact plant foliage to cause effects. Plants that are actively growing are most susceptible to oxyfluorfen. By forming a chemical barrier on the soil surface, oxyfluorfen affects plants at emergence. This barrier is formed with adequate spray coverage or irrigation following granule application (to partially dissolve granules and promote dispersion of oxyfluorfen over the soil surface). Because of the length of oxyfluorfen soil half-life, this barrier may last up to three months. Oxyfluorfen also affects plants through direct contact of spray or granules to exposed tissues.
Analytical standard (Technical grade)

Technical name : Oxyfluorfen
Nature of chemical : Herbicide
Purity (%) : 99.9 %
Physical appearance : White amorphous powder
Provided by : Sigma Aldrich, USA
Expiry date : 17-01-2014
Lot No. : SZE7017X
Storage condition : Dark & Cool (-10 °C)

3.2 Solvents and other chemicals

3.2.1 Acetone

Analytical grade acetone (2.5 L) was refluxed with KMnO₄ (0.5 g) till violet colour persisted. The acetone was then glass distilled at 58 °C.

3.2.2 Methylene chloride/Dichloromethane (DCM)

Analytical grade methylene chloride was glass distilled at 39 °C.

3.2.3 Petroleum spirit (40-60 °C)

Analytical grade petroleum spirit was distilled in glass assembly. The suitability of petroleum spirit was checked by concentrating 50 mL of solvent to 1.0 mL, which was subjected to Gas Liquid Chromatograph- Electron Capture Detector (GLC-ECD) for the detection of possible impurities.
3.2.4 Iso-octane

Analytical grade distilled iso-octane was used for the preparation of the working standards. Its suitability was checked on GLC-ECD.

3.2.5 Acetonitrile

Analytical grade acetonitrile (purity ≥ 99.9%) was used as such without distillation although its suitability was checked on GLC-ECD by completely evaporating 50 mL of solvent and then made up the volume to 1.0 mL with a mixture of petroleum spirit : acetone (1:1, v/v).

3.2.6 Cotton

Cotton was purified by Soxhlet extraction using petroleum spirit : acetone (1:1, v/v) for 6.0 h followed by drying in oven at 60 °C for 1.0 h. It was stored in a closed glass container.

3.2.7 Sodium chloride (NaCl)

Phthalate esters, present in reagent grade sodium chloride generally interfere in GLC-ECD analysis. Therefore, to remove the phthalate esters sodium chloride was subjected to heating at 540 °C in muffle furnace for 3 h and stored in a glass container.

3.2.8 Sodium sulfate (Na₂SO₄)

Anhydrous, granular, reagent grade sodium sulfate was purified in the same way as sodium chloride and stored in a glass container.
3.2.9 **Magnesium sulfate (MgSO₄)**

Anhydrous, fine powdered, reagent grade magnesium sulfate (>99.5%) was used as such for analysis and stored in a desiccator at room temperature.

3.2.10 **Primary Secondary Amine (PSA)**

Primary Secondary Amine (Ethylenediamine-N-propyl, Bond Elut™ PSA) was used as anion exchanger for dispersive column clean up and was kept in a desiccator at room temperature. PSA removes fatty acids, sugars and other H-bonding matrix co-extractives. Its particle size is 40 μm.

3.3 **Equipments and instruments**

3.3.1 **Equipments**

3.3.1.1 **Weighing balance**

‘Contcch’ make digital weighing balance (1000 g capacity and 0.01 g sensitivity) was used for weighing the samples and reagents while ‘Sartorious basic plus’ balance (maximum capacity 210 g and sensitivity 0.01 mg) was used for weighing pesticide standard.

3.3.1.2 **Vacuum rotary evaporator**

‘Heidolph’ make vacuum rotary evaporator was used to concentrate the large volume of the sample extract.

3.3.1.3 **TurboVap LV (Concentration workstation)**

‘Caliper’ make nitrogen based evaporator was used for reducing the final volume of the sample under inert atmosphere.
3.3.1.4 Centrifuge

A high speed digital controlled centrifuge model 'R-23' obtained from Remi Instruments Ltd. was used.

3.3.1.5 Vortex mixer

A simple Vortex mixer from Tarson Ltd. was used to mix the solvent in test tube.

3.3.2 Instruments

3.3.2.1 Gas Liquid Chromatograph (GLC)

Varian 450 GC equipped with Electron Capture Detector (ECD) was used for the qualitative and quantitative estimation of pesticide residues by using auto sampler AOC 201.

3.4 Certified Reference Material (CRM)

Certified reference material (purity 99.9%) of oxyfluorfen was used for comparison and iso-octane was used in the standard preparation.

3.4.1 Primary standard

A technical grade pesticide standard (10 mg), was accurately weighed on Sartorius basic plus balance (maximum capacity 210 g and sensitivity 0.01 mg). It was then transferred to 100 mL of volumetric flask (A grade). The standard was initially dissolved with 10 to 15 mL of distilled acetone and final volume was made up with iso-octane. This gave the concentration of 100 µg g⁻¹.
3.4.2 Secondary/Intermediate standard

From the primary standard, 5 mL aliquot was diluted to 50 mL with acetone in volumetric flask. This gave a concentration of 10 μg g⁻¹.

3.4.3 Final working standards

The intermediate standard was used for the preparation of final working standards. Suitable aliquots were diluted to required final volume using acetone, to obtain final concentrations of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 μg g⁻¹.

3.5 Linearity study

A linearity study was performed to determine the performance of ECD detector. For the linearity study, response (height) v/s concentration was plotted. To establish the linearity of gas liquid chromatograph, six different concentrations of the standards viz., 0.01, 0.025, 0.05, 0.1, 0.25 and 0.5 μg g⁻¹ were injected and their response (mV) was recorded. The volume of the standard used for the injection was 1.0 μL. A correlation coefficient and equation was determined by using best fit model of linear relationship.

3.6 Limit of detection (LOD) and limit of quantitation (LOQ)

Prior to quantitation of oxyfluorfen in two different matrices viz., soil and column leachates, the LOD and LOQ were worked out. This was carried out by injecting matrix-match oxyfluorfen in gas liquid chromatograph to get signal to noise ratio 1:3 for LOD and 1:10 for
LOQ. The results are discussed in the chapter-IV (Results and Discussion).

3.7 Experimental details

3.7.1 Depth wise distribution of oxyfluorfen in soils

3.7.1.1 Experimental set up

Nature of study : Laboratory study

Location : All India Network Project on Pesticide Residues

Centre for Organic Farming

ICAR, Unit-9

Anand Agricultural University

Anand-388 110 (Gujarat)

Year : 2013

Types of soil : i) Sandy

ii) Sandy loam

iii) Clayey

Test matrix : Soil

Design : FCRD

No. of repetition : Three
Column size: 36 cm long with 6.0 cm i.d.

Method of application: Fortification done by auto pipette

Number of application: One

Date of application: 07-02-2013

Dose rate:

1. Active ingredients: Oxyfluorfen

2. Treatments:
   - Control (T0): Untreated control
   - Standard dose (T1): 30 mg/column

Sampling details:

a. Number of sample collected: Three per treatment

b. Sampling interval and dates of soil leachates:

   - 0-Day: 07-02-2013
   - 1-Day: 08-02-2013
   - 3-Day: 10-02-2013
   - 5-Day: 12-02-2013
   - 7-Day: 14-02-2013
   - 10-Day: 17-02-2013
   - 15-Day: 22-02-2013
   - 30-Day: 09-03-2013
   - 45-Day: 24-03-2013
   - 60-Day: 08-04-2013
A laboratory study was conducted at All India Network Project on Pesticide Residues, Centre for Organic Farming, ICAR, Unit-9, Anand Agricultural University, Anand-388 110, Gujarat (India).

Three types of soils which were denoted as S₁, S₂ and S₃ for sandy, sandy loam and clayey soil, respectively were collected from Sardar Krushinagar Dantiwada Agricultural University, Dantiwada, Anand Agricultural University, Anand and Navsari Agricultural University, Navsari, respectively. Physico-chemical study of the soil reflects that the soil collected from Dantiwada was sandy in texture while that of Anand and Navsari were sandy loam and clayey, respectively. Sandy soils collected from Daintywada represent the North Gujarat while that of sandy loam and clayey soil represent the Middle Gujarat and South Gujarat, respectively. These soils were air dried passed through 2 mm sieve and used for the study. A 36 cm long PVC column with an internal diameter (i.d.) of 6 cm was used by marking into sections each of 6 cm. The bottom ring was tied up with muslin cloth with thread to avoid contamination of leachate with soil particles from bottom and 30 cm column from bottom was filled with soil. Before applying the herbicide, soil column was wetted to their apparent water holding capacity by applying 0.01 M CaCl₂ to the top of column at an interval of 24 h. After this initial equilibration 30 mg/column of oxyfluorfen was applied to the top of column. Polythene
Plate 1: Column leaching study of oxyfluorfen in sandy, sandy loam and clayey soils
sheet was placed on each column in order to prevent volatization. The entire experiment was conducted at room temperature.

The column was leached after 24 h of application of herbicide with 0.01 M CaCl₂ at 50% water holding capacity for all the soils per day during the first 10 days followed by 50 mL per day until the end of the experiment (60 days).

Leachates collected at an interval as mentioned in 3.7.1.1, were filtered, extracted and analyzed by GLC for herbicide residues. After end of experiment, each column was cut into 5 segments each of 6 cm and herbicide residues in soil from different depths i.e. 0-6 cm (D₁), 6-12 cm (D₂), 12-18 cm (D₃), 18-24 cm (D₄), 24-30 cm (D₅) was analyzed. The soil inside the column was dried under shade and 10 g of soil was used for residue analysis and it was extracted and estimated by GLC.

Physico-chemical properties of three different types of soils are mentioned in Table 3.1 Physico-chemical analysis was carried out at Department of Soil Science and Agricultural Chemistry, B. A. College of Agriculture, Anand Agricultural University, Anand.
Table 3.1: Physico-chemical properties of three different types of soils

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Physico-chemical properties</th>
<th>Sandy (S₁)</th>
<th>Sandy loam (S₂)</th>
<th>Clayey (S₃)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>pH (1:2.5) (Soil:Water ratio)</td>
<td>8.17</td>
<td>8.36</td>
<td>8.58</td>
<td>Jackson (1979)</td>
</tr>
<tr>
<td>2.</td>
<td>EC at 25 °C (dS m⁻¹) (1:2.5) (Soil:Water ratio)</td>
<td>0.25</td>
<td>0.21</td>
<td>0.29</td>
<td>Jackson (1979)</td>
</tr>
<tr>
<td>3.</td>
<td>MWHC (%)</td>
<td>22.41</td>
<td>41.30</td>
<td>56.38</td>
<td>Chopra and Kanwar (1976)</td>
</tr>
<tr>
<td>4.</td>
<td>Organic carbon (%)</td>
<td>0.20</td>
<td>0.39</td>
<td>1.12</td>
<td>Jackson (1979)</td>
</tr>
<tr>
<td>6.</td>
<td>Soil mechanical analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coarse sand (%)</td>
<td>39.70</td>
<td>0.15</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sand (%)</td>
<td>46.82</td>
<td>78.45</td>
<td>30.76</td>
<td>Piper (1966)</td>
</tr>
<tr>
<td></td>
<td>Silt (%)</td>
<td>1.19</td>
<td>1.67</td>
<td>20.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clay (%)</td>
<td>7.90</td>
<td>17.92</td>
<td>55.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Textural class</td>
<td>Sandy</td>
<td>Sandy loam</td>
<td>Clayey</td>
<td></td>
</tr>
</tbody>
</table>
3.7.1.2 **Solvents and reagents**

Dichloromethane, Sodium chloride, Sodium sulphate, Petroleum spirit, Acetone and Calcium chloride

3.7.1.3 **Apparatus**

Weighing balance, Vacuum rotary evaporator, Turbomax Low Volume, Vortex mixer

3.7.1.4 **Extraction of oxyfluorfen from column leachates**

The leachates collected from column were measured in measuring cylinder which ranged from 65 to 450 mL and taken into 1000 mL separatory funnel and NaCl was added until it dissolved. The residues were extracted twice with 50 mL methylene chloride each time and shaken vigorously. The lower organic phase was collected followed by passing through bed of anhydrous Na$_2$SO$_4$. The combined DCM phase was subject to complete evaporation using vacuum rotary evaporator (below 45 °C). The concentration step was repeated thrice in the presence of petroleum spirit to remove the traces of methylene chloride. Final volume was made up by petroleum spirit : acetone (1:1, v/v). Quantitative estimation was carried out by GLC with ECD (Electron Capture Detector). The stepwise photographs of oxyfluorfen extraction from column leachates are depicted in Plate-2.
Plate 2: Extraction of oxyfluorfen residues from leachates
### 3.7.1.5 Instrument and its parameters

**Instrument:** Varian 450 GC

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLC</td>
<td>Varian 450 GC</td>
</tr>
<tr>
<td>Auto sampler</td>
<td>CP 8400</td>
</tr>
<tr>
<td>Column</td>
<td>DB5MS, 30 m, 0.25 mm i.d., 0.25 µm film thickness</td>
</tr>
<tr>
<td>Detector</td>
<td>ECD- Ni63</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>Oven programming</td>
<td>185 °C → 290 °C (0.0 min) → 290 °C (5.0 min)</td>
</tr>
<tr>
<td>Column flow mode</td>
<td>Constant flow</td>
</tr>
<tr>
<td>Column flow</td>
<td>1.6 mL min⁻¹</td>
</tr>
<tr>
<td>Injection mode</td>
<td>Split</td>
</tr>
<tr>
<td>Split ratio</td>
<td>1:10</td>
</tr>
<tr>
<td>Injection volume</td>
<td>1.0 µL</td>
</tr>
<tr>
<td>Injection temperature</td>
<td>250 °C</td>
</tr>
<tr>
<td>Contact potential</td>
<td>400 mV</td>
</tr>
</tbody>
</table>
Materials and Methods

Detector temperature: 310 °C

Make up gas and flow: N₂ and 30 mL min⁻¹

Retention time: Columns (1) DB5MS: 10.78 min

(2) GSBP5MS: 11.20 min

3.7.1.6 Quantitative analysis

Quantitative estimation of oxyfluorfen was performed on Varian 450 GC equipped with ECD-Ni⁶³ using auto sampler AOC 20i. Quantitation was carried out by external standard method using different concentrations of working standard of oxyfluorfen. Hamilton make 10 μL capacity syringe was used for GLC injection.

\[
\text{Oxyfluorfen content (μg g}^{-1}) = \frac{H_1}{H_2} \times \frac{V}{W} \times C
\]

Where,

\(H_1\) = Peak height of sample

\(H_2\) = Peak height of standard

\(V\) = Volume of sample extract

\(W\) = Weight of soil sample for extraction

\(C\) = Concentration of oxyfluorfen (μg mL⁻¹) standard
3.7.1.7 Method validation

Validation of method was performed in terms of recovery studies before the analysis of experimental samples. The recovery study was carried out at 1.0 and 5.0 ng g\(^{-1}\) levels.

3.7.2 Persistence of oxyfluorfen in different soils

To study the persistence of oxyfluorfen in sandy, sandy loam and clayey soils a laboratory study was also conducted in Pesticide Residue Laboratory at All India Network Project on Pesticide Residues, Centre for Organic Farming, ICAR Unit-9, Anand Agricultural University, Anand, Gujarat (India). Each soil type (10 g) was taken in a 50 mL capacity of polypropylene tube separately in triplicate with a control. Each polypropylene tube was fortified @ 0.5 µg g\(^{-1}\). The details are as follow.

3.7.2.1 Experimental set up

Nature of study : Laboratory study
Location : All India Network Project on Pesticide Residues
Centre for Organic Farming
ICAR, Unit-9
Anand Agricultural University
Anand-388 110 (Gujarat)
### Type of soil

- Sandy
- Sandy loam
- Clayey

### Test matrix

- Soil

### Design

- CRD (Factorial)

### Date of application

- 20-02-2013, for all the three soils

### Sampling details

**a. Number of samples collected**

- Three, per treatment

**b. Sampling interval and dates:**

<table>
<thead>
<tr>
<th>Interval</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-Day</td>
<td>20-02-2013</td>
</tr>
<tr>
<td>1-Day</td>
<td>21-02-2013</td>
</tr>
<tr>
<td>3-Day</td>
<td>23-02-2013</td>
</tr>
<tr>
<td>5-Day</td>
<td>25-02-2013</td>
</tr>
<tr>
<td>10-Day</td>
<td>02-03-2013</td>
</tr>
<tr>
<td>20-Day</td>
<td>12-03-2013</td>
</tr>
<tr>
<td>30-Day</td>
<td>22-03-2013</td>
</tr>
<tr>
<td>45-Day</td>
<td>06-04-2013</td>
</tr>
<tr>
<td>60-Day</td>
<td>21-04-2013</td>
</tr>
</tbody>
</table>
Materials and methods

3.7.2.1 Dose rate

Control

\[ \text{T}_0 : \text{Untreated control} \]

Standard dose

\[ \text{T}_1 : 0.5 \mu g \text{ g}^{-1} \text{oxyfluorfen} \]

Number of samples

: Three collected per treatment

Sample weight for

: 10 g pesticide estimation

Sampling: Samples were drawn at 0 (1 h), 1, 3, 5, 10, 20, 30, 45 and 60 days after application.

3.7.2.2 Solvents and reagents

Acetonitrile, Petroleum spirit, Acetone, NaCl, MgSO\(_4\) and Primary Secondary Amine (PSA)

3.7.2.3 Apparatus

Weighing balance, Vacuum rotary evaporator, Turbovap Low Volume, Vortex mixer

3.7.2.4 Extraction (QuEChERS method)

A representative 10 g soil sample was taken in 50 mL polypropylene centrifuge tube, and 20 mL acetonitrile was added followed by mixture of 1 g NaCl + 4 g MgSO\(_4\) and shaken vigorously by
Plate 3: Extraction of oxyfluorfen residues from soil
Plate 4: Equipments used in residue analysis

(A) Nitrogen Evaporator

(B) Vacuum Rotary Evaporator- Heidolph
Plate 5: Equipments used in residue analysis
Plate 6: **Instrument used in residue analysis**
Materials and methods

hand for 1 minute before centrifuge. The tubes were centrifuged at 3500 rpm for 3 minutes. A 10 mL aliquot from the supernatant was transferred by auto pipette into a 15 mL polypropylene centrifuge tube containing 1.5 g MgSO₄ and 0.25 g PSA, followed by centrifugation at 2500 rpm for 2 minute. A 4.0 mL aliquot was transferred into a glass test tube and acetonitrile was completely evaporated on a TurboVap (AOAC, 2007). Final volume was made up to 1.0 mL using 1:1 (petroleum spirit:acetone) and subjected to ECD analysis on a Varian 450 GC-ECD. The schematic diagram of oxyfluorfen extraction from soil sample is shown in Plate-3.

3.8 Statistical analysis

The statistical analysis was carried out in the Microsoft Excel Version-2007 programme with the help of computer. The average, Standard Deviation (S.D.), regression equation, R², half-life and residues (µg g⁻¹) were calculated in excel software. Regression equation and other parameters were worked out as per Hoskins (1961).

The room temperature and relative humidity ranged from 28-31 °C and 61-64 %, respectively.
RESULTS
AND
DISCUSSION
IV. RESULTS AND DISCUSSION

In order to study the degradation and downward movement of oxyfluorfen in sandy, sandy loam and clayey soils, the experiment was carried out at Pesticide Residue Laboratory, All India Network Project on Pesticide Residues, ICAR, Unit-9, Anand Agricultural University, Anand (Gujarat).

To determine the downward movement of oxyfluorfen, a column study was conducted at All India Network Project on Pesticide Residues, ICAR, Unit-9, Anand Agricultural University, Anand (Gujarat). Three different types of soils viz. Sandy, Sandy loam and Clayey were filled in PVC columns.

The experiment was carried out during February to April, 2013. These soils were filled in 36 cm long PVC columns. Before applying the herbicide, soil columns were wetted to their apparent water holding capacity by applying 0.01 M CaCl₂ to the top of column at an interval of 24 h. After this initial equilibration 30 mg/column of oxyfluorfen was applied to the top of column. The column was leached after 24 h of application of herbicide with 0.01 M CaCl₂ @ 50 % water holding capacity of respective soil per day during the first 10 days followed by 50 ml per day until the end of the experiment. Leachates collected at an interval as mentioned in 3.7.1.1, were filtered, extracted and analyzed by GLC for herbicide residues. After end of experiment, each column was cut into 5 segments each of 6 cm and herbicide residues
in soil from different depth i.e. 0-6 cm (D₁), 6-12 cm (D₂), 12-18 cm (D₃), 18-24 cm (D₄), 24-30 cm (D₅) was analyzed. The soil inside the column was dried under shade and 10 g of soil was used for residue analysis and it was extracted and estimated by GLC.

A persistence study was also carried out in laboratory to know the persistence of oxyfluorfen in three different soils, viz., sandy, sandy loam and clayey @ 0.5 μg g⁻¹ in polypropylene tube. The experiment was carried out during February to April, 2013. Soil samples were collected on 0 (1h), 1, 3, 5, 10, 20, 30, 45 and 60 days after treatments and analysed for the residues of oxyfluorfen using QuEChERS method.

The detail results obtained from the present investigation are reported in this chapter along with statistical inferences. The results are presented and discussed under the following sub-heads.

4.1 Method validation studies of oxyfluorfen from different matrices

4.1.1 Linearity study

4.1.2 Validation studies (Accuracy, Precision, and LOD and LOQ)

4.1.2.1 Validation of oxyfluorfen extraction method from different soils

4.1.2.2 Validation of oxyfluorfen extraction method from column leachates
4.2 Depthwise distribution of oxyfluorfen in three different soils

4.2.1 Oxyfluorfen residues in column leachates

4.2.2 Depthwise distribution of oxyfluorfen in columns filled with sandy, sandy loam and clayey soils under laboratory conditions

4.3 Persistence of oxyfluorfen in different types of soils

4.3.1 Sandy soil

4.3.2 Sandy loam soil

4.3.3 Clayey soil

4.1 Method validation studies of oxyfluorfen from different matrices

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability, and consistency of analytical results; it is an integral part of any good analytical practice (Huber, 2007). Prior to analyzing unknown samples for the presence of oxyfluorfen, several validation parameters were determined viz., linearity, accuracy (mean % recovery), precision (% RSD\textsubscript{wr}), method detection-limits i.e. LOD and LOQ.

A linearity study was performed to determine the performance of ECD detector. For the linearity study, a graph of detector's response vs concentration of oxyfluorfen was plotted and correlation equation and coefficient were determined.

Accuracy of the methods employed for the analysis of oxyfluorfen from
different matrices was determined by recovery studies. Mean recovery obtained from such studies reflects the accuracy of the methods. Precision of the analytical method is reflected by % Relative Standard Deviation (% RSDWR).

Before quantitation of oxyfluorfen from soil samples, the LOD and LOQ were worked out. The limits of detection (LOD) and quantification (LOQ) were calculated in accordance with Taylor (1987). For this purpose, 7 independent analyses of different samples spiked with oxyfluorfen at different levels were performed. The LOD and LOQ were calculated from the standard deviation of these determinations. The limit of detection and limit of quantitation were worked out in order to quantify and detect the minimum amount of oxyfluorfen with optimum confidence level. The LOD and LOQ were calculated as below:

\[
\text{LOD (\mu g \, g^{-1})} = \frac{SD \times \text{Fortification level}}{100} \times 3.14
\]

\[
\text{LOQ (\mu g \, g^{-1})} = \text{LOD} \times 3.0
\]

4.1.1 Linearity study

A linearity study was performed to determine the performance of ECD detector. For the linearity study, a graph of detector's response vs. concentration was plotted (Figure 4.1). To establish the linearity of oxyfluorfen on electron capture detector, equal volume of six different concentrations of the standards of oxyfluorfen viz., 0.01, 0.025, 0.05, 0.10, 0.25 and 0.5 \mu g \, g^{-1} were injected and their corresponding responses were
Figure 4.1: Linearity study of oxyfluorfen

\[ y = 2596.0x - 51.09 \]

\[ R^2 = 0.988 \]
recorded. As per the data obtained in linearity study (Table 4.1), oxyfluorfen were found linear in the range of 0.025 to 0.5 µg g⁻¹. The R² value obtained from the correlation equation was calculated by adopting positive linear correlation model (Y = a + bC) was 0.988.

The response of oxyfluorfen on electron capture detector at different concentrations is presented in Table 4.1. Linearity was drawn on the basis of these data.

Table 4.1: Response of oxyfluorfen on ECD at different concentrations

<table>
<thead>
<tr>
<th>Concentration (µg g⁻¹)</th>
<th>Detector Response (Height, mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>15.8</td>
</tr>
<tr>
<td>0.025</td>
<td>42.5</td>
</tr>
<tr>
<td>0.05</td>
<td>84.2</td>
</tr>
<tr>
<td>0.1</td>
<td>174.7</td>
</tr>
<tr>
<td>0.25</td>
<td>507.5</td>
</tr>
<tr>
<td>0.5</td>
<td>1296.2</td>
</tr>
</tbody>
</table>

4.1.2 Validation studies

4.1.2.1 Validation of oxyfluorfen extraction method from different soils

Prior to dissipation study of oxyfluorfen, different types of soils viz., sandy, sandy loam and clayey were subjected to recovery study. Each soil type was spiked at 2 different levels i.e. 0.1 and 0.5 µg g⁻¹ and further analysed with method mentioned in section 3.7.2.4 of materials and methods chapter. The data obtained from the study are depicted in Table 4.2. The recovery experiment was performed with 7 replications along with a control and reagent blank.
Table 4.2: Recovery of oxyfluorfen in different types of soils

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Fortification level (µg g⁻¹)</th>
<th>Recovery (%)</th>
<th>Mean Recovery (%)</th>
<th>Standard deviation (SD)</th>
<th>% RSDWR</th>
<th>LOD (µg g⁻¹)</th>
<th>LOQ (µg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R-I</td>
<td>R-II</td>
<td>R-III</td>
<td>R-IV</td>
<td>R-V</td>
<td>R-VI</td>
</tr>
<tr>
<td>Sandy</td>
<td>0.1</td>
<td>110.02</td>
<td>106.35</td>
<td>102.61</td>
<td>107.01</td>
<td>105.73</td>
<td>106.32</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>95.36</td>
<td>97.36</td>
<td>96.45</td>
<td>99.36</td>
<td>98.34</td>
<td>89.23</td>
</tr>
<tr>
<td>Sandy loam</td>
<td>0.1</td>
<td>109.14</td>
<td>112.30</td>
<td>111.30</td>
<td>108.36</td>
<td>113.50</td>
<td>119.88</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>112.32</td>
<td>110.23</td>
<td>105.36</td>
<td>107.26</td>
<td>99.34</td>
<td>104.23</td>
</tr>
<tr>
<td>Clayey</td>
<td>0.1</td>
<td>125.16</td>
<td>117.55</td>
<td>124.60</td>
<td>122.30</td>
<td>127.36</td>
<td>119.17</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>92.56</td>
<td>96.23</td>
<td>95.12</td>
<td>97.57</td>
<td>99.12</td>
<td>102.85</td>
</tr>
</tbody>
</table>
Chromatogram: Recovery of oxyfluorfen in different types of soils- Reagent Blank
Chromatogram: Recovery of oxyfluorfen in sandy soil at 0.1 ppm level

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret. Time</th>
<th>Name</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.79</td>
<td>Oxyfluorfen</td>
<td>17093.1</td>
<td>365600.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>365600.3</td>
</tr>
</tbody>
</table>

Oxyfluorfen
Chromatogram: Recovery of oxyfluorfen in sandy loam soil at 0.1 ppm level

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret. Time</th>
<th>Name</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.78</td>
<td>Oxyfluorfen</td>
<td>16120.8</td>
<td>348420.8</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>348420.8</td>
</tr>
</tbody>
</table>
Chromatogram: Recovery of oxyfluorfen in clayey soil at 0.1 ppm level
The results obtained from the recovery study revealed that mean recovery of oxyfluorfen from sandy, sandy loam and clayey soils was 106.15, 112.85 and 119.56 % at 0.1 µg g\(^{-1}\) level of fortification, respectively. The corresponding values for 0.5 µg g\(^{-1}\) fortification level was 94.79, 106.05 and 96.72 %. Percent RSD\(_{WR}\) recorded for sandy, sandy loam and clayey soils at 0.1 µg g\(^{-1}\) spiking level was 2.10, 3.50 and 2.36 %. The corresponding values for 0.5 µg g\(^{-1}\) spiking level were 4.88, 4.10 and 3.63 %. LOD determined at 0.1 µg g\(^{-1}\) for sandy, sandy loam and clayey soils was 0.007, 0.011 and 0.007 µg g\(^{-1}\), respectively. The corresponding values for 0.5 µg g\(^{-1}\) spiking level were 0.007, 0.013 and 0.011 µg g\(^{-1}\). The LOQ at 0.1 µg g\(^{-1}\) fortification level for sandy, sandy loam and clayey soils were 0.021, 0.033 and 0.022 µg g\(^{-1}\), respectively. The corresponding values for 0.5 µg g\(^{-1}\) fortification levels were 0.020, 0.039 and 0.034 µg g\(^{-1}\).

The analytical method employed for the extraction of oxyfluorfen from soil was found accurate and precise as mean recovery was in the range of 94.79 to 119.56 % and RSD\(_{WR}\) was 2.10 to 4.88 % which was within the limits of ≤ 20 % as prescribed by the SANCO (2011). According to the SANCO (2011) guidelines, any analytical method which records mean recovery in the range of 70-120% and % RSD\(_{WR}\) ≤ 20% is the sufficiently accurate and precise. Hence the method employed for the extraction of oxyfluorfen from different soils is accurate and precise.
4.1.2.2 Validation of oxyfluorfen extraction method from column leachate

Prior to column leaching study of oxyfluorfen, aqueous solution of 0.05 M CaCl₂ was subjected to recovery study with 2 different spiking levels i.e. 1.0 and 5.0 ng g⁻¹ and further analysed with method mentioned in section 3.7.1.4 of materials and methods chapter. The data obtained from the study are depicted in Table 4.3. The recovery experiment was performed with 3 replications along with a control.

The result obtained from the recovery study revealed that mean recovery of oxyfluorfen from water was 95.34 % at 1.0 ng g⁻¹ level of fortification. The corresponding value for 5.0 ng g⁻¹ fortification level was 86.69 %. Percent RSD_WR recorded for water at 1.0 ng g⁻¹ spiking level was 3.14 %. The corresponding value for 5.0 ng g⁻¹ spiking level was 0.90 %. LOD determined at 1.0 ng g⁻¹ water was 0.094 ng g⁻¹. The corresponding value for 5.0 ng g⁻¹ spiking level was 0.024 ng g⁻¹. The LOQ at 1.0 ng g⁻¹ fortification level for water was 0.281 ng g⁻¹. The corresponding value for 5.0 ng g⁻¹ fortification levels was 0.070 ng g⁻¹.

4.2 Depth wise distribution of oxyfluorfen in three different soils

In order to study the downward movement of oxyfluorfen in soil columns, residues of the oxyfluorfen was determined from 0-6 (D₁), 6-12 (D₂), 12-18 (D₃), 18-24 (D₄), 24-30 (D₅) cm depths after 60 days of the experimentation as mentioned 3.7.2.4.
Table 4.3: Recovery of oxyfluorfen in water

<table>
<thead>
<tr>
<th>Fortification level (ng g⁻¹)</th>
<th>Recovery (%)</th>
<th>Mean Recovery (%)</th>
<th>Standard deviation (SD)</th>
<th>% RSD_WR</th>
<th>LOD (ng g⁻¹)</th>
<th>LOQ (ng g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92.89 98.67 94.45</td>
<td>95.34</td>
<td>2.99</td>
<td>3.14</td>
<td>0.094</td>
<td>0.281</td>
</tr>
<tr>
<td>5</td>
<td>86.61 85.96 87.51</td>
<td>86.69</td>
<td>0.78</td>
<td>0.90</td>
<td>0.024</td>
<td>0.070</td>
</tr>
</tbody>
</table>
Chromatogram: Recovery of oxyfluorfen in water- Reagent Blank
Chromatogram: Recovery of oxyfluorfen in water-Control
Chromatogram: 1.0 ppm Oxyfluorfen standard

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret.Time</th>
<th>Name</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.62</td>
<td>Oxyfluorfen</td>
<td>108665</td>
<td>2200306</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>2200306</td>
</tr>
</tbody>
</table>
Chromatogram: Recovery of oxyfluorfen in water at 5 ppb level
4.2.1 Oxyfluorfen residues in column leachates:

With a view to evaluate the risk of groundwater contamination following the herbicidal application, fortified soil columns were regularly irrigated with 0.01 M CaCl₂ solution and leachates were analysed as mentioned in section 3.7.1.

**Table 4.4: Oxyfluorfen residues (ng g⁻¹) in column leachates**

<table>
<thead>
<tr>
<th>Days of sampling</th>
<th>Column leachates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sandy (S₁)</td>
</tr>
<tr>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>1</td>
<td>1.367</td>
</tr>
<tr>
<td>3</td>
<td>6.013</td>
</tr>
<tr>
<td>5</td>
<td>7.771</td>
</tr>
<tr>
<td>7</td>
<td>13.759</td>
</tr>
<tr>
<td>10</td>
<td>4.398</td>
</tr>
<tr>
<td>15</td>
<td>2.452</td>
</tr>
<tr>
<td>30</td>
<td>1.629</td>
</tr>
<tr>
<td>45</td>
<td>0.246</td>
</tr>
<tr>
<td>60</td>
<td>0.084</td>
</tr>
</tbody>
</table>

BDL: Below determination limit, BDL of water: 0.281 ng g⁻¹

Data presented in Table 4.4 show that oxyfluorfen residues confirmed up to 60 days except 0 day in sandy soil. In sandy soil the residues showed increasing trend up to 7th day that is 13.759 ng g⁻¹ and indicated decreasing trend thereafter till the 60th day. Absence of the herbicide residues in leachates collected beyond 30 cm depth of the soil column in spite of the high dose of application, reveal no risk of groundwater contamination in case of the sandy loam and clayey soils. However, the presence of the metabolites of this herbicide in
Figure 4.2: Oxyfluorfen residues in column leachates from sandy soil
Chromatogram: Residue of oxyfluorfen in sandy soil-Control
Chromatogram: Residue of oxyfluorfen in sandy soil- 0 Day

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret. Time</th>
<th>Name</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.20</td>
<td>Oxyfluorfen</td>
<td>82047.6</td>
<td>1582287.1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>1582287.1</td>
</tr>
</tbody>
</table>
Chromatogram: Residue of oxyfluorfen in sandy soil- 60 Day

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret.Time</th>
<th>Name</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.20</td>
<td>Oxyfluorfen</td>
<td>39985.7</td>
<td>820212.9</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>820212.9</td>
</tr>
</tbody>
</table>
Chromatogram: Residue of oxyfluorfen in sandy loam soil-Control
Peak # | Ret.Time | Name       | Area    | Height   \\
-------|----------|------------|---------|----------
   1    | 11.20    | Oxyfluorfen| 72091.6 | 1542795.3\\nTotal  |          |            |         | 1542795.3

Chromatogram: Residue of oxyfluorfen in sandy loam soil - 60 Day
Chromatogram: Residue of oxyfluorfen in clayey soil-Control
Chromatogram: Residue of oxyfluorfen in clayey soil - 0 Day

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret.Time</th>
<th>Name</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.19</td>
<td>Oxyfluorfen</td>
<td>123379.6</td>
<td>2285923.7</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>2285923.7</td>
</tr>
</tbody>
</table>
Chromatogram: Residual of oxyfluorfen in clayey soil - 60 Day

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret. Time</th>
<th>Name</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.20</td>
<td>Oxyfluorfen</td>
<td>73012.2</td>
<td>1652490.8</td>
</tr>
</tbody>
</table>
The same data are graphically depicted in Figure 4.2.

The residues were detected from the leachates collected from sandy soil only. Organic matter is low in sandy soils compared to sandy loam and clayey soils. Porosity and saturated conductivity significantly affect herbicide movement and in the sandy soils, the water flux is greater than the higher clay content soils.

Soil column leaching study was conducted by Valliappan et al. (1990) using Poly Vinyl Chloride (PVC) tubes 30 cm long x 10 cm diameter. The columns were filled with sandy loam soil and oxyfluorfen @ 0.1 kg ha⁻¹ was applied to the opened ends. Oxyfluorfen was shown to be the least mobile, butachlor and benthiocarb to be moderately mobile and oxyfluorfen and pyrazosulfuron-ethyl to have leached furthest.

4.2.2 Depthwise distribution of oxyfluorfen residues in sandy, sandy loam and clayey soils

Data presented in Table 4.5 showed that oxyfluorfen mainly confined to the top layer (0-6 cm) in sandy soil. The residue data recorded at each depth were converted to percentage of total residues recovered from the column. The same data are graphically depicted in Figure 4.3.

The downward movement of oxyfluorfen in the column under laboratory conditions revealed that after 60 days of application, the
residues were mainly confirmed to the top three layers i.e. 0-6 cm, 6-12 cm and 12-18 cm in sandy soil. The residues were below determination limit in sandy loam and clayey soils even to the top layer of column.

Adsorption of pesticides to various soils usually follows the inverse generalization, supporting the contention that adsorption governs movement. Thus, those soil factors influencing pesticide adsorption—especially soil organic matter, clay and (sometime) soil pH have often been related to movement. Movement of organophosphorus insecticides was inversely related to their adsorption in soils (McCarty and King, 1966). Pesticide leaching was dependent on use pattern, soil texture, total organic carbon in soil, pesticide half-life and depth to water table.

Table 4.5 Depthwise distribution of oxyfluorfen residues in soils

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Oxyfluorfen residues (µg g⁻¹) in soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sandy (S₁)</td>
</tr>
<tr>
<td>0-6</td>
<td>0.057 (41.60)</td>
</tr>
<tr>
<td>6-12</td>
<td>0.042 (30.65)</td>
</tr>
<tr>
<td>12-18</td>
<td>0.038 (27.73)</td>
</tr>
<tr>
<td>18-24</td>
<td>BDL</td>
</tr>
<tr>
<td>24-30</td>
<td>BDL</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>0.137</td>
</tr>
</tbody>
</table>

BDL- Below determination limit (µg g⁻¹): Sandy- 0.021 Sandy loam- 0.033 Clayey- 0.022

*Figures in parentheses indicate percentage of total loss.
Figure 4.3: Depthwise distribution of oxyfluorfen in soil columns
In the present study, movement of oxyfluorfen in soil columns was not found beyond 18 cm in sandy soil, while in case of sandy loam and clayey soils, residues were below determination limit even in the top layer of the columns. This was in conformity with the findings of Ying and Williams (2000) who found that the leaching behaviour of oxyfluorfen in South Australian soils on packed soil columns and actual mobility using intact soil columns. On the packed soil columns, norflurazon was the most leachable herbicide. More of the herbicides were detected in the leachates from the sandy soils than from the sandy loam and clayey soils.

Leaching of sulfentrazone, isoxaflutole and oxyfluorfen in three soil profiles was studied by Melo et al. (2010). Oxyfluorfen @ 0.72 and 1.08 kg ha⁻¹ was applied on the surface of 30 cm PVC columns filled with sandy loam and clayey soils. Oxyfluorfen remained adsorbed in surface layers of soil only. When compared the types of soil, the highest herbicide activity and movement occurred in sandy loam soil compared to clayey soil. Oxyfluorfen showed low leaching potential.

Leaching potential of herbicides used in cotton crop under soil column conditions was studied by Inoue et al. (2010) to evaluate the influence of different irrigation depth on the leaching potential of four herbicides in sandy and clayey soils. Regardless of the irrigation depth applied in sandy soil, oxyfluorfen did not exceed the depth of 5-10 cm.
Chromatogram: Residue of oxyfluorfen in sandy soil - Depth I
In columns filled with clay soil, oxyfluorfen did not move beneath the surface layer, even under the highest water depths. A more intense downward movement of the herbicide molecules was found in sandy soil than in clay soil.

Movement and persistence of imazaquin, oxyfluorfen, flurochloridone and terbacil in a sandy loam soil under field conditions for 2 years was studied by Milanova and Grigorov (1996). Oxyfluorfen @ 1.2 kg a.i. ha\(^{-1}\) showed least mobility in the soil profile. It was retained in the 0.5 cm layer throughout the experimental period in both the years.

A field study was performed to evaluate the accuracy of six pesticide screening leaching indexes for herbicide movement by Kogan et al. (2007). Adsorption, dissipation and soil movement were studied in a vineyard in a sandy loam soil during 2005 season. They observed that simazine reached 120 cm, diuron 90 cm, flumioxazin 30 cm soil depth respectively. Pendimethalin and oxyfluorfen were retained up to 5 cm.

**4.3 Persistence of oxyfluorfen in different types of soils**

Persistence study of herbicides is important to determine potential water and soil contamination and verify the actual herbicide concentration in the soil to assure long term weed control. Taking this point under consideration, an investigation was carried out to determine the persistence of oxyfluorfen in three different soils viz.,
sandy, sandy loam and clayey. Prior to persistence study, physico-chemical properties of each soil were determined which are mentioned in Table 3.1 of Materials and Methods chapter. The detailed methodology of the experiment is mentioned in Chapter-3. The persistence of oxyfluorfen was determined at 0.5 \( \mu \text{g g}^{-1} \) and samples were collected on 0, 1, 3, 5, 10, 20, 30, 45 and 60 days after application. The data obtained from the study were subjected to regression analysis to determine the persistence and half-life (DT50) which is mentioned in Table 4.6.

While studying the degradation pattern of the oxyfluorfen it was observed that it follows the first order kinetics and mathematically represented as bellow:

\[ C_t = C_0 . e^{-kt} \]

Where, \( C_t \) = Residues (\( \mu \text{g g}^{-1} \)) of pesticide at time ‘t’
\( C_0 \) = Initial concentration of pesticide
\( k \) = Rate constant

The half-life of oxyfluorfen was also calculated by using the following formula (Hoskins, 1961 and Timme and Frehse, 1980).

\[ \text{Log} \ 2 \]
\[ DT_{50} = \frac{-}{k} \]

Where, \( DT_{50} \) denotes the dissipation half-life of the oxyfluorfen, and \( k \) is the slope of the regression equation
4.3.1 Sandy soil

The residues of oxyfluorfen recorded in sandy soil on 0 day was 0.408 μg g⁻¹. This was dissipated at faster rate in this soil. The residues of oxyfluorfen recorded on 1, 3, 5, 10, 20, 30, 45 and 60 days after treatment were 0.407, 0.404, 0.347, 0.346, 0.304, 0.271, 0.198 and 0.149 μg g⁻¹, respectively. The dissipation pattern is depicted in Figure 4.4. The persistence of oxyfluorfen in sandy soil followed first order kinetics. In order to work out the rate of degradation, BDL level was calculated as half of 0.021 μg g⁻¹ (BDL for sandy soil) and thus the degradation showed first order kinetics with half-life of 43.00 days.
<table>
<thead>
<tr>
<th>Days</th>
<th>To</th>
<th>T1 (0.5 μg g⁻¹)</th>
<th>T2 (0.5 μg g⁻¹)</th>
<th>Clayey (Control) (0.5 μg g⁻¹)</th>
<th>Sandy loam (Control) (0.5 μg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>ND</td>
<td>0.408</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>ND</td>
<td>0.407 (0.25)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>0.404 (0.98)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>0.347 (14.95)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>ND</td>
<td>0.346 (15.20)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>20</td>
<td>ND</td>
<td>0.304 (25.49)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>30</td>
<td>ND</td>
<td>0.271 (33.58)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>45</td>
<td>ND</td>
<td>0.198 (51.47)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>60</td>
<td>ND</td>
<td>0.149 (63.48)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>DT50 (Days)</td>
<td>43.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression equation</td>
<td>0.984</td>
<td>y = -0.0071x + 1.6132 (0-60 day)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Figures in parentheses indicate percentage of total loss.
ND = Not detected, BDL = Below Determination Limit
BDL = 0.021 μg g⁻¹, 0.033 μg g⁻¹ and 0.022 μg g⁻¹ for sandy, sandy loam and clayey soil, respectively.
Results and Discussion

4.3.2 Sandy loam soil

The residues of oxyfluorfen recorded in sandy loam soil on 0 day was 0.496 µg g⁻¹. The residues of oxyfluorfen recorded on 1, 3, 5, 10, 20, 30, 45 and 60 days after treatment were 0.442, 0.439, 0.437, 0.428, 0.422, 0.417, 0.361 and 0.225 µg g⁻¹, respectively (Table 4.6). The dissipation pattern is depicted in Figure 4.4. The limit of detection (LOD) and limit of quantification (LOQ) of oxyfluorfen determined for sandy loam soil was 0.011 and 0.033 µg g⁻¹, respectively. In order to work out the rate of degradation, BDL level was calculated as half of 0.033 µg g⁻¹ (BDL for sandy loam soil) and thus the degradation showed first order kinetics with half-life of 60.20 days.

4.3.3 Clayey soil

The residues of oxyfluorfen recorded in clayey soil on 0 day was 0.498 µg g⁻¹ for standard dose of oxyfluorfen. The residues of oxyfluorfen recorded on 1, 3, 5, 10, 20, 30, 45 and 60 days were 0.495, 0.488, 0.482, 0.479, 0.446, 0.440, 0.239 and 0.195 µg g⁻¹, respectively. The dissipation pattern is depicted in Figure 4.6. The limit of detection (LOD) and limit of quantification (LOQ) of oxyfluorfen determined for clayey soil was 0.010 and 0.030 µg g⁻¹, respectively (Table 4.2). In order to work out the rate of degradation, BDL level was calculated as half of 0.030 µg g⁻¹ (BDL for clayey soil) and thus the degradation showed first order kinetics with half-life of 75.25 days.
Figure 4.4: Persistence of oxyfluorfen in sandy soil
Figure 4.5: Persistence of oxyfluorfen in sandy loam soil
Figure 4.6: Persistence of oxyfluorfen in clayey soil

\[ y = -0.004x + 1.704 \]

\[ R^2 = 0.958 \]
The persistence of oxyfluorfen was found considerably low and dissipated rapidly in all three soils as dissipation half-life of oxyfluorfen varied between 43.00 to 75.25 days (Table 4.6). Maximum persistence of oxyfluorfen was observed in clayey soil (DT$_{50}$ 75.25 days) while lowest in sandy soil (DT$_{50}$ 43.00 days).

Clay and organic matter contents are the two major soil components of significance to herbicide behaviour and persistence in soil. The chemical properties of herbicides which affect the persistence in soil are solubility in water, vapour pressure and bio-chemical stability of surface. The composition of solid and liquid phase of soil medium defines the adsorption-desorption of herbicide from and into the liquid phase. The chemical mobility and reactivity is a function of natural variation of soil mineralogical and chemical composition (Yaron et al. 1985). Adsorption of pesticide is the key process in soil because detoxification mechanisms, such as degradation, metabolism, microbial uptake and mobilization involve only the nonsorbed fraction of molecules. The extent of adsorption depends on various soil properties, including organic matter contents, type and amount of clay, ion exchange capacity and pH. Moreover, various physiochemical parameters of these compounds, such as water solubility and octanol-water partition coefficient, also play an important role in determining adsorption extent (Singh et al. 1990; Barriuso et al. 1992). Generally,
Chromatogram: 1.0 ppm Oxyfluorfen standard

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret. Time</th>
<th>Name</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.21</td>
<td>Oxyfluorfen</td>
<td>86149</td>
<td>1656309.8</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>86149</td>
<td>1656309.8</td>
</tr>
</tbody>
</table>
Chromatogram: Residue of oxyfluorfen in sandy soil - 7 Day
it has been found that adsorption of pesticides is positively correlated with octanol-water partitioning coefficient and negatively correlated with their water solubility.

Persistence of oxyfluorfen at different temperature and moisture levels in Alfisol and Vertisol was studied by Sireesha et al. (2012) under laboratory conditions. The result showed that at high temperature and moisture level, the half-life values were lower compared to low temperature and moisture level.

Movement and persistence of imazaquin, oxyfluorfen, flurochloridone and terbacil in a sandy loam soil under field conditions for 2 years was studied by Milanova and Grigorov (1996). Oxyfluorfen @ 1.2 kg a.i. ha⁻¹ showed least mobility in the soil profile. It was retained in the 5 cm layer throughout the experimental period in both the years.

Under field conditions, oxyfluorfen was applied @ 240, 120 and 60 g ha⁻¹ on onion. Onion and soil samples from 0-5 cm depth were collected for residue studies. The results showed that herbicide residues in soil varied with application and sampling depth and initial concentration indicated that there was little or no loss during application. No residues of oxyfluorfen were found in the drainage water leaving the treated area (Frank et al., 1991).
Dumas et al. (2008) studied the dissipation rate of prometryn, oxyfluorfen and imazethapyr in the Molisol San Anton and the Vertisol both soils from the south semi-arid coastal region of Puerto Rico. Herbicide rates were 2.24, 0.28 and 0.070 kg a.i. ha\(^{-1}\) for prometryn, oxyfluorfen and imazethapyr, respectively. Soil samples at 15 cm depth were collected at 0, 28, 56, 112 and 168 days after application. Field half-lives between 2 and 43, 31 and 88, and 19 and 46 days were found for prometryn, oxyfluorfen and imazethapyr, respectively.

The dissipation and mobility of the herbicide oxyfluorfen in field soil of Taiwan were investigated in the laboratory with six tea garden soils by JuiHung et al. (2003). The results indicated that the half-life of oxyfluorfen ranged from 72 to 160 days.
Summary
and
conclusion.
Oxyfluorfen is used for the control of annual grasses and broad-leaved weeds in tropical and subtropical crops, by pre- or post-emergence application in maize, rice, gram, groundnut, soybean, cotton and onion crops. It is selective contact herbicide and absorbed more readily by the foliage (and especially the shoots) than by the roots, with very little translocation.

Looking to the above facts, laboratory study entitled “To study the degradation and downward movement of oxyfluorfen in sandy, sandy loam and clayey soils” was proposed to study the degradation and downward movement of oxyfluorfen under laboratory conditions with the following objectives:

1. To validate the pesticide residue method of oxyfluorfen for sandy, sandy loam and clayey soils.
2. To study the depth-wise distribution of oxyfluorfen in soil columns.
3. To study the degradation of oxyfluorfen in sandy, sandy loam and clayey soils under laboratory conditions.
4. To study the level of oxyfluorfen in column leachates.

The summary and conclusion drawn from these investigations are outlined under the following subheadings.
5.1 Oxyfluorfen residues in column leachates:

The oxyfluorfen residues confirmed up to 60 days except 0 day in sandy soil. In sandy soil the residues showed increasing trend up to 7th day that is 13.759 ng g⁻¹ and indicated decreasing trend till the 60th day. In case of sandy loam and clayey soils the residues were below determination limit until the termination of experiment.

5.2 Depthwise distribution of oxyfluorfen residues in columns:

The downward movement of oxyfluorfen in the column under laboratory conditions revealed that after 60 days of application, the residues were mainly detected to the top three layers i.e. 0-6 cm, 6-12 cm and 12-18 cm in sandy soil, while in case of the sandy loam and clayey soil the residues were below detection limit even to the top layer of column.

5.3 Persistence of oxyfluorfen in different types of soils

A laboratory trial was carried out to determine the persistence of oxyfluorfen in three different soils viz., sandy, sandy loam and clayey. Prior to persistence study, physico-chemical properties of each soil were determined. The persistence of oxyfluorfen was studied at 0.5 µg g⁻¹ level and samples were collected on 0, 1, 3, 5, 10, 20, 30, 45 and 60 days after application. The analytical method employed for the extraction of oxyfluorfen was found accurate and precise as mean recovery at two levels in three types of soil was in the range of 94.79 to
119.56 % and % RSDWR in the range of 2.10 to 4.88 %. The LOD and LOQ of the method for different soils were also determined.

5.3.1 Sandy soil

The residues of oxyfluorfen recorded in sandy soil on 0 day was 0.408 μg g⁻¹, which persisted up to 60th day i.e. 0.149 μg g⁻¹. The persistence of oxyfluorfen in sandy soil followed first order kinetics. In order to work out the rate of degradation, BDL level was calculated as half of 0.021 μg g⁻¹ (BDL for sandy soil) and thus the degradation showed first order kinetics with half-life of 43.00 days.

5.3.2 Sandy loam soil

The residues of oxyfluorfen recorded in sandy loam soil on 0 day was 0.496 μg g⁻¹, which persisted up to 60th day i.e. 0.225 μg g⁻¹. The limit of detection (LOD) and limit of quantification (LOQ) of oxyfluorfen determined for sandy loam soil was 0.011 and 0.033 μg g⁻¹, respectively. In order to work out the rate of degradation, BDL level was calculated as half of 0.033 μg g⁻¹ (BDL for sandy loam soil) and thus the degradation showed first order kinetics with half-life of 60.20 days.
5.3.3 Clayey soil

The residues of oxyfluorfen recorded in clayey soil on 0 day was 0.498 μg g⁻¹, which persisted up to 60th days i.e. 0.195 μg g⁻¹. The limit of detection (LOD) and limit of quantification (LOQ) of oxyfluorfen determined for clayey soil was 0.007 and 0.022 μg g⁻¹, respectively. In order to work out the rate of degradation, BDL level was calculated as half of 0.022 μg g⁻¹ (BDL for clayey soil) and thus the degradation showed first order kinetics with half-life of 75.25 days.

5.4 CONCLUSION

- The persistence of oxyfluorfen was the highest in clayey soil followed by sandy loam and sandy soils.
- The downward movement of oxyfluorfen in column study was up to 18 cm in sandy soil. In case of sandy loam and clayey soil residues were below determination limit. This confirms the low risk of sub-soil contamination in sandy loam and clayey soils compared to the sandy soil.
- High adsorption of oxyfluorfen on soil makes it less hazardous from groundwater contamination point of view.
Bibliography
VI. BIBLIOGRAPHY


